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# 13 Growth performance, haematological responses, intestinal microbiology and carcass traits of broiler chickens fed finisher diets containing two-stage fermented banana peel meal

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## Abstract

The study investigated the effect of finisher diets containing two-stage fermented banana peel (FBP) meal on growth performance, haematological responses, intestinal microbiology and carcass traits of broilers. From days 22 to 38, 200 broiler chicks were grouped to maize-soybean meal-based finisher diet as a control (CONT) and finisher diets containing either 5% FBP (FBP-5), 10% FBP (FBP-10) or 15% FBP (FBP-15). Birds were blood sampled and slaughtered at day 38. For microbial population and villi height measurement, digesta and intestinal segments were collected. Breast meat was obtained for meat colour determination. Data collected were analysed according to analysis of variance followed by Duncan's multiple-range test when there was significant effect. There was no effect of treatments on broiler growth performance. The relative duodenum weight tended ( $P = 0.08$ ) to be lower in FBP than in CONT. The mean corpuscular haemoglobin concentration (MCHC) was higher ( $P < 0.05$ ) in FBP-5 than in CONT and FBP-15. Uric acid concentration was lower ( $P < 0.05$ ) in FBP-10 and FBP-15 than in CONT. Feeding FBP tended ( $P = 0.08$ ) to decrease coliform population in the ileum. Compared to CONT, ileal villi heights were higher ( $P < 0.05$ ) and lower ( $P < 0.05$ ) in FBP-10 and FBP-5, respectively. The relative weight of thigh ( $P < 0.05$ ) and wings ( $P = 0.07$ ) were higher in CONT than in FBP-fed birds, but the eviscerated carcass was not different ( $P > 0.05$ ) among groups. The lightness ( $L^*$ ) values of meat were higher ( $P < 0.05$ ) in FBP-10 and FBP-5 than in CONT and FBP-5. Compared with others, breast meat from FBP-10 birds had lower ( $P < 0.05$ ) redness ( $a^*$ ) values. In conclusion, dietary inclusion of FBP at the levels of up to 15% had no detrimental effect on growth and health performances of broiler chickens.

**Keywords** Broiler · Carcass · Fermented banana peel meal · Growth · Health

## Introduction

The price of conventional feed ingredients, such as maize, has increased substantially over the years. This condition may increase the production cost and reduce efficiency of the broiler industry. To reduce the cost of feed, there has been an increasing interest to use by-products from the agro-industries as feedstuffs in poultry rations (Kasapidou et al. 2015). Among the agro-industrial by-products, banana peel may be used as an alterna-

7  
tive energy source in broiler diets. Banana peel contains 10% crude protein (CP) and 2932 kcal/kg metabolizable energy (ME) (Blandon et al. 2015). Compared with other agro-industrial by-products (such as palm kernel, cassava pulp, rice bran, copra meal and orange peel and pulp), banana peel seems to be less utilised while the production continuously increases (Sugiharto et al. 2018a). As a consequence, banana peel has no economic value. It was shown by Siyal et al. (2016) that dietary inclusion of 1.5% and 3.0% of banana peels resulted in higher final body weight (BW) and dressing percentage, when compared with control. Concomitantly, Blandon et al. (2015) revealed that feeding banana peels up to 25% had no adverse effect on the average BW of broiler chicks at day 42. In contrast to the above-mentioned studies, dietary inclusion of 10% banana peel resulted in lower BW gain, feed consumption (Hernawan and Sun 2014) and feed efficiency ratio of broiler chickens (Fas et al. 2015). Moreover, Duwa et al. (2014) reported that feeding 5%,

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10% or 15% banana peel meal as a replacement for maize resulted in lower final BW of broilers. Several factors might be responsible for the inconsistent data highlighted above, two of which were the variation in the nutritional compositions and the contents of antinutritive factors in banana peels used in the studies. Note that the nutritional traits (Koni 2013; Blandon et al. 2015) and antinutritive factors (Anhwange et al. 2009; Romelle et al. 2016) in banana peel vary among cultivars. Regarding the antinutritive factors, the compounds such as hydrogen cyanide, oxalate, phytates and other antinutritional factors in banana peel may adversely affect nutrient digestibility and thus growth performance of broilers.

