OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

Acidification of Papaya Leaf and Seed Meal using *Averrhoa bilimbi* L. Fruit Filtrate and their Effect on Growth and Carcass Traits of Broiler Chickens

Endang Widiastuti, Tri Agus Sartono, Hanny Indrat Wahyuni, Retno Murwani, Turrini Yudiarti, Anugrah Robby Pratama, Sugiharto Sugiharto*

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Central Java, Indonesia.

Abstract | The aim of the study was to determine the impact of graded levels of *A. bilimbi*-acidified papaya leaf and seed meal (APLS) on growth performance, physiological conditions and intestinal ecology of broilers. Two hundred broiler chicks were grouped into CONT (chicks provided control diet), ACID1 (chicks provided with diet containing 1% APLS), ACID25 (diet containing 2.5% APLS) and ACID5 (diet containing 5% APLS). The ratio between the acidified papaya leaf meal and seed meal in the mixture was 3:1. Live body weight and feed consumption were weekly recorded. At day 35, the birds were blood sampled and slaughtered. The use of APLS in diets had no substantial effect ($P > 0.05$) on final weight and weight gain of broilers. Dietary inclusion of APLS linearly increased ($P < 0.05$) the accumulative feed consumption of broilers. Inclusion of APLS, particularly at the level of 5%, compromised ($P < 0.05$) feed conversion ratio (FCR) of broilers when compared to that of control. The graded levels of APLS in diets linearly increased ($P < 0.05$) the gizzard weight. Total cholesterol and low-density lipoprotein (LDL)-cholesterol were higher ($P < 0.05$) in ACID1 than in other treatment groups. High-density lipoprotein (HDL)-cholesterol tended ($P = 0.08$) to be higher in ACID1 than in other groups. The increased levels of APLS in feed linearly increased ($P < 0.05$) HDL to LDL ratio, while linearly decreased ($P = 0.06$) cholesterol to HDL ratio of broilers. The elevated levels of APLS in feed tended ($P = 0.08$) to decrease the pH values of duodenum. There was no significant effect of APLS on final body weight and weight gain, intestinal bacterial populations, complete blood counts, carcass and commercial cuts of broilers. In conclusion, dietary inclusion of APLS at 5% compromised FCR, but improved serum lipid profile of broilers. The high fibre content of APLS may limit the use of such alternative feed ingredients in broiler feeds. Overall, the APLS can be used up to 2.5% in broiler chicken diets without causing harm to their growth, physiological conditions, and intestinal ecology.

Keywords | Acidifier, Averrhoa bilimbi fruit filtrate, Broiler, Intestinal ecology, Feed conversion, Lipid profile

Received | April 29, 2021; **Accepted** | June 06, 2021; **Published** | July 28, 2021
***Correspondence** | Sugiharto Sugiharto, Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Central Java, Indonesia; **Email:** sgh_undip@yahoo.co.id
Citation | Widiastuti E, Sartono TA, Wahyuni HI, Murwani R, Yudiarti T, Pratama AR, Sugiharto S (2021). Acidification of papaya leaf and seed meal using *Averrhoa bilimbi* L. fruit filtrate and their effect on growth and carcass traits of broiler chickens. Adv. Anim. Vet. Sci. 9(9): 1368-1375.
DOI | http://dx.doi.org/10.17582/journal.aavs/2021/9.9.1368.1375
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

The broiler industry is currently facing the problem of expensive and volatile feed prices. Particular attention has been paid to protein-rich feedstuffs like soybean meal as well as energy-rich feed stuff like yellow maize as both feedstuffs occupy more than two-thirds of broiler rations. During the COVID-19 pandemic, the price of soybean meal and yellow maize had further increased as the supply chain for both commodities was disturbed. To reduce the dependence of the broiler industry on soybean meal and maize, nutritionists are now searching for the alternative

