Effect of *Averrhoa bilimbi* Fruit Filtrate and Shrimp Paste Mixture on Performance, Gut Microbes and Blood Profile of Broilers

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ABSTRAK

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Penelitian bertujuan mengevaluasi pengaruh kombinasi filtrat buah belimbing wuluh (*Averrhoa bilimbi* L) dan terasi (*Mysis* sp.) terhadap performa pertumbuhan, profil darah, populasi mikroba usus dan pH saluran pencernaan ayam broiler. Campuran filtrat buah belimbing wuluh dan terasi diinkubasi selama 4 hari dan digunakan dalam penelitian. Penelitian *in vivo* menggunakan 40 ekor ayam broiler strain Lohmann umur sehari yang didistribusikan secara acak pada dua kelompok perlakuan, meliputi kontrol (T1) dan ayam yang diberikan kombinasi filtrat buah belimbing wuluh dan terasi sebanyak 10% dalam air minum (T2). Bobot badan dan konsumsi pakan dicatat setiap minggu. Pada hari ke-42, 2 ekor ayam dari setiap pen (8 ayam per kelompok perlakuan) diambil untuk pengambilan darah dan digesta. Bobot organ internal dan karkas ditimbang setelahnya. Hasil penelitian menunjukkan bahwa nilai konversi pakan (FCR) lebih rendah ($P<0,05$) pada ayam yang diberi kombinasi filtrat buah belimbing wuluh dan terasi dibandingkan dengan kontrol. Hemoglobin dan volume rata-rata sel darah merah (MCV) ayam perlakuan lebih tinggi ($P<0,05$) dari ayam kontrol. Total kolesterol darah ayam perlakuan lebih tinggi ($P<0,05$) dari kontrol. Total *coliform* dalam sekum ayam perlakuan lebih rendah ($P<0,05$) dari kontrol. Nilai pH saluran pencernaan (jejunum, ileum dan sekum) ayam yang diberikan kombinasi filtrat buah belimbing wuluh dan terasi lebih rendah ($P<0,05$) dari ayam kontrol. Kesimpulan pada penelitian ini bahwa pemberian campuran filtrat buah belimbing wuluh dan terasi ke dalam air minum memperbaiki FCR, meningkatkan nilai hemoglobin dan MCV, menurunkan pH usus dan bakteri *coliform* pada sekum ayam broiler.

Kata Kunci: Belimbing Wuluh, Ayam Broiler, Filtrat, Performa, Terasi

ABSTRACT

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This study was aimed to evaluate effect of a mixture of *Averrhoa bilimbi* fruit filtrate and shrimp paste (*Mysis* sp.) on the growth performance, blood profile, selected intestinal bacterial number and pH value of broiler digestive tract. The mixture of *A. bilimbi* fruit filtrate and shrimp paste were incubated for 4 days and were then used in the experiment. For *in vivo* experiment, 40 day-old Lohmann broiler chicks were distributed randomly to two treatment groups, i.e., control (T1) and chickens given the mixture of 10% *A. bilimbi* fruit filtrate and shrimp paste in drinking water (T2). Body weight and feed intake were recorded weekly. At day 42, 2 birds from each pen (8 chicks per treatment group) were taken for blood and digesta collection. Internal organ weight and carcass traits were determined thereafter. Feed conversion ratio (FCR) was lower ($P<0.05$) on the treatment group than the control. Hemoglobin and mean corpuscular volume (MCV) of the treatment group were higher ($P<0.05$) than that of the control. Total cholesterol was higher ($P<0.05$) in the treatment group than that in control. Total cecum coliform was lower ($P<0.05$) in the treatment group than that in the control. The pH values of the small intestinal segments (jejunum, ileum, cecum) were lower ($P<0.05$) in the treatment group than that in the control group. In conclusion, administration of the blends of *A. bilimbi* fruit filtrate and shrimp paste into drinking water improved FCR, increased hemoglobin and MCV values, decreased gut pH and cecal coliform of broiler chickens.