Fermentation has been conducted to improve the nutritional properties of banana peel. Koni et al. (2013) fermented banana peel using the fungus *Rhizopus oligosporus*, and found an increase and decrease in CP and crude fibre (CF) contents, respectively, after fermentation. For broiler diets, however, Koni et al. (2013) and Koni et al. (2013) recommended that fermented banana peel by *R. oligosporus* may only be included at the maximum of 10%, as higher inclusion levels impaired growth performance of broilers. In general, although fungal fermentation can increase CP content, it did not substantially improve protein solubility and amino acid profile of the substrates (Koni et al. 2013; Mukherjee et al. 2016). The latter condition may, therefore, limit protein utilisation of the fungal-fermented products by broiler chickens (Koni et al. 2013). Besides single-stage fermentation, two-stage fermentation has often been conducted to produce better nutritional characteristics in the products. Compared to single fermentation with *Bacillus subtilis*, two-stage fermentation using *R. oligosporus* followed by *B. subtilis* resulted in higher protein content in soybean in the study of Weng and Chen (2011). Concomitantly, two-stage fermentation with *Aspergillus oryzae* and *Lactobacillus casei* produced higher soluble protein, available lysine and protein digestibility of soybean meal, when compared with single fermentation using *A. oryzae* (Chen et al. 2010). In the present study, we firstly fermented banana peel meal with the fungus *Chrysonilia crassa* and then followed by *B. subtilis*. First-stage fermentation with *C. crassa* was particularly expected to lower the CF content of banana peel meal due to the remarkable fibrolytic activity of the fungus (Sugiharto et al. 2017). *B. subtilis* was preferred as a starter for the second-stage fermentation given that such bacteria were considerably able to improve protein solubility/digestibility and amino acid profile of the substrates (Mukherjee et al. 2016). Overall, two-stage fermentation of banana peel meal with *C. crassa* and *B. subtilis* was expected to produce better nutritional traits and thus increase the inclusion level of banana peel meal in broiler rations. Moreover, considering the probiotic potentials of *C. crassa* (Sugiharto et al. 2017) and *B. subtilis* (Sugiharto et al. 2018b), the two-stage fermentation using both microorganisms was therefore expected to improve not only the nutritional qualities, but also the functional properties of banana

peels. The latter features may exert health benefits that are of importance for broiler production following the ban of antibiotic growth promoters. The aim of the present study was to investigate the effect of finisher diets containing two-stage fermented banana peel (FBP) meal on growth performance, haematological responses, intestinal microbiology and carcass traits of broiler chickens.

## Materials and methods

### Preparation of starter cultures

Starter culture of *C. crassa* was prepared according to Sugiharto et al. (2017) with few modifications. It was conducted by rejuvenating the stock culture of *C. crassa* (maintained on a potato dextrose agar [PDA; Merck KGaA, Darmstadt, Germany] and stored at 4 °C) on chloramphenicol-supplemented PDA. The mycelia of the fungi were dislodged from the PDA after aerobic incubation at 38 °C for 48 h. It was then diluted in 200 ml of sterilised distilled water. To produce the fungal starter culture, the mycelia suspension was used for inoculating the sterilised dry cassava pulp (200 g; 87.5% dry matter). The mixture was then incubated aerobically at room temperature. Cassava pulp was used for the production of starter culture as our preliminary study showed that *C. crassa* grew better on cassava pulp than on banana peel meal. After 4 days of incubation, the colonies of *C. crassa* were counted based on the colony counting method. The fungal starter culture produced was further used to ferment (first-stage fermentation) the banana peel meal. Starter culture *B. subtilis* (used for the second-stage fermentation) was kindly provided by PT. Bayer Indonesia, Jakarta, Indonesia. It was in the form of concentrate and contained minimum  $10^{10}$  spores/g.

### Preparation of two-stage fermented cassava pulp

Fermentation of banana peel meal was conducted based on the solid-state fermentation method. In brief, 10 kg of sterilised banana peel meal was soaked in sterile water (1:1) to achieve the water content of about 40% in the substrates. The banana peel meal was then inoculated with 110 g/kg starter culture of *C. crassa* (containing  $3.6 \times 10^{10}$  cfu/g) and 41 g/kg urea as a source of nitrogen. After being mixed thoroughly, the inoculated banana peel meal was incubated aerobically at room temperature (25–26 °C) for 4 days. The substrate was turned every 2 days to ensure that the fungi grew evenly throughout the substrate. After 4 days of incubation with *C. crassa*, the substrate was subsequently inoculated with 1 mg/g *B. subtilis* inoculum and further incubated aerobically for 2 days at room temperature. After being sun-dried, sample of the FBP was obtained for proximate analysis (Table 1), and the rest of the FBP was used for the *in vivo* experiment.