for protein- and energy-rich feedstuffs for broilers. Among the alternative feed ingredients, papaya leaf has been known to contain a high level of crude protein, which is 26.7% on a dry matter basis (Sugiharto et al., 2020). In line with this, papaya seed also contains crude protein ranging from 24 to 30% (Sugiharto, 2020). Apart from their potency as broiler feed ingredients (Oloruntola et al., 2018), the high proportion of crude fiber in papaya leaf (34.2%) and seed (23.6%) may confine their inclusion level in broiler diets (Sugiharto et al., 2020). To deal with this, fermentation has been conducted. Similar to fermentation, acidification has been demonstrated to enhance the physical and nutritional characteristics of plant-derived materials. Nikinmaa et al. (2020) have recently reported that acidification using lactic acid reduced the content of insoluble fiber and increased soluble fiber and protein in rye bran. Besides nutritional properties, acidification (using chloride acid) also increased the contents of functional properties like lycopene, polyphenols, vitamin C, and antioxidant activities of tomato juice in the study of Sarr and Tsai (2008). In agreement, Bayliak et al. (2016) documented that acid conditions favors the enhanced antioxidative activity of some medicinal herbs.

Averrhoa bilimbi L., a member of the Oxalidaceae family, has long been recognized as an acidic fruit that contains a variety of natural organic acids, including citric acid, acetic acid, and oxalic acid, with citric acid being the most prominent (Renatami et al., 2018; Sugiharto, 2020b). Taking advantage of the fruit's acidic properties, the juice of the *A. bilimbi* fruit has been used as a natural acid coagulant during the production of tofu (Sitanggang et al., 2020). The fruit has also been exploited as an acidifier for pigs (Silalahi et al., 2015) and layers (Wijayanti et al., 2019) to improve animal performance and health. In this study, the *A. bilimbi* fruit filtrate was employed to acidify the leaf and seed meals of papaya as feed ingredients for broiler chickens. The *A. bilimbi* fruit filtrate was selected as an acidifying agent since such fruit contain a high concentration of citric acid (Renatami et al., 2018), which was directed to increase the acidity of papaya leaf and seed meals during the acidification process (Mani-López et al., 2012). In this regard, the acidified product may then serve as an acidifier for the chickens. The high content of organic acid (Renatami et al., 2018; Sugiharto, 2020b) in *A. bilimbi* fruit filtrate was also subjected to improve the nutritional qualities of papaya leaf and seed meals through acidification (Nikinmaa et al., 2020). In Asia and other tropical countries, the *A. bilimbi* is widely grown. While *A. bilimbi* fruit can be harvested all year round, such fruit is still underutilized (not commercial) in most countries including Indonesia. The latter condition makes the *A. bilimbi* fruit has no economic interest so far (Sugiharto, 2020b). Overall, this is a pioneer study using *A. bilimbi* fruit filtrate as an acidifying agent to produce *A. bilimbi*-acidified papaya leaf and seed meal (APLS) for broiler diets. The objective of the present study was to investigate the effect of graded levels of APLS on growth performance, physiological conditions, and intestinal ecology of broilers.

MATERIALS AND METHODS

Preparation of acidified papaya leaf and seed meals

Papaya leaves were collected from the garden, while the seeds were obtained from the fruit street vendors around the campus of Universitas Diponegoro. The papaya leaves used in the study were mature leaves from the papaya variety "Bangkok". The papaya leaves were air-dried, whereas the seeds were sun-dried. They were ground into a meal and then stored until use. The ripe *A. bilimbi* has fruits were collected from the garden close to the campus. After being cleaned with running water, the *A. bilimbi* fruit was juiced using an electric blender. No water was included during juicing and the cheese cloth was used to filter the juice of *A. bilimbi* fruit during the production of fruit filtrate. The acidification was carried out by mixing the fruit filtrate and papaya leaf or seed meal (3:1, mL:g). The mixture was put in an anaerobic jar for three days at about 25°C, and then sun-dried thereafter. The samples of APLS were collected for proximate determination and the remaining stuff was stored for *in vivo* trial. The production of APLS was conducted in several batches with the same protocol for each batch. The proximate proportions of APLS is shown in Table 1.