Key Words: *Averrhoa bilimbi* L, Broiler Chicken, Filtrate, Performance, Shrimp Paste

INTRODUCTION

Antibiotic growth promoters (AGP) have long been used to boost the growth rate and control pathogenic bacteria in broiler production. However, despite its advantageous effects, the continuous use of AGP may cause microbial resistance and leave residues in the meat of broilers (Singh et al. 2014). Among the alternatives that can be used to replace AGP in poultry production are organic acids and probiotics.

Organic acids may serve as acidifier, which may consist of citric acid, lactic acid, propionate, acetic acid or the mixture of some organic acids. Organic acid is applied as feed additive into feed or drinking water in order to enhance digestive enzyme activities, decrease the pH of the gut and maintain microbial balance in digestive tract of broilers (Octavia et al. 2018). Probiotic is a feed additive in the form of beneficial living microbes. It functions to improve ecosystem of the digestive tract of chickens (Sugiharto et al. 2017).

Organic acids may be combined with probiotics to increase the effectiveness of organic acid as an alternative to AGP (Sugiharto 2016). Abudabos et al. (2016) noticed that the combination of organic acid and *Bacillus* sp. probiotic could effectively replace AGP in terms of improving growth rate, nutrient absorption and controlling pathogenic bacteria in the gut of broilers such as *Salmonella* sp., *Escherichia coli* and *Clostridium perfringens* in the intestine of broilers.

Averrhoa bilimbi L. fruit is a sour fruit that has a potential to be used as an alternative antibiotic for broilers. *A. bilimbi* belongs to the Oxalidaceae family and is easily cultivated in tropical countries. In Indonesia, *A. bilimbi* fruit is called as *belimbing wuluh* and has traditionally been used to cure many diseases such as fever, hypertension and inflammation (Dewi et al. 2019). The fruit may also be used as a source of natural organic acids due to its high contents of acetic acid, citric acid, lactic acid, propionic acid and formic acid as well as other active substances such as phenol, vitamin C and tannin (Patil et al. 2013). Nakyinsige et al. (2016) further reported that *A. bilimbi* fruit filtrate contains antibacterial substance, which can inhibit the growth of pathogenic microbes. *A. bilimbi* filtrate also contains nutrients such as amino acid and glucose. Glucose contained in *A. bilimbi* fruit filtrate can be used by lactic acid bacteria as the source of nutrient for bacterial growth and development (Kumar et al. 2013). For this reason, *A. bilimbi* fruit filtrate has potential to be the substrates for lactic acid bacteria.

Shrimp (*Mysis* sp.) paste is a traditional food additive with special aroma and is made from the fermentation of shrimp. As with other fermented products, shrimp paste can be a natural source of lactic acid bacteria and can thus be a source of probiotics (Amalia et al. 2018). Kobayashi et al. (2003) reported that in general, the total colonies of lactic acid bacteria in Indonesian shrimp paste ranged from 4 to 6 log cfu/g. There are several lactic acid bacteria species isolated from shrimp paste, including *Lactobacillus plantarum, Lactococcus lactis, Vagococcus fluvialis* and *Lactococcus garvieae* (Maeda et al. 2014), with *L. plantarum* being the most dominant lactic acid bacteria species (Amalia et al. 2018).

Considering all facts about *A. bilimbi* fruit filtrate and shrimp paste, a mixture of *A. bilimbi* fruit filtrate and shrimp paste was therefore expected to improve the productivity and ecosystem of broiler's digestive tract. To date, publications or reports regarding the use of a mixture of *A. bilimbi* fruit filtrate and shrimp paste in broiler chicken have not been found in the literature. Therefore, this study was aimed to evaluate the effect of the mixture of *A. bilimbi* fruit filtrate and shrimp paste on growth performance, blood profile, selected intestinal bacterial number and pH value of broiler digestive tract.