**Table 1** Chemical compositions of fermented banana peel meal

Compositions (% as dry basis, unless otherwise stated)	Unfermented banana peel meal	Fermented banana peel meal
Moisture	18.3	22.9
Crude protein	7.96	11.8
Crude fat	3.24	2.39
Crude fibre	19.6	12.2
Ash	12.4	8.51
Metabolisable energy (kcal/kg) <sup>1</sup>	2660	3023

<sup>1</sup>Metabolisable energy was calculated based on formula (Bolton 1967) as follows: 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}

**5 In vivo experiment**

The animal trial was conducted complying with the standard rearing and handling of livestock stated in law of the Republic of Indonesia number 18, 2009, concerning animal husbandry, health and welfare. Two hundred Lohmann broiler day-old chicks (unsexed, with average BW of 35.6 ± 0.85 g) obtained from a local hatchery were raised in an open-sided broiler house during the study. All birds were provided with starter diet (Table 2) from days 0 to 21. The chicks were weighed at day 22 (average BW of 392 ± 3.66 g) and then randomly allocated to four dietary treatment groups of 50 birds each (5 replicates of 10 birds). These dietary groups were maize-soybean meal-based finisher diet as a control (CONT), finisher diet containing 5% FBP (FBP-5), finisher diet containing 10% FBP (FBP-10) and finisher diet containing 15% FBP (FBP-15). The treatment diets (in mash form) were prepared to be isonitrogenous and isocaloric and met the Indonesian National Standards for Broiler Feed (SNI 2006) (Table 3). For the entire trial period, the birds were allowed free access to feed and water. Vaccinations with commercial Newcastle disease virus (NDV) vaccine were done through spraying and drinking water on days 0 and 18, respectively. At day 38, BW gain, feed intake and feed conversion ratio (FCR) were recorded. The chick was weighed individually. A Camry electronic digital scale EK3650 with maximum capacity of 5-kg and 1-g graduation was used to weigh the chick and feed. FCR was determined as the total amount of feed consumed per unit BW gain from days 22 to 38.

At day 38, blood was obtained from the bird's wing veins of one chick from each replicate (5 chicks per treatment). For the determination of full blood counts, blood was collected in containers containing ethylenediaminetetraacetic acid, and the rest of the blood was collected in anticoagulant-free vacutainers to make serum (for the analyses of antibody titre and serum biochemistry). Immediately, the same chicks as blood sampled were slaughtered by neck cut, defeathered and eviscerated. Quickly, the segments of the duodenum,

**Table 2** Ingredients and chemical compositions of starter diet (days 0–21)

Items	Composition (%)
Maize	45.5
Soybean meal	17.0
Wheat flour	10.0
Bread flour	5.00
Rice bran	4.45
Crude palm oil	3.50
Corn gluten meal	3.00
Distiller dried grains	3.00
Meat bone meal	2.80
Chicken feather meal	2.00
Bone meal	1.50
Lysine	0.55
Methionine	0.37
L-Threonine	0.08
Salt	0.15
Premix <sup>1</sup>	0.50
Analysed compositions	
Crude protein	21.4
Crude fat	4.39
Crude fibre	7.63
Ash	7.71

<sup>1</sup>Premix contained (per kg of diet) of Ca 2.250 g, P 0.625 g, Fe 3.570 mg, Cu 0.640 mg, Mn 5.285 mg, Zn 0.003 mg, Co 0.001 mg, Se 0.013 mg, I 0.016 mg, vitamin A 375 IU, vitamin D 150 IU, vitamin E 0.080 mg

jejunum and ileum (each about 2 cm) were taken and put in 10% neutral formalin buffer solution (Leica Biosystems Richmond, Inc., Richmond, VA, USA) for the histological analysis. For the microbiological analysis, samples of digesta were collected from ileum and caecum by gently squeezing into the sterile sample bottle; the internal organs, carcass and commercial cuts of broilers were obtained and weighed. The samples of skinless breast meat were obtained for the determination of meat colour.

The full blood count analysis was performed using a haematology analyser based on the manufacturer's protocols (Prima Fully-auto Haematology Analyser, PT. Prima Alkesindo Nusantara, Jakarta, Indonesia). For the histological analyses of the intestine, 5-µm slices of the duodenum, jejunum and ileum were prepared and stained with haematoxylin and eosin. The villus height of each intestinal segment was measured with the aid of an optical microscope fitted with a digital camera (Leica Microsystems GmbH, Wetzlar, Germany). The populations of selected bacteria in the intestine were determined according to Sugiharto et al. (2017) with few modifications. The red and colourless colonies growing on MacConkey agar (Merck KGaA, Darmstadt, Germany) after aerobic incubation at 38 °C for 24 h were counted as total coliform and lactose-negative enterobacteria, respectively.

