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Administration of Fermented *Averrhoa bilimbi* L. Fruit Filtrate on Growth, Hematological, Intestinal, and Carcass Indices of Broilers

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ABSTRACT

The study investigated the effects of administration of graded levels of fermented *Averrhoa bilimbi* L. fruit filtrate in drinking water on the growth performance, hematological variables, intestinal ecology, and carcass characteristics of broilers. The experiment was arranged based on a completely randomized design. Two hundred day-old-Lohmann broiler chicks were randomly assigned into 4 treatment groups, each consisting of 5 replications with 10 chicks in each replication. The treatments were administration of fermented *A. bilimbi* L. fruit filtrate through drinking water at concentrations of 0% (CONT), 0.5% (FAB05), 1.0% (FAB1), and 2.0% (FAB2). Blood was sampled on days 21 and 33, while intestinal segments and digesta were collected on day 33. Feed conversion ratio (FCR) was improved ($p<0.05$) with the increased concentrations of fermented filtrate in drinking water. Body weight, cumulative feed intake, and mortality of broilers were not affected by the treatments. On day 21, thrombocytes decreased ($p<0.05$) with the increased concentrations of fermented filtrate. On day 33, leukocytes and lymphocytes were lower ($p<0.05$) in treated chicks than in control. On day 21, the high-density lipoprotein (HDL) and aspartate aminotransferase (AST) increased ($p<0.05$) with the enhanced fermented filtrate concentrations. On day 33, creatinine and alanine aminotransferase (ALT) increased ($p<0.05$) following the increased fermented filtrate concentration in drinking water. Fermented fruit filtrate increased ($p<0.05$) jejunal villi height and ileal crypt depth. Fermented filtrate also resulted in higher ($p<0.05$) pH values of jejunum. The *Enterobacteriaceae* counts in the ileum decreased ($p<0.05$) with the increased fermented filtrate concentration in drinking water. Fermented fruit filtrate decreased ($p<0.05$) the liver and caeca weights and increased ($p<0.05$) the proventriculus weight. In conclusion, administration of 2% of fermented *A. bilimbi* L. fruit filtrate (pH 1.83) through drinking water improved FCR, physiological condition, and intestinal ecology of broilers. The addition of fermented fruit filtrate up to 2% in drinking water did not exert a negative effect on the carcass characteristics of broilers.

Keywords: broiler; fermented fruit filtrate; lactic acid bacteria; organic acids

INTRODUCTION

Antibiotic growth promoter (AGP) has been banned for use in the poultry industry since 2018 in Indonesia. The continued use of AGP has been proposed to be the increase in microbial resistance to antibiotics in poultry. Indeed, the negative effects are not only evident in livestock but also in humans (Abudabos *et al*., 2017; Sugiharto *et al*., 2018). The ban on the use of AGP has an impact on reducing the productivity and health status of broilers. Therefore, an AGP alternative is needed to ensure the sustainability of the broiler industry. Organic acids and probiotics are among alternatives for AGP that are widely applied in broiler chicken production in the current time.

Organic acids are additives that can be included in the feed or drink of broiler chicken (Yang *et al*., 2019). Based on previous literature, organic acids can lower pH values, thereby supporting the growth of positive bacteria in chicken's digestive tract (Palamidi & Mountzouris, 2018; Sabour *et al*., 2019). In addition, administration of organic acids can improve the immune responses of chickens against pathogenic bacteria, increase protein absorption, as well as feed consumption (Widiastuti *et al*., 2019). Several investigators have shown that giving probiotics based on lactic acid bacteria can increase the productivity and immune system of broilers (Bai *et al*., 2013, Olnood *et al*., 2015, Forte *et al*., 2016). Also, probiotics could improve intestinal morphology and modify bacterial population of broilers (Awad *et al*., 2009; Bai *et al*., 2013; Sugiharto *et al*., 2017). Although many studies have reported the positive effects of organic acids and probiotics on chicken productivity and health, many studies have reported contradicting results. One study reported that administration of formic acid and butyric acid did not

increase the population of lactic acid bacteria in ileum of broilers (Boroojeni *et al*., 2014). Brzóska *et al*. (2013) reported that administration of commercial organic acid Acidomix AFG did not reduce the mortality rate of broiler chickens. With regard to probiotics, Gao *et al*. (2017) noticed that probiotic *Lactobacillus plantarum* did not increase lactic acid bacteria counts in the digestive tract of broiler chickens compared to control. Recently, nutritionists have combined organic acids and probiotics to improve their efficacy as AGP alternatives. Rodjan *et al*. (2018) documented that the blends of organic acids and probiotics resulted in a better intestinal morphology relative to the administration of organic acid or probiotic alone. Other studies also reported that combination of butyric acid and probiotic *Bacillus subtilis* was attributed to a better weight gain, feed consumption, and feed conversion in chickens when compared with the single administration of butyric acid or probiotic (Widiastuti *et al*., 2019).