MATERIALS AND METHODS

Preparation of the mixture of *A. bilimbi* fruit filtrate and shrimp paste

In this study, ripe (yellowish peel and soft texture) of *A. bilimbi* and shrimp paste which obtained from the district of Rembang were used. *A. bilimbi* fruit was crushed using electric blender and filtrated using cheesecloth. The filtrate of *A. bilimbi* fruit was placed into an anaerobe jar, and shrimp paste was then added (1 g/1000 ml) into it. The mixture of *A. bilimbi* fruit filtrate and shrimp paste was incubated for 4 days at room temperature in anaerobic conditions. After the incubation, the sample pH was determined and enumerated for lactic acid bacteria content. The preparation protocol was based on our preliminary *in vitro* study for assessing the best ripening stages of *A. bilimbi* fruit, concentration and brands of shrimp paste and time and condition of incubation (data published elsewhere). The number of lactic acid bacteria was determined based on total plate count method on *de Man*, *Rogosa* and *Sharpe agar* media (MRS; Merck KGaA, Darmstadt, Germany). The enumeration of bacteria was conducted after the sample was incubated in an anaerobic condition at 38°C for 48 hours. The pH value of the unincubated *A. bilimbi* fruit filtrate and after incubation with shrimp paste for 4 days were 1.5 and 1.2, whereas the number of lactic acid bacteria of the unincubated *A. bilimbi* fruit filtrate and after incubation with shrimp paste for 4 days were 5.30 log cfu/mL, and 30.56 log cfu/mL, respectively. The mixture was prepared for *in vivo* study and stored at -10ºC until used.

In vivo experiment and performance analysis

The *in vivo* experiment complied with the standard broiler rearing protocols established by the Laboratory of Poultry Production, Faculty of Animal and Agricultural Sciences, Diponegoro University. The trial was carried out on February to March 2020. The experiment was conducted on 40 day-old Lohmann broiler chicks with the average initial body weight of 47.3±0.78 g. During the rearing period, the chicks were fed with starter, grower and finisher rations. The mixture of *A. bilimbi* fruit filtrate and shrimp paste was added into drinking water as much as 10%. The feeds (in mash form) and drinking water were provided *ad libitum* throughout the study period. The chickens were vaccinated with Newcastle disease (ND) vaccine at 4 days of age through eye drop, IBD/Gumboro vaccine at 10 days of age and ND vaccine at the age of 18 days, both through drinking water. In this study, the chicks were divided into 2 treatment groups with 4 pens consisting of 5 chickens in each group. These groups were T1 (control, i.e., chickens given drinking water without the mixture) and T2 (chickens given drinking water containing 10% of the mixture).

During *in vivo* trial, the birds were raised in an open-sided broiler house with rice husk as bedding materials. They (5 chicks) were placed in 1×1 m^2 pen equipped with manual feeder and water container. The temperature and humidity inside the broiler house were adjusted using plastic curtains and electric fan. Temperature was adjusted at 32±1ºC on initial brooding period and then gradually reduced according to the age of broilers (around 27±1ºC on day 21 onward).

Body weight and feed consumption were recorded at weekly basis. At the end of experiment (day 42), 2 chickens from each pen (8 chicks per treatment group) were taken for sample collection. The chickens were weighed and then their blood was withdrawn through brachialis veins using 3 mL syringe. One mL blood was put into a tube given anticoagulants (ethylene diamine tetra acetic acid, EDTA) while the other 2 mL blood was put into a tube without anticoagulants. The non-anticoagulant blood was then centrifuged at 5,000 rpm for 15 minutes to obtain the serum. After blood sampling, the chicken was slaughtered and the digestive tract was taken. The digesta in the small intestine and ceca was collected. Some of the digesta was put into zip lock plastic bag for the determinations of selective bacteria enumerations. The rest of the intestinal digesta was used for pH measurement. The weight of internal organ, carcass and commercial cuts were also determined.