**Table 3** Ingredients and chemical composition of finisher diet (days 22–38)

Items	Treatment groups (% unless otherwise stated)			
	CONT	FBP-5	FBP-10	FBP-15
Maize	64.0	59.0	54.0	49.0
Soybean meal	20.0	20.0	20.0	20.0
Poultry meat meal	10.7	10.8	10.9	11.0
Rice	3.00	2.40	1.80	1.20
Crude coconut oil	1.00	1.50	2.00	2.50
FBP	–	5.00	10.0	15.0
Methionine	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20
Dicalcium phosphate	0.30	0.30	0.30	0.30
Premix <sup>1</sup>	0.50	0.50	0.50	0.50
<b>Calculated composition</b>				
Metabolisable energy (kcal/kg) <sup>2</sup>	3030	3030	3030	3030
Crude protein	20.0	20.0	20.0	20.0
Crude fibre	5.10	5.80	6.60	7.30
Methionine	0.70	0.70	0.70	0.70
Lysine	1.40	1.40	1.40	1.40
Ca	1.00	1.00	1.00	1.00
P	0.70	0.70	0.70	0.70
<b>Analysed compositions</b>				
Dry matter	87.7	84.6	85.4	85.4
Crude protein	22.5	20.8	21.8	18.5
Crude fat	3.49	3.54	2.89	3.52
Crude fibre	8.22	9.09	9.43	9.83
Ash	3.49	3.68	3.95	4.25

<sup>1</sup> Premix contained (per kg of diet) of Ca 2.250 g, P 0.625 g, Fe 3.570 mg, Cu 0.640 mg, Mn 5.285 mg, Zn 0.003 mg, Co 0.001 mg, Se 0.013 mg, I 0.016 mg, vitamin A 375 IU, vitamin D 150 IU, vitamin E 0.080 mg

<sup>2</sup> Metabolisable energy was calculated according to formula (Bolton 1967) as follows:  $40.81 \{0.87 [\text{crude protein} + 2.25 \text{ crude fat} + \text{nitrogen-free extract}] + 2.5\}$

The numbers of enterobacteria were determined as the sum of coliform and lactose-negative enterobacteria. The colonies of lactic acid bacteria (LAB) were enumerated on de Man, Rogosa and Sharpe (MRS; Merck KGaA) agar after anaerobic incubation at 38 °C for 48 h. The measurement of serum antibody titres against NDV was carried out according to the haemagglutination inhibition (HI) test (Villegas 1987), from which the titres were presented as geometric mean titres (Log<sub>2</sub>). The levels of total triglyceride, total cholesterol, high-density lipoprotein (HDL), low density lipoprotein (LDL), uric acid and creatinine in serum were determined according to the enzymatic colorimetric/colour methods. The concentrations of total protein, albumin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were determined based on spectrophotometric/photometric

tests. The serum globulin values were obtained from the difference between total protein and albumin. These above-mentioned chemical analyses in serum were performed using kits (DiaSys Diagnostic System GmbH, Holzheim, Germany) according to the producer's instructions. The colour values of broiler breast meats were measured with a digital colour meter in Mac OS X (set to CIE Lab) and expressed as L\* (lightness), a\* (redness) and b\* (yellowness) values. The L\* value of breast meat below 50 was categorised as dark-coloured meats, L\* value of 50 to 56 as normal-coloured meats and L\* value above 56 as light-coloured meats (Kaewthong et al. 2017).

All data obtained were analysed based on the analysis of variance using the general linear model procedure in SAS (SAS Inst. Inc., Cary, NC, USA). Data are presented as least square means (LSMEANS) and pooled standard error of the mean (SEM). Duncan's multiple-range test was performed when there was significant difference ( $P < 0.05$ ) among the treatment groups.

## Results

Data on the growth performance of broilers are presented in Table 4. In general, there was no substantial effect ( $P > 0.05$ ) of dietary treatments on BW gain, feed intake, FCR, and feed cost per kilogramme live BW gain of broiler chicks during the finishing phase. With regard to the relative weight of internal organs, there was a tendency ( $P = 0.08$ ) that the relative weight of the duodenum was lower in the treated birds as compared to that in control birds (Table 5). The other internal organs were not significantly different across the treatment diets.

Data on the full blood counts of broilers are presented in Table 6. The values of MCHC were higher ( $P < 0.05$ ) in FBP-5 when compared with those in CONT and FBP-15, but the difference was not different as compared to that in FBP-10. The other parameters did not differ among the treatment diets. Data on serum antibody titres against NDV and biochemical parameters of broilers are presented in Table 7. The concentration of uric acid was lower ( $P < 0.05$ ) in FBP-10 and FBP-15 as compared to that in CONT birds. There was no significant difference observed with regard to the other biochemical parameters.