Broiler experiment

The broiler study was approved by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (No. 57-03/A3/KEP/FPP). The experiment was set up in accordance with a completely randomized arrangement. A number of 200 unsexed day-old broilers were raised in pre-starter diets having 14% moisture, 20% crude protein, 5% crude fat, 5% crude fiber, and 8% crude ash (based on the feed label) for the first seven days. From day 7 onward, the birds (average body weight of 167.45±2.58 g; means ± standard deviations) were grouped into 4, each with 5 replications (10 chicks per replicate). These groups included CONT (chicks provided control diet containing no APLS), ACID1 (chicks provided with diet containing 1% APLS), ACID25 (a diet containing 2.5% APLS) and ACID5 (a diet containing 5% APLS). The ratio between the acidified papaya leaf meal and seed meal in the mixture was 3:1. Formulated starter feeds (Table 2) were offered to broiler chicks from day 7 to 21, while finisher feeds (Table 3) from day 22 to 35. The diets and water were served *ad libitum* for the entire trial.

Table 1: Proximate proportions of APLS[1].

Items (% dry matter)	Moisture	CP	Crude fat	CF	Ash
Papaya leaf meal	3.99	19.4	8.18	22.4	16.8
Papaya seed meal	7.19	21.7	22.9	38.7	10.8
Acidified papaya leaf meal	6.20	20.3	7.53	19.8	16.3
Acidified papaya seed meal	7.09	18.9	18.1	28.3	9.98

[1]Analysis was conducted in duplicate; CP: crude protein; CF: crude fiber.

Table 2: Chemical components of starter feed (days 7-21).

Items (%, unless otherwise noticed)	CONT	ACID1	ACID25	ACID5
Yellow maize	53.4	52.7	51.6	49.7
Palm oil	2.31	2.41	2.51	2.61
Soybean meal	40.2	39.8	39.3	38.6
APLS	-	1.00	2.50	5.00
DL-methionine, 990 g	0.19	0.19	0.19	0.19
Bentonite	0.75	0.75	0.75	0.75
Limestone	1.00	1.00	1.00	1.00
MCP	1.30	1.30	1.30	1.30
Premix	0.38	0.38	0.38	0.38
Chlorine chloride	0.07	0.07	0.07	0.07
Salt	0.40	0.40	0.40	0.40
Calculated chemical components				
ME, (kcal/kg)[1]	2,900	2,900	2,900	2,900
Crude protein	22.0	22.0	22.0	22.0
Crude fiber	5.46	5.62	5.85	6.24
Ca	1.14	1.13	1.13	1.11
P	0.57	0.57	0.56	0.55
Analyzed chemical composition				
Moisture	14.2	14.6	14.1	14.1
Crude protein	24.4	23.8	22.4	23.9
Crude fat	2.28	3.83	4.13	4.17
Crude fiber	2.42	2.26	3.06	2.97
Ash	7.12	8.48	8.78	8.98

[1]ME (metabolizable energy) was predicted based on formula (Bolton, 1967): 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}. CONT: chicks provided control diet with no APLS, ACID1: chicks provided with diet having 1% APLS, ACID25: diet having 2.5% APLS, ACID5: diet having 5% APLS, APLS: a mixture of acidified papaya leaf and seed meal, MCP: monocalcium phosphate.

Vaccination with Newcastle disease vaccine (Medivac ND La Sota, PT. Medion Ardhika Bhakti, Bandung, Indonesia) via eye drops and water was conducted on days 4 and 17, respectively. On day 12, the chicks were also given the Gumboro vaccine (Medivac Gumboro A, PT. Medion Ardhika Bhakti, Bandung, Indonesia) via drinking water. An open-sided broiler house was used and rice husk was employed as litter during the experiment. Throughout the trial, the continuous light program was used. Light bulbs and plastic curtains were used to adjust the temperature and humidity in the broiler house.