Underutilized fruits such as *Averrhoa bilimbi* L. appears to have potency as a source of organic acids due to its high contents of acetic acid and citric acid (Renatami *et al*., 2018). Given the beneficial impacts of these acids (Chowdhury *et al*., 2009; Fazayeli-Rad *et al*., 2014), *A. bilimbi* L. can therefore function as an acidifier for broiler chickens (Wiradimadja *et al*., 2015). In the form of fruit filtrate, *A. bilimbi* L. has low pH values ranging from 1.3 to 1.8 (Wijayanti *et al*., 2019). Aside from being an acidifier, *A. bilimbi* L. filtrate may also be a source of natural probiotics. This is actually inspired by the study of Rodriguez *et al*. (2019) revealing that, naturally, the acid fruits contain lactic acid bacteria that can serve as probiotics. Other studies also noticed that *A. bilimbi* L. was able to act as an antibacterial agent (Alhassan & Ahmed, 2016; Aziz, 2016). Owing to the above-mentioned facts, the application of *A. bilimbi* L. filtrate as a feed additive was therefore expected to exert growth- and health-promoting effects on broiler chickens due to the combined effects of organic acids and probiotic microorganisms in the fruit filtrate. Fermentation is a process that is commonly attributed to the increased lactic acid bacteria counts as well as decreased pH values of substrates (Sugiharto & Ranjitkar, 2019). Considering the presence of natural lactic acid bacteria in the fruit (Rodriguez *et al*., 2019), in the present study *A. bilimbi* L. filtrate was spontaneously fermented to increase the numbers of lactic acid bacteria as well as organic acid production in the filtrate. To the best of our knowledge, there is no study exploring the use of fermented *A. bilimbi* L. fruit filtrate in broiler chickens so far.

The purpose of this study was to investigate the effects of addition of graded concentrations of fermented *A. bilimbi* L. fruit filtrate in drinking water on growth performance, hematological parameters, intestinal ecology, and carcass characteristics of broiler chickens.

MATERIALS AND METHODS

Preparations of Fermented *A. bilimbi* L. Fruit Filtrate

The filtrate was prepared from the ripe *A. bilimbi* L. fruit collected from the gardens around the campus. The *A. bilimbi* L. fruit was firstly washed with running water, drained, and then blended using a medium-speed blender without adding water. The juice obtained was filtered with cheesecloth to obtain fruit filtrate. The *A. bilimbi* L. filtrate was placed in anaerobic jars and then spontaneously fermented for 0, 2, 4, and 6 days at room temperature (±25ºC). Samples of fermented-fruit filtrate were collected from each incubation period and then determined for the total lactic acid bacteria and pH values (using automatic pH meter; Eco Test pH 1). Each treatment consisted of 3 replicates. The numbers of lactic acid bacteria were counted on the selective agar medium (MRS agar; Merck KGaA, Darmstadt, Germany) after anaerobic incubation at 38ºC for 48 h. For the subsequent *in vivo* experiment, *A. bilimbi* L. fruit filtrate was anaerobic-spontaneously fermented for 4 days at room temperature.

Broiler Chicken Trial

The *in vivo* experiment was approved by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (No. No. 57-01/A-2/KEP-FPP). The study was arranged according to a completely randomized design. A total of 200 day-old-Lohmann broiler chicks (average body weight of 50.23±0.47 g; means ± standard deviations) were divided into 4 treatment groups, each consisting of 5 replications (with 10 chicks in each replication; about 5 males and 5 females). Throughout the rearing, the chicks were fed commercial starter and finisher diets (Table 1). The fermented *A. bilimbi* L. fruit filtrate (fermented anaerobically for 4 days at room temperature) was daily administrated in drinking water (water and fruit filtrate were replaced daily) and provided *ad libitum* from the initial until the end of the experiment. The fermented fruit filtrate was administrated at the following concentrations: i.e., 0% (CONT, drinking water containing 0% fermented *A. bilimbi* L. fruit filtrate), 0.5% (FAB05, drinking water containing 0.5% fermented *A. bilimbi* L. fruit filtrate), 1.0% (FAB1, drinking water containing 1.0% fermented *A. bilimbi* L. fruit filtrate), and 2.0% (FAB2, drinking water containing 2.0% fermented *A. bilimbi* L. fruit filtrate).

The birds were vaccinated on days 4 and 17 using Newcastle disease vaccine through eye drops and drinking water, respectively. The chicks were also vaccinated

Table 1. Analyzed chemical compositions of commercial rations

Compositions	Starter (day 1-21)	Finisher (day 22-33)
Crude protein (%)	18.5	16.5
Crude fiber (%)	7.55	8.86
Fat (%)	7.06	7.26
Moisture (%)	10.3	10.5
Ash (%)	11.7	7.66
Metabolizable energy (kcal/kg)[1]	3,284	3,388

Note: [1]Metabolizable energy was predicted based on the formula (Bolton, 1967): 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen - free extract] + 2.5}.

on day 12 with an infectious bursal disease vaccine through drinking water. During the study, the chicks were raised in an opened-sided broiler house using rice husk as bedding. The birds were raised under a continuous light program throughout the experimental period. Light bulbs and plastic curtains were used to control the temperature and humidity in the broiler house during the experiment.

Sample Collection and Analysis

The weight of birds, feed consumption, and feed conversion ratio (FCR) were weekly recorded during the experiment. On days 21 and 33, one male chick representing the average body weight from each pen/replicate was taken, and blood sampled through their brachial veins on the wing. The use of male chicks with body weight representing the body weight of each pen for the sample collection was to avoid the gender- and body weight-related physiological variations. The collected blood sample was placed in a tube containing EDTA for the determination of a complete blood profile and the rest of blood was placed in a tube without anticoagulants for the production of serum. To make serum, the blood was allowed to stand at room temperature for 2 h and then centrifuged at 5,000 rpm for 10 min. The produced serum was stored (at -10°C) until analysis. On day 33, the same chicks used for blood samplings were slaughtered, de-feathered, and dissected. The internal organs of chickens were obtained and weighed (empty condition) using analytical balance. The digesta were collected from ileum and caecum for pH measurement and enumerations of bacteria in the intestine. The segments (about 2 cm) of duodenum, jejunum, and ileum were taken and placed in a 10% neutral formalin buffer solution for the assessment of gut morphology. The pH of intestinal digesta was measured using a digital pH meter (Eco Test pH 1). The carcass and commercial pieces of each chicken were also determined at the end of the trial.