The effects of dietary treatments on the population of selected bacteria in the ileum and caecum of broilers are presented in Table 8. There was a tendency ( $P = 0.08$ ) that feeding finisher diet containing FBP decreased the numbers of coliform bacteria in the ileum of broilers. The numbers of other bacteria were not significantly different among the treatment diets. Compared to CONT, the ileal villi heights were higher ( $P < 0.05$ ) and lower ( $P < 0.05$ ) in FBP-10 and FBP-5 birds, respectively (Table 9). The villi heights in the

**Table 4** Growth performance of broilers fed FBP during days 22–38

Days	Treatment groups				SEM	P value
	CONT	FBP-5	FBP-10	FBP-15		
BW gain (g/bird/day)	48.6	52.8	55.5	52.0	2.11	0.19
Feed intake (g/bird/day)	77.0	87.0	80.6	87.8	3.61	0.14
FCR	1.61	1.66	1.46	1.69	0.09	0.29
Feed cost per kg live BW gain <sup>1</sup>	11,777	11,743	9971	11,132	627	0.18

<sup>1</sup> Values are expressed in IDR (Indonesian currency) as at the time of trial, and was counted by the cost of feed consumed to obtain a kilogramme live BW gain

CONT maize-soybean meal-based finisher diet, FBP-5 finisher diet containing 5% FBP, FBP-10 finisher diet containing 10% FBP, FBP-15 finisher diet containing 15% FBP, FBP fermented banana peel meal, BW body weight, FCR feed conversion ratio, SEM pooled standard error of the mean

duodenum and jejunum were not different ( $P > 0.05$ ) among the treatment groups.

Table 10 shows the effect of feeding finisher diets containing FBP on the carcass characteristics of broiler chicks. The relative weights of the thigh ( $P < 0.05$ ) and wing ( $P = 0.07$ ) were higher in CONT than in the treated birds. The eviscerated carcass of broilers was not different ( $P > 0.05$ ) among the treatment diets. With the organoleptic characteristics, values obtained showed that meats on the FBP-10 and FBP-15 had lighter ( $L^*$ ) colour as compared to those on the control diets and 5% FBP diets (Table 11). Compared to other birds, breast meat from birds in the FBP-10 group had lower ( $P < 0.05$ ) values of redness ( $a^*$ ). No significant difference was observed

with regard to yellowness ( $b^*$ ) values of breast meat across the treatment groups.

### Discussion

One of the purposes in the use of FBP was to reduce the proportion of maize as an energy source in broiler rations. This is of importance as the price of maize is increasing over the years. In the present study, inclusion of FBP in the finisher diets up to 15% had no deleterious effect on the growth performance of broilers. This result, therefore, disagreed with that of Hemawan and Abun (2014) and Duwa et al. (2014) showing an adverse effect of feeding banana peel meal on the growth performance of broilers. In the present study, two-stage fermentation decreased and increased the CF and CP contents of banana peel meal by 37.8% and 32.5%, respectively. On this basis, we incorporated either 5, 10 or 15% of FBP and formulated the finisher diets to be isonitrogenous and isocaloric. Divergent from our calculation, the proximate data showed that the CP contents of FBP-incorporated feeds (particularly FBP-15 diet) were lower than those of control diet. In spite of that, the growth performance did not vary between the FBP-treated and control birds, suggesting that protein utilisation was better in the FBP-fed than in control birds. This may be reasonable as Mukherjee et al. (2016) noticed better protein digestibility when feeding diets containing fermented feed to poultry. This inference should, however, be interpreted with caution as we did not measure protein digestibility of broiler during the present trial. It was seen in the present study that feed cost per kilogramme live BW gain was lower (by 15.3%, as compared to control) in FBP-10 than in other dietary groups, although the value was not statistically significant. Compared to feeding maize-soybean meal-based finisher diet, feeding finisher diet containing FBP at the level of 10% may therefore reduce the cost of feed resulting in improved production efficiency and profitability of broiler enterprises.