Data gathering and analysis

The data on weight of birds, amount of feed consumed and FCR were gathered weekly-during study. Two chicks from each pen were blood sampled from the brachial vein on the wing at day 34. The collected blood was placed in an EDTA-containing tube for determining the complete blood profile, and the remaining blood was placed in an anti-coagulant-free tube for serum processing. To make the serum, the blood was allowed to sit at room temperature for 2 hours before being centrifuged for 10 minutes at 5,000 rpm. The serum was kept cold (at -10°C) until it was analyzed. On day 35, one male chick reflecting the average weight of each replicate was killed, defeathered and dissected. The internal organs of chickens were collected and weighed using an analytical balance (empty condition). The carcass and commercial yields of each bird were also decided. For the determination of the selected bacterial population, the samples of intestinal content were collected from the ileum and cecum of broilers. They were placed in sterile tubes and brought to the microbiology laboratory. The intestinal content was also obtained from duodenum, jejunum, ileum and cecum to measure pH values of digesta (using Portable pH Meter OHAUS ST300, OHAUS Instruments (Shanghai) Co., Ltd., China).

The erythrocyte and leucocyte counts are calculated using the dilution flask technique, and the cell count is calculated using the Burker space. The amount of hemoglobin was measured using Sahli's method, and the hematocrit value was determined using the microhaematocrit technique. Using an immersion lens and a light microscope, differential leukocytes were counted. An eye patch is worn when preparing the blood smear. Glycerol-3-phosphatoxidase (GPO) was used to determine total serum triglycerides using the enzyme calorimetry process Total cholesterol was determined after enzymatic hydrolysis and oxidation, while triglycerides were determined after enzymatic separation using lipoprotein lipases. Both use a quinoneimine indicator made from 4-aminoantipyrine and 4-chlorophenol using hydrogen peroxide and peroxidase as a catalyst (DiaSys Diagnostic System GmbH, Holzheim, Germany). Heparin was used to precipitate low-density lipoprotein (LDL). After centrifugation, high-density lipoprotein (HDL) remained in the supernatant and was treated enzymatically using the Cholesterol Oxidase-Peroxsidase Aminoantypirin (CHOD-PAP) method. The difference between total cholesterol and cholesterol in

the supernatant is used to measure LDL concentration. Following aerobic incubation at 38°C for 24 hours, the number of coliforms was counted as red colonies on MacConkey agar (Merck KGaA, Darmstadt, Germany). Lactose-negative enterobacteriaceae were also counted as other colorless colonies, and the number of coliforms and lactose-negative enterobacteriaceae was referred to as *Enterobacteriaceae*. After anaerobic incubation at 38°C for 48 hours, the number of lactic acid bacteria (LAB) was determined on De Man, Rogosa, and Sharpe (MRS) agar (Merck KGaA).

Analysis of variance (ANOVA, SPSS 16.0 version) was used to statistically examine the data. Duncan multi-range test was used after the substantial impact ($P < 0.05$) of treatments was discovered. The effect of increasing the proportions of APLS in diets on the assessed parameters was also investigated using regression (linear) analysis.

Table 3: Chemical components of finisher feed (days 22-35).

Items (%, unless otherwise noticed)	CONT	ACID1	ACID25	ACID5
Yellow maize	61.0	60.3	59.1	57.3
Palm oil	2.95	3.05	3.15	3.25
Soybean meal	32.0	31.6	31.2	30.4
APLS	-	1.00	2.50	5.00
DL-methionine, 990 g	0.19	0.19	0.19	0.19
Bentonite	0.75	0.75	0.75	0.75
Limestone	1.00	1.00	1.00	1.00
MCP	1.30	1.30	1.30	1.30
Premix	0.38	0.38	0.38	0.38
Chlorine chloride	0.07	0.07	0.07	0.07
Salt	0.40	0.40	0.40	0.40
Chemical components				
ME, (kcal/kg)[1]	3,025	3,025	3,025	3,025
Crude protein	19.0	19.0	19.0	19.0
Crude fiber	5.53	5.69	5.92	6.31
Ca	1.12	1.11	1.10	1.09
P	0.58	0.58	0.57	0.56
Analyzed chemical composition				
Moisture	14.8	14.9	14.7	15.1
Crude protein	20.3	20.4	21.0	22.2
Crude fat	3.68	4.13	4.55	4.78
Crude fiber	2.59	3.59	2.82	3.11
Ash	7.16	6.26	7.12	7.31