Blood profile analysis

Complete blood analysis was conducted using hematology analyzer (Prima Fully-auto Hematology Analyzer) and total counts of bacteria in the intestinal digesta were performed according to Sugiharto et al. (2018). Total triglycerides in serum was measured using enzyme calorimetric method with glycerol-3-phosphateoxidase (GPO). The triglycerides were determined after enzymatic separation using lipoprotein lipase, whereas total cholesterol was determined after enzymatic hydrolysis and oxidation. Both used indicator of quinoneimine produced from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase (DiaSys Diagnostic System GmbH, Holzheim, Germany). The LDL was precipitated using heparin. The HDL remained in the supernatant after centrifugation and was enzymatically processed by the CHOD-PAP method. The LDL concentration was calculated as the difference between total cholesterol and cholesterol in the supernatant. Coliform bacteria were determined in MacConkey agar (Merck KGaA) as red colonies after aerobic incubation at the temperature of 38°C for 24 hours. The number of lactic acid bacteria was enumerated on MRS (Merck KGaA) after anaerobic incubation at the temperature of 38°C for 48 hours.

Statistical analysis

Data were analyzed using independent-samples t-test with 5% accuracy rate (Sudjana 1989). The analysis was conducted using SPSS 22.0 software.

RESULTS AND DISCUSSION

Performances of broilers

Results of the present study show that addition of the mixture of *A. bilimbi* fruit filtrate and shrimp paste at 10% to drinking water decreased ($P<0.05$) FCR of broilers (Table 1). Nevertheless, the treatments did not significantly affect the body weight gain (BWG) and feed consumption of broilers. The improvement of FCR in the treated birds was possible because the administration of the mixture, as source of organic acid and probiotic improves the digestive process particularly protein through the increase in digestive enzyme activities (Salgado-Tránsito et al. 2011). Marín-Flamand et al. (2013) suggested that administration of organic acid mixtures of ascorbic, citric, malic, sorbic and tartaric acids would trigger the secretion of hormones such as gastrin and cytokinin, which will improve the digestion and absorption of feed protein, so that FCR of broilers would consequently improve. Likewise, Jin et al. (2000) reported that supplementation of *Lactobacillus* culture increased digestive enzyme activities, which thereby improved the digestion and utilization of nutrients by broilers.

Table 1. Performances of broilers

Items	T1	T2	SEM	P value
Body weight gain (g/bird)	1,839	1,913	70.0	0.64
Feed consumption (g/bird)	3,762	3,374	107	0.08
Feed conversion ratio (FCR)	2.05	1.76	0.06	<0.01

SEM: standard error of the means

Table 2. Blood profile of broilers

Items	T1	T2	SEM	P value
Leukocyte (10^9/L)	78.6	74.5	2.11	0.36
Erythrocyte (10^{12}/L)	2.84	3.32	0.13	0.07
Hemoglobin (g/dL)	9.25	12.0	0.54	0.01
Hematocrit (%)	37.8	41.4	1.42	0.21
Thrombocyte (10^9/L)	8.87	9.75	0.42	0.32
Lymphocyte (%)	67.3	76.8	3.03	0.12
Neutrophil (10^9/L)	3.56	4.37	0.52	0.46
Mean corpuscular volume (fl)	116	127	1.97	<0.01
Mean corpuscular hemoglobin (pg)	31.5	33.7	0.71	0.12
Mean corpuscular hemoglobin concentration (g/dL)	25.3	27.6	0.61	0.06

SEM: standard error of the means

Table 3. Lipid profile in serum of broilers

Items (mg/dL)	T1	T2	SEM	P value
Total cholesterol	96.4	120	5.68	0.04
Low-density lipoprotein (LDL)	26.7	36.1	4.11	0.27
High-density lipoprotein (HDL)	59.0	68.6	4.37	0.28
Total triglycerides	53.3	63.7	4.67	0.28

SEM: standard error of the means

Blood profile of broilers

Results showed that addition of the mixture into drinking water increased (P<0.05) hemoglobin and MCV values of broilers (Table 2). However, the treatments did not affect (P>0.05) the numbers of leukocytes, erythrocyte, hematocrit, thrombocyte, lymphocyte, neutrophil, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). In this study, the increased values of hemoglobin and MCV seemed to be attributed to the antioxidant components in the mixture in the form of saponin, tannin, flavonoids, terpenoids and steroids, which can prevent oxidative stress and improve blood profile of broiler chickens (Asna & Noriham 2014) Surai (2016) suggested that antioxidants possess the ability to minimalize free radical as well as to prevent the negative effect of free radicals by completing electron lacking in cell which is caused by exposure to free radicals.