**Table 5** Relative internal organ weights of broilers fed FBP

Items (% live BW)	Treatment groups				SEM	P value
	CONT	FBP-5	FBP-10	FBP-15		
Heart	0.56	0.48	0.50	0.48	0.03	0.17
Liver	2.91	2.57	2.56	2.58	0.23	0.66
Proventriculus	0.62	0.60	0.60	0.59	0.04	0.94
Gizzard	1.91	1.63	1.83	1.78	0.14	0.58
Duodenum	0.86	0.62	0.67	0.71	0.06	0.08
Jejunum	1.41	1.15	1.29	1.15	0.09	0.16
Ileum	1.37	1.17	1.19	1.20	0.08	0.27
Pancreas	0.35	0.32	0.34	0.37	0.02	0.28
Caeca	0.40	0.37	0.33	0.42	0.40	0.31
Spleen	0.12	0.17	0.11	0.16	0.03	0.56
Thymus	0.32	0.28	0.27	0.21	0.03	0.11
Bursa of Fabricius	0.07	0.07	0.08	0.07	0.01	0.83
Abdominal fat	1.12	0.85	1.15	0.78	0.14	0.17

CONT maize-soybean meal-based finisher diet, FBP-5 finisher diet containing 5% FBP, FBP-10 finisher diet containing 10% FBP, FBP-15 finisher diet containing 15% FBP, FBP fermented banana peel meal, BW body weight, SEM pooled standard error of the mean

**Table 6** Complete blood counts of broilers fed FBP

Items	Treatment groups				SEM	P value
	CONT	FBP-5	FBP-10	FBP-15		
Haemoglobin (g/dL)	10.4	10.7	9.50	9.80	0.70	0.61
Erythrocytes (10 <sup>6</sup> /μL)	2.55	2.54	2.24	2.40	0.17	0.55
Haematocrit (%)	26.9	26.9	24.2	25.6	1.74	0.66
MCV (fl)	107	108	109	108	1.38	0.71
MCH (pg)	40.8	42.2	42.3	40.9	0.64	0.20
MCHC (g/dL)	38.6 <sup>b</sup>	39.7 <sup>a</sup>	39.2 <sup>ab</sup>	38.2 <sup>b</sup>	0.34	0.03
Leukocytes (10 <sup>3</sup> /μL)	68.0	61.4	65.6	65.4	6.92	0.92
Heterophils (10 <sup>3</sup> /μL)	7.30	6.70	5.80	7.70	1.38	0.78
Eosinophils (10 <sup>3</sup> /μL)	4.30	4.00	4.70	4.90	0.68	0.79
Lymphocytes (10 <sup>3</sup> /μL)	56.4	50.7	55.1	52.8	5.87	0.91
Thrombocytes (10 <sup>3</sup> /μL)	9.40	9.00	7.80	8.00	0.98	0.61

CONT maize-soybean meal-based finisher diet, FBP-5 finisher diet containing 5% FBP, FBP-10 finisher diet containing 10% FBP, FBP-15 finisher diet containing 15% FBP, FBP fermented banana peel meal, MCV mean corpuscular volume, MCH mean corpuscular haemoglobin, MCHC mean corpuscular haemoglobin concentration, SEM pooled standard error of the mean

<sup>a,b</sup> Means in the same row with different letters show significant differences (P < 0.05)

Data from the current study showed no substantial effect of dietary treatments on the relative organ weight of broiler chicks, although there was a tendency that duodenum relative weight was lower in the treated than in control broilers. The definite explanation for the lower duodenum relative weight of broilers in the present study was not known, but such decrease seemed to be advantageous for broiler chicks, as Wang

et al. (2016) suggested that the decrease in intestinal weight may imply better nutrient utilisation as more energy partitioned into growth than maintenance.

In the present study, the value of MCHC was higher in FBP-5 than in CONT and FBP-15 birds. In line with our finding, Olorunmila et al. (2016) reported an increased MCHC value in rabbits with feeding rumen liquor fermented cassava peels. The

**Table 7** Serum antibody titres against NDV and biochemical parameters of broilers fed FBP

Items	Treatment groups				SEM	P value
	CONT	FBP-5	FBP-10	FBP-15		
Antibody titre against NDV (Log <sub>2</sub> GMT)	2.40	2.75	2.33	4.25	0.93	0.34
AST (U/L)	168	186	195	205	19.2	0.59
ALT (U/L)	1.70	0.95	1.77	1.24	0.29	0.19
Uric acid (g/dL)	12.2 <sup>a</sup>	9.57 <sup>ab</sup>	5.78 <sup>b</sup>	7.69 <sup>b</sup>	1.52	0.04
Creatinine (g/dL)	0.06	0.07	0.07	0.08	0.01	0.16
Total triglyceride (g/dL)	53.1	45.1	57.8	65.5	6.99	0.21
Total cholesterol (g/dL)	116	122	125	124	8.95	0.89
LDL (g/dL)	47.4	32.9	34.3	30.4	12.5	0.76
HDL (g/dL)	67.6	74.0	79.0	80.2	6.28	0.49
Total protein (g/dL)	2.45	2.40	2.30	2.81	0.41	0.83
Albumin (g/dL)	1.11	1.08	1.14	1.16	0.06	0.81
Globulin (g/dL)	1.34	1.32	1.16	1.67	0.38	0.81
A/G ratio	1.47	0.97	1.17	0.77	0.28	0.36