[1]ME (metabolizable energy) was predicted based on formula (Bolton, 1967): 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}. CONT: chicks provided control diet with no APLS, ACID1: chicks provided with diet having 1% APLS, ACID25: diet having 2.5% APLS, ACID5: diet having 5% APLS, APLS: a mixture of acidified papaya leaf and seed meal, MCP: monocalcium phosphate.

RESULTS AND DISCUSSION

The use of APLS in diets had no substantial effect ($P > 0.05$) on the final body weight and weight gain of birds in the present trial (Table 4). Accumulative feed intake was higher ($P < 0.05$) in the APLS fed broilers than that in control. Dietary inclusion of APLS linearly increased ($P < 0.05$) the accumulative feed consumption of broilers. The feed conversion ratio (FCR) was higher ($P < 0.05$) in ACID5 than that in CONT, but did not differ from ACID1 and ACID25 birds. Regression analysis further showed that the increased levels of APLS linearly ($P < 0.05$) increased the FCR of broilers.

Our data showed no notable impact ($P > 0.05$) of dietary inclusion of APLS on the internal organs relative weight of broilers (Table 5). However, the graded levels of APLS in diets linearly increased ($P < 0.05$) gizzard relative weight of chickens. Dietary incorporation of APLS had no significant impact ($P > 0.05$) on the carcass yield and commercial proportions of broilers (Table 6).

Data on complete blood counts and total plasma protein of broilers are presented in Table 7. There was no significant effect ($P > 0.05$) of dietary treatments on the blood profiles as well as and total plasma protein of broiler chickens. Serum lipid profiles of broilers are shown in Table 8. Total cholesterol and LDL-cholesterol were higher ($P < 0.05$) in ACID1 than in other treatment groups. Likewise, HDL-cholesterol tended ($P = 0.08$) to be higher in ACID1 than in other groups. Regression analysis showed that the increased levels of APLS in feed linearly increased ($P < 0.05$) HDL/LDL ratio, while linearly decreased ($P = 0.06$) cholesterol/HDL ratio of broilers.

There was a clear tendency ($P = 0.06$) that pH values of the duodenum were lower in APLS fed chickens than that in control. Regression analysis further showed that the elevated levels of APLS in the feed tended ($P = 0.08$) to decrease the pH values of the duodenum (Table 9). The pH values of jejunum, ileum, and cecum were not different ($P > 0.05$) among the treatment groups of chickens. Table 10 shows the numbers of selected bacteria in the ileum and cecum of broilers. Both in ileum, and cecum, the numbers of coliform, *Enterobacteriaceae* and LAB did not differ ($P > 0.05$) across the treatment groups of broilers.

The inclusion of APLS in diets had no substantial effect on the final body weight and weight gain of birds in the present trial. It was clear that dietary inclusion of APLS linearly increased the accumulative feed consumption of broilers. The definite reason for such a condition was not determined, but it was most possible that the increased dietary fibre resulted in increased feed consumption of broilers. Inline, Mpofu et al. (2016) demonstrated that a

greater amount of fibre in diets containing *Lippia javanica* leaf meal resulted in higher feed intake of broilers during the finishing phase. In this regard, broilers on high-fibre diets have been found to maximize feed consumption to compensate for the lower nutrient contents in the diets (Walugembe et al., 2014; Mpofu et al., 2016). In respect to feed conversion, dietary inclusion of APLS, particularly at the level of 5%, compromised the FCR of broilers when compared to that of control. Indeed, the increased levels of APLS linearly increased FCR of broilers. It was most likely that the enhanced fibre content in diets due to the incorporation of APLS reduced the feed digestibility and thus limited the utilization of feed by the chickens (Tejeda and Kim, 2021), as reflected by the increased FCR values.