The complete blood profile analysis was performed using a hematology analyzer (Prima Fully-auto Hematology Analyzer, PT. Prima Alkesindo Nusantara, Jakarta, Indonesia) following the procedures by Sugiharto *et al*. (2018). The lipid parameters, uric acid, and creatinine levels in the serum were analyzed according to the enzymatic colorimetric test. The spectrophotometric/photometric tests were applied to measure the total serum of protein, albumin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). The concentrations of globulin in the serum was obtained from the total protein subtracted by albumin in the serum. The biochemistry analysis of serum was carried out on the basis of manufacturer's instructions (DiaSys Diagnostic System GmbH, Holzheim, Germany).

The histological measurement of the small intestine of experimental birds was carried out based on the procedure described by Tunç *et al*. (2019). In brief, a 5 μm intestinal slice was stained using hematoxylin and eosin. An optical microscope connected to a digital camera was used to measure villus height and crypt depth. For each bird, the average villi height and crypt depth were obtained from 5 measurements. The bacterial populations in the intestine was enumerated as previously described by Sugiharto *et al*. (2018). The numbers of coliform and lactose-negative *enterobacteriaceae* were counted on MacConkey agar (Merck KGaA, Darmstadt, Germany) as red and colorless colonies following aerobic incubation at 38°C for 24 h. *Enterobacteriaceae* were the total of coliform and lactose-negative *Enterobacteriaceae*. The numbers of lactic acid bacteria were determined on MRS agar (Merck KGaA) after anaerobic incubation at 38°C for 48 h.

Data were analyzed using analysis of variance (ANOVA, SPSS 16.0 version). Once the significant effects ($p<0.05$) of treatments was found, Duncan multi-range test was subsequently conducted. Data were also analyzed based on the linear, quadratic, and cubic regressions to determine the effect of increasing concentrations of fermented fruit filtrate in drinking water on the measured parameters.

RESULTS

pH Values and Lactic Acid Bacteria Counts of the Fermented *Averrhoa bilimbi* L. Fruit Filtrate

Spontaneous anaerobic fermentation decreased ($p<0.05$) pH values and increased ($p<0.05$) the numbers of lactic acid bacteria of *A. bilimbi* L. fruit filtrate. The longer period of fermentation was associated with the linear reduction ($p<0.05$) in pH and increase ($p<0.05$) in lactic acid bacteria counts in *A. bilimbi* L. fruit filtrate (Table 2).

Performance of Broilers

Data on the production performances of broilers are presented in Table 3. It appeared that FCR linearly ($p<0.05$) improved with the increased concentrations of fermented *A. bilimbi* L. fruit filtrate in the drinking water of broilers. Body weight and cumulative feed intake were not affected ($p>0.05$) by the treatments.

Blood Profiles of Broilers

On day 21, the values of thrombocytes decreased quadratically ($p<0.05$) in the blood of broilers with the increased concentrations of fermented *A. bilimbi* L. fruit filtrate in drinking water. On day 33, the numbers of leukocytes and percentage of lymphocytes were lower ($p<0.05$) in the blood of the treated chicks than that in control. Regression analysis showed that the numbers of leukocytes decreased quadratically ($p<0.05$) with the elevated levels of fermented fruit filtrate (Table 4).

Serum Biochemistry of Broilers

At the determination on day 21, the levels of high-density lipoprotein (HDL)-cholesterol increased quadratically ($p<0.05$) while AST linearly increased ($p<0.05$) with the enhanced concentrations of fermented *A. bilimbi* L. fruit filtrate in the drinking water. On day 33, the concentrations of creatinine linearly increased ($p<0.05$),

Table 2. pH values and lactic acid bacteria counts of the fermented *Averrhoa bilimbi* L. fruit filtrate

Items	Fermentation time				SEM	p value			
	day 0	day 2	day 4	day 6		A	L	Q	C
pH	2.30[a]	2.10[b]	1.83[c]	1.80[c]	0.06	<0.01	<0.01	<0.01	0.66
LAB (log cfu/mL)	8.75[c]	16.3[b]	24.8[a]	25.3[a]	2.06	<0.01	<0.01	<0.01	0.03

Note: LAB= lactic acid bacteria; A= analysis of variance; L= linear regression; Q= quadratic regression; C= cubic regression; SEM= standard error of the means. Means in the same row with different superscripts differ significantly (p<0.05).

Table 3. Performances of broilers administrated with fermented *Averrhoa bilimbi* L. fruit filtrate through drinking water

Items	Treatments				SEM	p value			
	CONT	FAB05	FAB1	FAB2		A	L	Q	C
Day 21									
BW (g)	768	746	746	763	7.78	0.67	0.82	0.22	0.75
Cumulative FI (g)	1399	1257	1180	1178	55.3	0.48	0.14	0.38	0.88
FCR	1.96	1.81	1.70	1.66	0.09	0.63	0.19	0.59	0.96
Day 33									
BW (g)	1501	1457	1446	1526	21.1	0.52	0.78	0.17	0.99
Cumulative FI (g)	3297	3004	2973	2891	82.8	0.35	0.09	0.35	0.55
FCR	2.26	2.14	2.13	1.96	0.05	0.20	0.04	0.94	0.54
Mortality (%)	22.0	2.00	8.00	2.00	0.37	0.19	0.11	0.22	0.17

Note: CONT= drinking water containing 0% fermented *A. bilimbi* L. fruit filtrate; FAB05= drinking water containing 0.5% fermented *A. bilimb*i L. fruit filtrate; FAB1= drinking water containing 1.0% fermented *A. bilimbi* L. fruit filtrate; FAB2= drinking water containing 2.0% fermented *A. bilimbi* L. fruit filtrate; BW= body weight; FI= feed intake; FCR= feed conversion ratio; A= analysis of variance; L= linear regression; Q:= quadratic regression; C= cubic regression; SEM= standard error of the means.