CONT maize-soybean meal-based finisher diet, FBP-5 finisher diet containing 5% FBP, FBP-10 finisher diet containing 10% FBP, FBP-15 finisher diet containing 15% FBP, FBP fermented banana peel meal, NDV Newcastle disease virus, GMT geometric mean titre, AST aspartate aminotransferase, ALT alanine aminotransferase, LDL low-density lipoprotein, HDL high-density lipoprotein, A/G ratio albumin to globulin ratio, SEM pooled standard error of the mean

<sup>a,b</sup> Means in the same row with different letters show significant differences (P < 0.05)

**Table 8** Selected bacterial populations in the ileum and caecum of broilers fed FBP

Items	Treatment groups				SEM	P value
	CONT	FBP-5	FBP-10	FBP-15		
<b>Ileum (log cfu/g)</b>						
Coliform	8.39	6.25	6.70	6.69	0.64	0.08
Lactose-negative enterobacteria	8.85	8.91	8.54	8.41	0.48	0.84
Enterobacteria	9.07	8.91	8.92	8.45	0.49	0.80
LAB	10.9	10.4	10.7	11.0	0.35	0.56
<b>Caecum (log cfu/g)</b>						
Coliform	9.21	9.63	9.50	9.13	0.26	0.54
Lactose-negative enterobacteria	8.57	9.21	9.22	8.74	0.33	0.44
Enterobacteria	9.45	9.66	9.66	9.33	0.18	0.43
LAB	11.4	11.6	11.5	11.5	0.12	0.58

CONT maize-soybean meal-based finisher diet, FBP-5 finisher diet containing 5% FBP, FBP-10 finisher diet containing 10% FBP, FBP-15 finisher diet containing 15% FBP, FBP fermented banana peel meal, cfu colony-forming units, LAB lactic acid bacteria, SEM pooled standard error of the mean

latter authors further confirmed that feeding such fermented feed improved the haematopoiesis in rabbits. To date, there has been no definite explanation for the elevated value of MCHC in broilers with feeding FBP. A recent study by Aureli et al. (2018) showed an efficacy of glucuronoxylan hydrolase (produced by fermentation using *Bacillus* sp.) in increasing MCHC value in broiler. In this regard, glucuronoxylan hydrolase in FBP seemed to be responsible for the increased haematopoietic activity in FBP-fed broilers. The latter speculation should, however, be noted with caution as we had no data of glucuronoxylan hydrolase in FBP and, also, there was no increase in the values of haemoglobin and erythrocytes in FBP-fed birds in the present study.

Data in our present study showed that the concentration of uric acid decreased with feeding FBP. Considering that uric acid is the ultimate product of protein catabolism, the lower level of uric acid may reflect the lower protein degradation in FBP-fed broilers (Swennen et al. 2005). This may be beneficial for protein deposition in broilers.

**Table 9** Height of intestinal villi of broilers fed FBP

Items (µm)	Treatment groups				SEM	P value
	CONT	FBP-5	FBP-10	FBP-15		
Duodenum	1277	1193	1107	1135	73.3	0.38
Jejunum	1277	1275	1209	1358	56.5	0.33
Ileum	863.9 <sup>b</sup>	706.8 <sup>c</sup>	1036 <sup>a</sup>	945.2 <sup>ab</sup>	52.9	<0.01

CONT maize-soybean meal-based finisher diet, FBP-5 finisher diet containing 5% FBP, FBP-10 finisher diet containing 10% FBP, FBP-15 finisher diet containing 15% FBP, FBP fermented banana peel meal, SEM pooled standard error of the mean

<sup>a,b,c</sup> Means in the same row with different letters show significant differences ( $P < 0.05$ )

In the present study, feeding FBP tended to decrease the number of coliform bacteria in the ileum of broilers. Studies have revealed the antibacterial activities of *C. crassa* (Sugiharto et al. 2018b) and *B. subtilis* (Jeong and Kim 2014). In this respect, the probiotic activity of both microorganisms used to ferment banana peel meal may be responsible for lowering the number of ileal coliform in broiler chickens. In this study, we believed that both *C. crassa* and *B. subtilis* remained alive in the feeds as they were able to form spores that are resistant to heat and chemicals. Apart from the probiotic effect, the antimicrobial activities of banana peel itself may take an action in lowering the population of coliform in the ileum of broilers. Indeed, Sirajudin et al. (2014) demonstrated the