Table 4: Performances of broilers fed experimental feeds.

Items	Treatment groups				SEM	P value	
	CONT	ACID1	ACID25	ACID5		A	L
Final BW (g)	1543	1512	1513	1459	15.5	0.30	0.07
Weight gain (g)	1373	1345	1346	1294	15.3	0.34	0.09
Accumulative FI (g)	2325[b]	2562[a]	2551[a]	2663[a]	38.3	<0.01	<0.01
FCR	1.69[b]	1.92[ab]	1.90[ab]	2.06[a]	0.04	0.02	<0.01

[a,b]Values with different letters in the same row are significantly (P < 0.05) different. CONT: chicks provided control diet having no APLS, ACID1: chicks provided with diet having 1% APLS, ACID25: diet having 2.5% APLS, ACID5: diet having 5% APLS, A: analysis of variance (ANOVA), L: linear regression, BW: body weight, FI: feed intake, FCR: feed conversion ratio, SEM: standard error of the means.

Table 5: Internal organ relative weight of broilers.

Items (% live BW)	Treatment groups				SEM	P value	
	CONT	ACID1	ACID25	ACID5		A	L
Heart	0.45	0.45	0.40	0.34	0.03	0.42	0.11
Liver	2.85	2.14	2.39	2.15	0.12	0.11	0.08
Proventriculus	0.47	0.48	0.53	0.45	0.02	0.65	0.84
Gizzard	1.47	1.60	1.75	1.74	0.05	0.09	0.02
Pancreas	0.25	0.29	0.25	0.28	0.11	0.49	0.62
Duodenum	0.60	0.82	0.76	0.73	0.04	0.10	0.28
Jejunum	1.71	1.61	1.62	1.85	0.05	0.28	0.33
Ileum	1.15	1.05	1.07	1.21	0.04	0.43	0.56
Caeca	0.71	0.66	0.61	0.62	0.03	0.79	0.33
Spleen	0.14	0.18	0.08	0.24	0.05	0.72	0.63
Thymus	0.13	0.23	0.21	0.20	0.02	0.15	0.26
Bursa of Fabricius	0.17	0.13	0.13	0.13	0.13	0.51	0.25

CONT: chicks provided control diet having no APLS, ACID1: chicks provided with diet having 1% APLS, ACID25: diet having 2.5% APLS, ACID5: diet having 5% APLS, BW: body weight, A: analysis of variance (ANOVA), L: linear regression, SEM: standard error of the means.

Table 6: Carcass and commercial proportions of broilers.

Items	Treatment groups				SEM	P value	
	CONT	ACID1	ACID25	ACID5		A	L
Eviscerated carcass (% live BW)	66.8	67.1	65.6	66.2	0.48	0.73	0.45
	% eviscerated carcass						
Breast	33.5	35.9	34.1	32.6	0.68	0.40	0.48
Wings	10.6	11.9	11.0	11.5	0.26	0.32	0.46
Thigh	15.8	16.3	15.5	16.7	0.31	0.61	0.52
Drumstick	15.1	14.8	14.3	15.5	0.23	0.32	0.72
Back	25.0	21.1	25.1	23.7	0.75	0.21	0.99

CONT: chicks provided control diet having no APLS, ACID1: chicks provided with diet having 1% APLS, ACID25: diet having 2.5% APLS, ACID5: diet having 5% APLS, BW: body weight, A: analysis of variance (ANOVA), L: linear regression, SEM: standard error of the means.

Table 7: Complete blood counts and total plasma protein of broilers.