Table 4. Blood profiles of broilers administrated with fermented *Averrhoa bilimbi* L. fruit filtrate through drinking water

Items	Treatments				SEM	p value			
	CONT	FAB05	FAB1	FAB2		A	L	Q	C
Day 21									
Leukocytes (10^9/L)	63.6	66.2	73.7	77.4	4.29	0.68	0.21	0.88	0.86
Erythrocytes (10^{12}/L)	2.58	2.97	2.72	2.84	0.89	0.49	0.52	0.41	0.49
Hemoglobin (g/dL)	9.20	10.4	9.80	10.2	0.33	0.63	0.43	0.48	0.31
Hematocrit (%)	34.8	39.4	35.9	37.6	1.25	0.62	0.67	0.54	0.23
Thrombocytes (10^9/L)	12.4	10.0	9.60	10.4	0.42	0.08	0.09	0.02	0.48
Lymphocytes (%)	61.3	63.7	70.3	74.6	4.05	0.67	0.21	0.91	0.88
Heterophils (10^9/L)	2.30	2.50	3.40	2.80	0.33	0.70	0.44	0.49	0.59
MCV (fl)	136	133	133	133	0.47	0.12	0.06	0.07	0.52
MCH (pg)	35.6	35.0	35.9	35.8	0.38	0.87	0.64	0.82	0.52
MCHC (g/dL)	26.4	26.4	27.2	27.0	0.26	0.65	0.28	0.71	0.55
Day 33									
Leukocytes (10^9/L)	85.9[a]	69.5[b]	68.8[b]	72.8[b]	2.35	0.02	0.05	0.01	0.23
Erythrocytes (10^{12}/L)	2.69	2.54	2.57	2.72	0.05	0.52	0.79	0.16	0.68
Hemoglobin (g/dL)	9.70	9.10	9.10	9.60	0.20	0.63	0.87	0.19	0.75
Hematocrit (%)	33.1	31.9	32.1	34.0	0.67	0.70	0.64	0.31	0.88
Thrombocytes (10^9/L)	13.0	11.0	9.00	7.80	0.79	0.09	0.01	0.44	0.98
Lymphocytes (%)	79.0[a]	64.8[b]	63.0[b]	67.6[b]	2.12	0.02	0.05	0.05	0.33
Heterophils (109/L)	6.90	4.70	5.80	5.20	0.45	0.36	0.33	0.31	0.17
MCV (fl)	124	127	126	126	3.67	0.74	0.50	0.41	0.50
MCH (pg)	36.0	35.8	35.4	35.2	0.30	0.78	0.29	0.83	0.88
MCHC (g/dL)	29.3	28.5	28.3	28.2	0.35	0.68	0.26	0.47	0.78

Note: MCV= mean corpuscular volume; MC= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; CONT= drinking water containing 0% fermented *A. bilimbi* L. fruit filtrate; FAB05= drinking water containing 0.5% fermented *A. bilimb*i L. fruit filtrate; FAB1= drinking water containing 1.0% fermented *A. bilimbi* L. fruit filtrate; FAB2= drinking water containing 2.0% fermented *A. bilimbi* L. fruit filtrate; A= analysis of variance; L= linear regression; Q:= quadratic regression; C= cubic regression; SEM= standard error of the means. Means in the same row with different superscripts differ significantly (p<0.05).

and ALT quadratically increased (p<0.05) following the increased proportion of fermented fruit filtrate in the drinking water. Other serum biochemical parameters were not affected (p>0.05) by the treatments (Table 5).

Gut Morphology of Broilers

Data on the intestinal morphology of birds are presented in Table 6. Administration of fermented *A. bilimbi* L. fruit filtrate in drinking water increased (p<0.05) villi height of jejunal segments of broilers. In ileum, the treatments enhanced (p<0.05) crypt depth of broilers. Regression analysis further showed that crypt depth linearly and quadratically increased (p<0.05) with the elevated levels of fermented fruit filtrate.

pH Values of Intestine of Broilers

Application of fermented *A. bilimbi* L. fruit filtrate through drinking water resulted in higher (p<0.05) pH values of jejunum of broilers. The increased levels of fermented fruit filtrate were also attributed to the quadratic and cubic increases (p<0.05) in pH values of jejunum (Table 7).

Intestinal Bacterial Populations of Broilers

The numbers of *Enterobacteriaceae* in ileal digesta linearly decreased (p<0.05) with the increased concentrations of fermented *A. bilimbi* L. fruit filtrate in the drinking water. The treatment did not affect the bacterial populations in the caecum of broilers (Table 8).

Carcass Traits of Broilers

The data on carcass characteristics of broilers are presented in Table 9. Overall, the administration of fermented *A. bilimbi* L. fruit filtrate in the drinking water had no impact (p>0.05) on the carcass characteristics of broiler chickens.