The addition of electrons by antioxidant compound in cells can prevent the damage of nucleic acid, protein, fat and deoxyribonucleic acid (DNA). It has been understood that amino acids are the main components necessary for the process of blood synthesis. This means that the more nutrient components such amino acids and protein are available, the more optimum the process of blood synthesis. Optimum process of hematopoiesis will improve the blood profiles (Adil et al. 2011). Hemoglobin plays an important role in loading and binding oxygen in the lung and releasing it to parts of the body which need it. Considering that oxygen is needed in the process of metabolism, high total of hemoglobin and mean corpuscular volume

(MCV) will therefore make the metabolic process more optimum in broilers (Ugwuene 2011).

Lipid profile of broilers

The result showed that administration of the mixture into drinking water increased (P<0.05) total cholesterol in the serum of broilers (Table 3). However, the treatments did not influence (P>0.05) the levels of LDL, HDL and triglycerides in the serum. In this work, the increase in serum total cholesterol was in parallel with the increased LDL, HDL and total triglycerides values in the serum, though the differences were not statistically significant. Formerly, Ndelekwute et al. (2016) documented that organic acids administration was associated with the enhanced fat digestibility and utilization by broilers.

Considering the positive correlation between fat utilization (and metabolism) and blood cholesterol levels (Saleh et al. 2020), the possible increased fat digestibility and utilization in broilers treated with the mixture of *A. bilimbi* fruit filtrate and shrimp paste may therefore be attributed to the increased total serum cholesterol in the respective chicks. However, the latter inference should be noted with caution as we did not conduct the digestibility trial in this present study. Also, our finding was different from that of Taherpour et al. (2009) who reported that combination of organic acid and probiotic decreased serum total cholesterol in broiler chickens. Other study by Yakhkeshi et al. (2011) also reported that the use of probiotic as feed additive in broilers ration decreased total cholesterol of broilers. The differences in the natures and levels of organic acids as well as lactic acid bacteria between our product and the products used by other investigators were most likely be responsible for the divergent results above.

pH values and bacterial populations of intestine of broilers

The present study showed that addition of the mixture into drinking water decreased (P<0.05) pH values of jejunum, ileum and cecum (Table 4), but did not significantly affect pH value of duodenum of broilers. Sugiharto (2016) documented that the use of organic acid separately or in combination with probiotic decreased the pH values of the entire intestinal segments and may function as antibacterial substance to inhibit the growth of pathogenic microbes in the digestive tract of broilers. *A. bilimbi* fruit filtrate as the source of organic acids and shrimp paste as the source of lactic acid bacteria-based probiotic may promote the production of lactic acid and other short chain fatty acids (SCFA; acetic, butyric and propionic acids), which are generally known to decrease the pH values of the digestive tract of broilers (Dittoe et al. 2018).

The results of this study showed that addition of the mixture into drinking water decreased (P<0.05) total coliform in the cecum of broilers (Table 4). However, the treatment had no significant effect on coliform count in ileum and lactic acid bacteria populations in ileum and cecum of broilers. Nakyinsige et al. (2016) reported that *A. bilimbi* fruit filtrate contained some bioactive compounds including phenol and tannin, which can inhibit the growth of pathogenic microbes in the digestive tract of broilers. The latter study was confirmed by Tosi et al. (2013) documenting that tannin can function as antibacterial substance by coagulating bacteria protoplasm so that pathogenic bacteria cannot replicate and finally lysis. Sugiharto (2016) reported that lactic acid bacteria-based probiotic can perform competitive exclusion, which is a competition between pathogenic microbes such as coliform and non-pathogenic microbe such as lactic acid bacteria. Both types of bacteria compete to obtain nutrition and reside in small intestine of broilers. Moreover, lactic acid bacteria were able to produce lactic acid, which can inhibit the growth of pathogenic microbes in the digestive tract of broilers. Furthermore, Sumarsih et al. (2012) reported that lactic acid bacteria may produce natural antibiotic compound, which able to kill pathogenic microorganisms in broiler digestive tract.