Items	Treatment groups				SEM	P value	
	CONT	ACID1	ACID25	ACID5		A	L
Erythrocytes (10^6/µL)	2.26	2.23	2.39	2.40	0.44	0.42	0.14
Leukocytes (10^3/µL)	9.35	9.44	10.2	8.77	0.31	0.47	0.73
Hemoglobin (g/dL)	7.39	7.44	7.71	7.41	0.06	0.15	0.52
PCV (%)	21.2	20.7	21.3	21.5	0.34	0.87	0.63
Heterophils (%)	36.4	34.3	35.0	29.7	1.75	0.58	0.22
Eosinophils (%)	0.50	0.50	0.40	0.20	0.11	0.74	0.30
Lymphocytes (%)	55.1	57.4	56.9	63.1	1.83	0.46	0.15
Monocyte (%)	8.00	7.80	7.70	7.00	0.41	0.85	0.40
H/L ratio	0.72	0.65	0.68	0.54	0.06	0.69	0.29
Total plasma protein (g/dL)	2.52	2.74	2.74	2.72	0.08	0.71	0.39

CONT: chicks provided control diet having no APLS, ACID1: chicks provided with diet having 1% APLS, ACID25: diet having 2.5% APLS, ACID5: diet having 5% APLS, PCV: packed cell volume, H/L ratio: heterophils to lymphocytes ratio, A: analysis of variance (ANOVA), L: linear regression, SEM: standard error of the means.

Table 8: Serum lipid profile of broilers.

Items	Treatment groups				SEM	P value	
	CONT	ACID1	ACID25	ACID5		A	L
Total cholesterol (mg/dL)	129[b]	174[a]	131[b]	116[b]	7.54	0.03	0.25
Triglycerides (mg/dL)	82.3	139	100	98.2	10.4	0.26	0.93
HDL-cholesterol	73.7	98.5	86.7	85.8	3.40	0.08	0.43
LDL-cholesterol	124[b]	169[a]	126[b]	111[b]	7.58	0.04	0.24
HDL/LDL ratio	0.64	0.63	0.75	0.93	0.05	0.13	0.03
Cholesterol/HDL ratio	1.74	1.95	1.51	1.37	0.09	0.13	0.06

[a,b]Values with different letters in the same row are significantly ($P < 0.05$) different. CONT: chicks provided control diet having no APLS, ACID1: chicks provided with diet having 1% APLS, ACID25: diet having 2.5% APLS, ACID5: diet having 5% APLS, HDL: high-density lipoprotein, LDL: low-density lipoprotein, A: analysis of variance (ANOVA), L: linear regression, SEM: standard error of the means.

Table 9: pH values of intestinal segments.

Items	Treatment groups				SEM	P value	
	CONT	ACID1	ACID25	ACID5		A	L
Duodenum	6.70	6.25	5.93	6.26	0.11	0.06	0.08
Jejunum	5.29	5.40	5.09	5.48	0.09	0.53	0.77
Ileum	5.81	6.17	6.03	6.11	0.13	0.78	0.51
Cecum	7.18	7.35	7.03	7.67	0.13	0.36	0.33

CONT: chicks provided control diet having no APLS, ACID1: chicks provided with diet having 1% APLS, ACID25: diet having 2.5% APLS, ACID5: diet having 5% APLS, A: analysis of variance (ANOVA), L: linear regression, SEM: standard error of the means.

Table 10: Selected intestinal bacterial populations of broilers.

Items (log cfu/g)	Treatment groups				SEM	P value	
	CONT	ACID1	ACID25	ACID5		A	L
Ileum							
Coliform	7.51	8.27	7.95	8.69	0.31	0.61	0.25
Enterobacteriaceae	7.95	9.12	8.51	8.83	0.29	0.56	0.45
LAB	11.6	11.4	11.7	11.7	0.06	0.09	0.21
Cecum							
Coliform	8.12	8.74	6.68	8.21	0.33	0.13	0.55
Enterobacteriaceae	8.22	9.26	9.54	8.59	0.23	0.15	0.51
LAB	11.7	11.7	11.7	11.7	0.01	0.42	0.18

CONT: chicks provided control diet having no APLS, ACID1: chicks provided with diet having 1% APLS, ACID25: diet having 2.5% APLS, ACID5: diet having 5% APLS, cfu: colony forming units, LAB: lactic acid bacteria, A: analysis of variance (ANOVA), L: linear regression, SEM: standard error of the means.