Internal Organs of Broilers

Table 10 presents data on the internal organ weight of broilers. The relative weight of liver quadratically decreased (p<0.05) with the increased levels of fermented *A. bilimbi* L. fruit filtrate in drinking water. The proventriculus weight increased significantly (p<0.05) following the enhanced levels of fermented fruit filtrate in drinking water of experimental broilers. The caeca

Table 5. Serum biochemical variables of broilers administrated with fermented *Averrhoa bilimbi* L. fruit filtrate through drinking water

Items	Treatments				SEM	p value			
	CONT	FAB05	FAB1	FAB2		A	L	Q	C
Day 21									
Total cholesterol (g/dL)	121	118	125	120	3.97	0.94	0.89	0.85	0.59
LDL (g/dL)	23.6	13.0	11.3	15.5	2.63	0.38	0.28	0.12	0.67
HDL (g/dL)	73.6	86.8	90.4	84.0	0.17	2.80	0.17	0.04	0.69
Triglycerides (g/dL)	117	90.7	88.7	102	6.48	0.47	0.45	0.13	0.66
Total protein (g/dL)	2.75	2.90	3.03	2.79	3.97	0.94	0.89	0.85	0.59
Albumin (g/dL)	1.26	1.34	1.39	1.26	0.04	0.61	0.94	0.20	0.89
Globulin (g/dL)	1.48	1.55	1.64	1.57	0.06	0.88	0.73	0.47	0.85
Uric acid (mg/dL)	7.22	5.71	6.53	6.86	0.30	0.34	0.93	0.14	0.19
Creatinine (mg/dL)	0.07	0.07	0.05	0.06	0.01	0.33	0.33	0.20	0.43
AST (U/L)	221	245	254	230	7.50	0.43	0.59	0.10	0.96
ALT (U/L)	0.52	1.13	1.82	1.63	0.30	0.09	0.02	0.14	0.79
Day 33									
Total cholesterol (g/dL)	115	102	113	120	4.19	0.52	0.53	0.30	0.37
LDL (g/dL)	23.0	15.3	12.8	18.8	2.40	0.50	0.50	0.14	0.88
HDL (g/dL)	77.8	73.8	86.8	86.8	3.05	1.19	0.15	0.95	0.28
Triglycerides (g/dL)	71.8	70.6	75.0	64.8	2.63	0.61	0.50	0.50	0.50
Total protein (g/dL)	3.04	3.12	3.43	3.02	0.07	0.13	0.71	0.08	0.25
Albumin (g/dL)	1.24	1.25	1.36	1.27	0.03	0.41	0.38	0.32	0.34
Globulin (g/dL)	1.80	1.87	2.07	1.74	0.05	0.91	0.96	0.05	0.27
Uric acid (mg/dL)	4.76	5.14	6.45	5.45	0.24	0.06	0.11	0.08	0.21
Creatinine (mg/dL)	0.06	0.06	0.07	0.08	0.01	0.07	0.01	0.83	0.35
AST (U/L)	309	244	256	316	14.7	0.20	0.82	0.09	0.52
ALT (U/L)	1.59	1.01	1.28	2.02	0.15	0.10	0.26	0.04	0.47

Note: LDL= low-density lipoprotein; HDL= high-density lipoprotein; ALT= alanine aminotransferase; AST= aspartate aminotransferase; CONT= drinking water containing 0% fermented *A. bilimbi* L. fruit filtrate; FAB05= drinking water containing 0.5% fermented *A. bilimbi* L. fruit filtrate; FAB1= drinking water containing 1.0% fermented *A. bilimbi* L. fruit filtrate; FAB2= drinking water containing 2.0% fermented *A. bilimbi* L. fruit filtrate; A= analysis of variance; L= linear regression; Q:= quadratic regression; C= cubic regression; SEM= standard error of the means.

Table 6. Gut morphology of broilers administrated with fermented *Averrhoa bilimbi* L. fruit filtrate through drinking water

Items	Treatments				SEM	p value			
	CONT	FAB05	FAB1	FAB2		A	L	Q	C
Duodenum									
Villi height (μm)	1588	1972	1480	1620	89.2	0.24	0.63	0.58	0.06
Crypt depth (μm)	234	236	223	298	12.0	0.10	0.09	0.21	0.44
VH/CD	6.98	8.49	6.85	5.50	0.42	0.08	0.11	0.14	0.20
Jejunum									
Villi height (μm)	474^{b}	1001^{a}	819^{ab}	908^{a}	73.2	0.04	0.09	0.07	0.05
Crypt depth (μm)	230	288	208	283	24.5	0.62	0.73	0.91	0.23
VH/CD	7.11	4.78	6.45	5.02	0.48	0.26	0.29	0.53	0.88
Ileum									
Villi height (μm)	1475	1179	1282	1317	67.4	0.51	0.55	0.21	0.33
Crypt depth (μm)	126^{b}	182^{a}	197^{a}	180^{a}	9.89	0.04	0.04	0.02	0.55
VH/CD	3.95	5.50	4.04	5.25	0.35	0.29	0.45	0.72	0.07

Note: VH= villi height; CD= crypt depth; VH/CD= villi height to crypt depth rasio; CONT= drinking water containing 0% fermented *A. bilimbi* L. fruit filtrate; FAB05= drinking water containing 0.5% fermented *A. bilimb*i L. fruit filtrate; FAB1= drinking water containing 1.0% fermented *A. bilimbi* L. fruit filtrate; FAB2= drinking water containing 2.0% fermented *A. bilimbi* L. fruit filtrate; A= analysis of variance; L= linear regression; Q:= quadratic regression; C= cubic regression; SEM= standard error of the means.