Internal organ weights and carcass characteristics of broilers

Results of the experiment showed that administration of the mixture into drinking water did not affect (P>0.05) internal organ relative to weights of broilers (Table 5). This finding was in accordance with Youssef et al. (2017) confirming that administration of the combination of organic acid and probiotic into ration did not affect the relative weights of internal organ of broilers. In concurrence with this, Sugiharto et al. (2018) showed no impact of dietary administration of multi-strains probiotic combined with vitamins and minerals on the relative weights of internal organs of broilers.

The results showed that addition of the mixture into drinking water did not affect (P>0.05) the carcass weight and commercial cuts of broilers (Table 5). Malik et al. (2016) also reported that supplementation of the combination of organic acid and probiotic into rations did not make significant difference in carcass weight and commercial cuts of broilers. In general, some factors may affect the carcass characteristics of broilers, including genetic, sex, physiological status, age, final live body weight and nutrients (Hidayat et al. 2017).

Table 4. pH values and intestinal bacterial populations of intestine of broilers

Items	T1	T2	SEM	P value
pH values				
Duodenum	5.82	5.45	0.12	0.13
Jejunum	5.70	4.77	0.18	0.01
Ileum	6.00	4.95	0.20	<0.01
Cecum	6.54	6.07	0.10	0.02
Bacterial populations				
Coliform in ileum	5.50	5.06	0.28	0.20
Coliform in cecum	5.37	4.55	0.15	<0.01
Lactic acid bacteria in ileum	8.19	8.54	0.06	0.09
Lactic acid bacteria in cecum	8.70	8.85	0.27	0.06

SEM: standard error of the means

Table 5. Internal organ weights and carcass traits of broilers

Items	T1	T2	SEM	P value
Internal organ weights (% live body weight)				
Heart	0.33	0.31	0.01	0.40
Liver	1.72	1.63	0.08	0.63
Proventriculus	0.41	0.36	0.01	0.11
Gizzard	1.63	1.58	0.09	0.78
Pancreas	0.18	0.17	0.01	0.80
Abdominal fat	1.03	1.30	0.09	0.19
Duodenum	0.26	0.33	0.02	0.15
Jejunum	0.67	0.70	0.04	0.71
Ileum	0.49	0.58	0.03	0.15
Cecum	0.43	0.35	0.05	0.45
Spleen	0.09	0.08	0.01	0.35
Thymus	0.11	0.10	0.01	0.63
Bursa of Fabricius	0.03	0.03	0.00	0.25
Carcass traits				
Carcass weight (% live body weight)	54.2	54.8	1.55	0.87
Breast (% carcass)	35.8	35.2	0.51	0.56
Wings (% carcass)	11.6	11.3	0.24	0.42
Thigh (% carcass)	16.9	17.1	0.29	0.74
Drumstick (% carcass)	14.6	14.7	0.28	0.81
Back (% carcass)	21.1	21.7	0.54	0.56

SEM: standard error of the means

CONCLUSION

Administration of the mixture of *A. bilimbi* fruit filtrate and shrimp paste into drinking water improved FCR, increased hemoglobin and MCV values, decreased the pH of the gut and the number of faecal coliform of broiler chickens. In conclusion, the mixture was beneficial in improving feed efficiency, physiological condition and intestinal ecology of broiler chickens which inhibited the growth of pathogenic microbes in the digestive tract of broilers.

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