Data in the present study showed no notable impact of dietary inclusion of APLS on the internal organs relative weight of broilers. However, the graded levels of APLS in diets linearly increased gizzard relative weight of chickens. Such increase in the weight of the gizzard seemed to be closely associated with the elevated fibre contents in the diets due to the inclusion of APLS. This inference was in line with Sacranie et al. (2012) demonstrating that the inclusion of fibre into diets increased the gizzard weight of broilers. The latter investigators also suggested that high dietary fibre may induce muscular hypertrophy, which in turn increased the gizzard weight. Note that dietary fibre is difficult to digest and thus accumulates in the gizzard of chicks. In this study, dietary incorporation of APLS had no significant impact on the carcass yield and commercial proportions of broilers. In this regard, feeding APLS to broilers had no detrimental consequences on the edible portions of broilers.

Our present finding showed no notable effect of dietary treatments on the blood profiles as well as and total plasma protein of broiler chickens. Considering that blood profiles and total plasma protein may reflect the physiological and inflammatory conditions of broilers (Bueno et al., 2017), the absent effect of feeding APLS on such indices could therefore indicate that dietary APLS did not compromise the physiological and health conditions of broiler chickens.

Data in the current experiment showed that the increased levels of APLS in feed linearly increased HDL/LDL ratio, and on the other hand decreased cholesterol/HDL ratio of broilers. This condition was actually favorable for the physiological conditions of broilers; since the increased HDL/LDL ratio and the decreased cholesterol/HDL ratio would prevent broiler chickens from cardiovascular problems (Bueno et al., 2017). Also, the decreased cholesterol/HDL ratio may alleviate the cholesterol deposition in broiler meats. It was very possible that organic acid and LAB content in *A. bilimbi* fruit filtrate lowered cholesterol synthesis as well as reducing the absorption of cholesterol derived from feed, which was also reported by Taherpour et al. (2009) when feeding butyric acid and probiotic *Lactobacillus* to broiler chickens. Also, the high level of fibers in papaya leaf and seed may contribute to the reduced cholesterol and LDL synthesis in broilers (Oloruntola et al., 2018; Tejeda and Kim, 2021).

It is generally expected that dietary administration of acidifiers would improve the intestinal ecology and bacterial ecosystem of broilers (Sugiharto, 2020b). In agreement with this, our present findings showed that the elevated levels of APLS in the feed decreased the pH values of the duodenum of broilers. However, no significant effect of APLS on the numbers of coliform, *Enterobacteriaceae*, and LAB was observed in both ileum and cecum of broilers. Indeed, there are several factors influencing the bacterial populations in the intestine of broilers, one of which is the availability of nutrients or substrates for bacteria. In this study, the high fiber content in APLS administrated diets seemed to compromise the nutrient digestibility of birds, and hence reduce the nutrient or substrate availability for the intestinal bacteria.

CONCLUSIONS AND RECOMMENDATIONS

Dietary inclusion of APLS at 5% compromised FCR, but improved serum lipid profile of broilers. The high fibre content of APLS may limit the use of such alternative feed ingredients in broiler feeds. Overall, APLS may safely be used up to 2.5% in the diets without detrimental effects on the growth, physiological conditions and intestinal ecology of broiler chickens.

ACKNOWLEDGEMENTS

The research was financed by the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro for the fiscal year of 2021.

NOVELTY STATEMENT

Acidification is a novel technique to lower crude fiber content of papaya leaf and seed meal. However, APLS can only be included in broiler diets at 2.5%, as greater inclusion level may harmful for broiler performance.

AUTHOR'S CONTRIBUTION

EW conducted experiments and drafted the manuscript, TAS, HIW, RM, and TY revised the manuscript, ARP conducted lab and data analysis, SS designed and revised the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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