Table 7. pH values of intestine of broilers administrated with fermented *Averrhoa bilimbi* L. fruit filtrate through drinking water

Items	Treatments				SEM	p value			
	CONT	FAB05	FAB1	FAB2		A	L	Q	C
Duodenum	6.30	6.68	5.58	6.86	0.06	0.19	0.34	0.14	0.09
Jejunum	4.94^{b}	6.16^{a}	6.72^{a}	6.54^{a}	0.15	0.02	0.09	0.02	0.02
Ileum	5.58	5.78	6.14	7.02	0.17	0.13	0.79	0.05	0.08
Caeca	6.86	5.84	5.92	6.84	0.08	0.22	0.58	0.76	0.05

Note: CONT= drinking water containing 0% fermented *A. bilimbi* L. fruit filtrate; FAB05= drinking water containing 0.5% fermented *A. bilimb*i L. fruit filtrate; FAB1= drinking water containing 1.0% fermented *A. bilimbi* L. fruit filtrate; FAB2= drinking water containing 2.0% fermented *A. bilimbi* L. fruit filtrate; A= analysis of variance; L= linear regression; Q:= quadratic regression; C= cubic regression; SEM= standard error of the means. Means in the same row with different superscripts differ significantly ($p<0.05$)

Table 8. Gut bacterial populations of broilers administrated with fermented *Averrhoa bilimbi* L. fruit filtrate through drinking water

Items	Treatments				SEM	p value			
	CONT	FAB05	FAB1	FAB2		A	L	Q	C
Ileum (log cfu/g)									
Coliform	7.67	7.17	6.26	6.66	0.25	0.20	0.07	0.21	0.58
Enterobacteriaceae	8.18	7.76	7.19	6.56	0.26	0.12	0.01	0.05	0.99
LAB	10.9	10.9	7.01	10.9	0.92	0.99	0.79	0.81	0.94
Caecum (log cfu/g)									
Coliform	9.21	7.92	8.25	8.29	0.25	0.34	0.30	0.15	0.28
Enterobacteriaceae	9.53	8.37	8.34	8.44	0.24	0.22	0.12	0.11	0.43
LAB	11.6	11.5	11.7	11.7	0.05	0.37	0.13	0.70	0.44

Note: LAB= lactic acid bacteria; CONT= drinking water containing 0% fermented *A. bilimbi* L. fruit filtrate; FAB05= drinking water containing 0.5% fermented *A. bilimb*i L. fruit filtrate; FAB1= drinking water containing 1.0% fermented *A. bilimbi* L. fruit filtrate; FAB2= drinking water containing 2.0% fermented *A. bilimbi* L. fruit filtrate; A= analysis of variance; L= linear regression; Q:= quadratic regression; C= cubic regression; SEM= standard error of the means.

weights decreased ($p<0.05$) with the increased proportions of fermented fruit filtrate in drinking water.

DISCUSSION

It was shown in this study that *A. bilimbi* L. fruit filtrate contained a substantial number of lactic acid bacteria, which was in agreement with Rodriguez *et al*. (2019). Interestingly, spontaneous anaerobic fermentation resulted in the increased lactic acid bacteria populations in the filtrate. This result may confirm that *A. bilimbi* L. fruit filtrate contained some essential nutrients capable of supporting the growth of lactic acid bacteria including glucose and amino acids. The increased numbers of lactic acid bacteria were attributed to the reduced pH values of fermented filtrate. This could be understood

Table 9. Carcass characteristics of broilers administrated with fermented *Averrhoa bilimbi* L. fruit filtrate through drinking water

Items	Treatments				SEM	p value			
	CONT	FAB05	FAB1	FAB2		A	L	Q	C
Eviscerated carcass (% live BW)	68.8	69.7	67.56	68.9	0.43	0.37	0.67	0.76	0.12
			% eviscerated carcass						
Breast	37.2	37.7	36.64	36.4	0.41	0.42	0.37	0.82	0.54
Wings	10.8	10.8	10.89	11.0	0.13	0.90	0.49	0.85	0.89
Thigh	16.2	16.2	16.29	15.6	0.20	0.58	0.33	0.49	0.68
Drumstick	13.8	13.8	14.33	14.1	1.47	0.79	0.31	0.56	0.36
Back	22.0	21.6	21.85	22.9	0.29	0.43	0.26	0.30	0.93

Note: BW= body weight; CONT= drinking water containing 0% fermented *A. bilimbi* L. fruit filtrate; FAB05= drinking water containing 0.5% fermented *A. bilimb*i L. fruit filtrate; FAB1= drinking water containing 1.0% fermented *A. bilimbi* L. fruit filtrate; FAB2= drinking water containing 2.0% fermented *A. bilimbi* L. fruit filtrate; A= analysis of variance; L= linear regression; Q:= quadratic regression; C= cubic regression; SEM= standard error of the means.

Table 10. Internal organ weights of broilers administrated with fermented *Averrhoa bilimbi* L. fruit filtrate through drinking water

Items (% live BW)	Treatments				SEM	p value			
	CONT	FAB05	FAB1	FAB2		A	L	Q	C
Heart	0.45	0.44	0.45	0.42	0.09	0.70	0.34	0.71	0.67
Liver	2.54	2.43	2.34	2.14	0.09	0.37	0.07	0.02	0.80
Proventriculus	0.47	0.41	0.50	0.48	0.01	0.07	0.33	0.54	0.02
Gizzard	1.80	1.82	1.80	1.73	0.48	0.93	0.61	0.72	0.96
Pancreas	0.30	0.28	0.31	0.26	0.01	0.31	0.24	0.61	0.24
Abdominal fat	1.30	0.93	1.31	1.44	0.09	0.20	0.32	0.23	0.14
Duodenum	0.60	0.59	0.58	0.58	0.02	0.97	0.61	0.85	0.93
Jejenum	1.14	1.28	1.20	1.32	0.06	0.70	0.38	0.81	0.41
Ileum	0.82	0.89	0.98	0.92	0.42	0.61	0.32	0.34	0.81
Caeca	0.76	0.66	0.65	0.57	0.03	0.21	0.03	0.29	0.51
Spleen	0.08	0.12	0.09	0.08	0.31	0.21	0.60	0.29	0.18
Thymus	0.18	0.13	0.19	0.18	0.01	0.45	0.55	0.57	0.16
Bursa of fabricius	0.13	0.10	0.15	0.13	0.12	0.63	0.64	0.95	0.24

Note: BW= body weight; CONT= drinking water containing 0% fermented *A. bilimbi* L. fruit filtrate; FAB05= drinking water containing 0.5% fermented *A. bilimb*i L. fruit filtrate; FAB1= drinking water containing 1.0% fermented *A. bilimbi* L. fruit filtrate; FAB2= drinking water containing 2.0% fermented *A. bilimbi* L. fruit filtrate; A= analysis of variance; L= linear regression; Q:= quadratic regression; C= cubic regression; SEM= standard error of the means.

as lactic acid bacteria can convert glucose into organic acids that can thereby decrease pH of the fermented products (Widyastuti, 2016; Ngasotter, 2020).

Throughout the *in vivo* experiment, FCR linearly reduced with the increased concentrations of fermented *A. bilimbi* L. fruit filtrate in drinking water of experimental broilers. In this study, the fermented *A. bilimbi* L. filtrate probably served as both acidifiers as well as lactic acid bacteria-based probiotics for broilers. Indeed, both substances have been reported to improve feed digestibility and utilization, and thus lower feed conversion of broiler chickens. Also, the improved intestinal bacterial ecology, enzyme activity, and gut morphology have been associated with the organic acids and probiotic treatments, which are then eventually attributed to the improved intestinal functions of broilers (Sugiharto, 2016).

On days 21 and 33, the numbers of thrombocytes decreased with the increased levels of fermented *A. bilimbi* L. fruit filtrate in the drinking water of experimental broilers. As part of the innate effector cells, thrombocytes may take part in inflammation process in poultry (Ferdous *et al*., 2008). In this regard, any increase in thrombocyte concentrations may therefore be associated with the increased potential infection in broilers. Taken together, the decreased thrombocyte concentrations in the experimental birds treated with fermented *A. bilimbi* L. filtrate may therefore be associated with the reduced potential infections in these respective birds. Our inference was also supported by the reduced levels of leukocytes and lymphocytes in the blood of broilers. Note that the increase in leukocytes and lymphocytes levels may indicate the infections and inflammation in broiler chickens (Hidanah *et al*., 2018). It was most likely that organic acids and lactic acid bacteria in the fermented *A. bilimbi* L. filtrate acted as antibacterial agents in the intestine of broilers in the present study. This assumption was actually supported by the linear decrease in *Enterobacteriaceae* numbers in the ileum of experimental broilers.

The increase in HDL level was observed on day 21 in the serum of broilers treated with fermented

filtrate. Formerly, Hedayati *et al*. (2015) reported that administrations of organic acid and probiotics increased the concentration of HDL in the serum of broilers. In general, HDL has been associated with good cholesterol. Our finding, therefore, suggested that fermented *A. bilimbi* L. fruit filtrate may improve the physiological conditions of birds. The mechanism by which fermented fruit filtrate increased serum HDL levels was not exactly known, but it was most likely that probiotic activity of lactic acid bacteria in the fermented fruit filtrate modified the lipoprotein metabolism resulting in the increased levels of apolipoprotein A-V (apo A-V) levels, which is the potent modulator of HDL (Ahn *et al*., 2015). With regard to the acids contained in the fermented fruit filtrate, organic acids may improve gut ecology, digestion, and absorption of protein and amino acids. The latter condition may thereby increase protein anabolism and thus increase lipoprotein synthesis (Fouladi *et al*., 2018), which is the major component of HDL. At both times of measurements, the increased levels of fermented fruit filtrate in drinking water were associated with increased serum ALT levels. In poultry, the serum levels of AST and ALT are associated with liver function and health. Any increase in both biochemical indices may be attributed to the deteriorated liver function. In this study, although the levels of AST increased following the increased concentration of fermented fruit filtrate, its values were still within the normal range, as Sugiharto *et al*. (2019) documented that ALT concentrations of broilers were about 1.00 to 1.81 U/L. Linear regression showed that creatinine levels increased with the elevated proportion of fermented filtrate in drinking water. In general, creatinine may be used to indicate proteolytic activity and also kidney functions. In this study, although the fermented filtrate increased serum creatinine levels yet, the increase was still within the acceptable range. Indeed, broiler study by Sugiharto *et al*. (2019) previously showed that creatinine might range from 0.05 to 0.10 g/dL.

Administration of fermented *A. bilimbi* L. fruit filtrate enhanced villi height of jejunum and crypt depth of ileum of experimental broilers. This present finding was in accordance with Agboola *et al*. (2015), who documented that dietary supplementation of organic acid (formic and propionic acids), probiotics (*Lactobacillus sporogenes* and *Saccharomyces cerevisiae*) or the blends of both increased villi height and crypt depth of broiler intestine. Similarly, Rodjan *et al*. (2018) showed that feeding the blends of organic acid and probiotics resulted in higher duodenal villi height and crypt depth when compared with control. Considering the essential role of villus and crypt in the absorption process, our findings therefore suggested that fermented *A. bilimbi* L. fruit filtrate improved the absorptive capacity of experimental broilers. Previously, Xu *et al*. (2003) and Agboola *et al*. (2015) suggested that the increased villus height and crypt depth were associated with improved nutrient absorption and thus growth performance of broiler chickens, and *vice versa*. It was most likely that organic acids and lactic acid bacteria in the fermented *A. bilimbi* L. fruit filtrate improved the microbial balance as well as ecology of the intestine and thereby improved the gut morphology of broilers (Sugiharto, 2016).

The acidification effect of organic acids and probiotics on the gastrointestinal tract of broilers has widely been noticed (Sugiharto, 2016). In contrast, we reported in this study that fermented *A. bilimbi* L. fruit filtrate increased pH values of jejunum. In this regard, organic acids and lactic acid bacteria in the fermented fruit filtrate seemed not to exert an acidification effect on the jejunum of experimental broilers. We could not explain this definitely, but the strong or excessive buffering action of the intestine (in response to the acid) may result in the increased pH values of jejunum (Pearlin *et al*., 2020). Aside from the acidification effect, the pH values of intestine of the experimental birds treated with fermented *A. bilimbi* L. fruit filtrate were actually within the normal range. Mabelebele *et al*. (2014) suggested that normal pH value of broiler chickens is about 6.43. In respect, particularly to the control birds without supplementation of fermented *A. bilimbi* L. fruit filtrate, the jejunal pH values of these respective birds (pH 4.94) were by far lower from the normal jejunal pH values of broilers (pH 6.2; Recoules *et al*., 2019). So far, we could not identify the exact explanation for the latter condition.

The counts of *Enterobacteriaceae* in ileum decreased with the increased proportion of fermented *A. bilimbi* L. fruit filtrate in drinking water of experimental broilers. The mechanism by which the fermented fruit filtrate reduced intestinal population of *Enterobacteriaceae* was not exactly known, but it seemed that the presences of organic acids and lactic acid were responsible for the antibacterial activity of the fermented fruit filtrate. Rodjan *et al*. (2018) noticed that dietary supplementation of organic acids, probiotics, or combination of both improved the intestinal bacterial ecology by decreasing intestinal population of *Escherichia coli* and increasing lactic acid bacteria counts. Yet, our treatment did not substantially affect the population of lactic acid bacteria in the intestine of experimental broilers, which was in accordance to Agboola *et al*. (2015) showing no effect of organic acids, probiotics, or combination of both on intestinal lactic acid bacteria counts.

In this study, administration of fermented *A. bilimbi* L. fruit filtrate through drinking water had no impact on the carcass characteristics of broilers. It could therefore be assumed that the presence of organic acids and lactic acid bacteria did not exert any effect on the carcass traits of broilers. In agreement with our assumption, Nosrati *et al*. (2017) did not find any impact of organic acids and probiotics on carcass quality of broilers. With regards to internal organs, the relative weight of the liver decreased with the increased levels of fermented fruit filtrate. Previously, Agboola *et al*. (2015) and Nosrati *et al*. (2017) noted the absent effect of organic acids and probiotics on the relative liver weight of broilers. For this reason, it was difficult to relate between the presence of organic acids and lactic acid bacteria in the fermented filtrate and the relative weight of liver. In the infection trial, Isroli *et al*. (2018) reported a higher relative weight of liver (due to the inflammation and necrotic lesion) in broilers infected with avian pathogenic

Escherichia coli as compared to that of uninfected broilers. In our present case, fermented filtrate was capable of reducing the *Enterobacteriaceae* load in the intestine and thereby avoided liver enlargement. The relative weight of proventriculus increased with the increased levels of fermented filtrate in the drinking water. In respect, particularly to the content of the organic acid in the fermented filtrate, Nourmohammadi & Khosravinia (2015) reported that dietary supplementation of citric acid resulted in higher weights of proventriculus in broiler chickens. Owing to the crucial role of proventriculus in digesting feed protein, the increased relative weight of this organ may therefore improve the protein digestion and utilization by the chicks. This inference was supported by the improved FCR in the experimental birds treated with fermented filtrate. The increased levels of fermented filtrate were also associated with the decrease in the relative weight of caeca. Previously, Saki *et al*. (2012) reported that feeding organic acid mixture resulted in the decreased cecal relative weight of broilers. Considering the positive relationship between the fiber content of diets and cecal relative weight (Wu *et al*., 2019), the decrease in cecal weight in the experimental chickens treated with fermented filtrate may be attributed to the improved fiber digestion in the upper part of the digestive tract and hence less fiber reaching the caeca of broilers. In support to our inference, Rodjan *et al*. (2018) reported that dietary administration of organic acids and probiotics increased the ability of broilers to degrade crude fiber. Khan and Iqbal (2016) documented that organic acids could increase the activity of fiber-degrading enzymes resulting in increased crude fiber digestibility. Likewise, lactic acid bacteria may produce cellulolytic enzymes that can degrade the crude fiber in the feed as reported by Herdian *et al*. (2018).

CONCLUSION

Administration of 2% of fermented *A. bilimbi* L. fruit filtrate (pH 1.83) through drinking water improved feed conversion, physiological condition, and intestinal ecology of broilers. The addition of fermented *A. bilimbi* L. fruit filtrate (pH 1.83) up to 2% in drinking water did not exert negative effect on the carcass characteristics of broilers.

CONFLICT OF INTEREST

The authors clarify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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