**Table 10** Carcass characteristics of broilers fed FBP

Items	Treatment groups				SEM	P value
	CONT	FBP-5	FBP-10	FBP-15		
% live BW						
Eviscerated carcass	64.3	65.5	66.7	64.4	0.99	0.31
% eviscerated carcass						
Breast	32.4	34.8	34.6	33.0	0.96	0.26
Thigh	17.6 <sup>a</sup>	16.2 <sup>b</sup>	16.5 <sup>b</sup>	16.5 <sup>b</sup>	0.31	0.03
Drumstick	16.0	15.4	15.2	15.9	0.31	0.25
Wing	12.0	11.5	11.0	11.4	0.24	0.07
Back	21.9	22.1	22.6	23.2	0.55	0.36

CONT maize-soybean meal-based finisher diet, FBP-5 finisher diet containing 5% FBP, FBP-10 finisher diet containing 10% FBP, FBP-15 finisher diet containing 15% FBP, FBP fermented banana peel meal, BW body weight, SEM pooled standard error of the mean

<sup>a,b</sup> Means in the same row with different letters show significant differences ( $P < 0.05$ )



**Table 11** Colour values of breast meats of broilers fed FBP

Items	Treatment groups				SEM	P value
	CONT	FBP-5	FBP-10	FBP-15		
L* (lightness)	49.4 <sup>c</sup>	51.7 <sup>bc</sup>	56.1 <sup>a</sup>	53.1 <sup>ab</sup>	1.16	< 0.01
a* (redness)	10.6 <sup>a</sup>	8.13 <sup>a</sup>	4.53 <sup>b</sup>	9.27 <sup>a</sup>	0.96	< 0.01
b* (yellowness)	24.2	22.0	25.4	24.5	1.02	0.12

CONT maize-soybean meal-based finisher diet, FBP-5 finisher diet containing 5% FBP, FBP-10 finisher diet containing 10% FBP, FBP-15 finisher diet containing 15% FBP, FBP fermented banana peel meal, SEM pooled standard error of the mean

<sup>a,b,c</sup> Means in the same row with different letters show significant differences ( $P < 0.05$ )

antimicrobial activities of banana (*Musa paradisiaca* variety Nangka and *M. paradisiaca* variety Tanduk) peel extracts against food borne pathogens (*Staphylococcus aureus*, *Candida krusei*, *Vibrio parahaemolyticus* and *Candida albicans*).

In the present study, FBP-10 and FBP-15 had a relatively higher ileal villi height as compared to CONT birds. This suggests that these respective birds may have better absorptive capacity than control birds. In contrast to FBP-10 and FBP-15, birds in the FBP-5 group had a lower ileal villi height. It may be speculated that these birds had reduced absorptive capacity and thus lower growth rate than other birds. However, this was not the case in our present study, as there was no difference in terms of BW gain of broiler. Perhaps, the lower ileal villi height in FBP-5 birds was compensated by the increased crypt depth (not observed in the current study) as previously reported by Bogucka et al. (2016). As a result, the difference in absorptive capacity and thus growth performance in birds was not observed in this study. In general, the morphology of ileal villi is greatly affected by the presence of digested nutrients in the ileal lumen. In this regard, the lower ileal villi height in FBP-5 birds may be due to most of the nutrients being already absorbed in the duodenum and jejunum of broiler chicks resulting in less nutrients reaching the ileum (Yamauchi et al. 2010).

In this study, feeding finisher diet containing FBP did not affect the relative weight of eviscerated carcass of broiler chicks. Our present finding was in accordance with that of Koni et al. (2013) showing no effect of feeding FBP up to 15% on the relative weight of eviscerated carcass. This may suggest that FBP may be included in broiler ration without any deleterious effect on the carcass weight of broilers. However, it should be noted that feeding such stuff may reduce thigh and wing relative weights in broilers. Overall, the numerical increase in breast weight may explain why the increased thigh and wing relative weights did not affect the carcass weight of broilers.

The colour of breast meat has been used to estimate the quality of meat of broiler chicks. With regard especially to L\* values, the increase in L\* values may be associated with reduced water holding capacity and pH, which corresponded with pale-soft-exudative (PSE) meat. Indeed, data in the present study showed that feeding FBP at the levels of 10% and 15% increased the L\* values of broiler breast meats. However, it seemed that breast meats in the present study still had normal lightness, as Kaewthong et al. (2017) categorised the lightness of broiler meats as follows: dark-coloured meats ( $L^* < 50$ ), normal-coloured meats ( $50 \leq L^* \leq 56$ ) and light-coloured meats ( $L^* > 56$ ). In this study, it was observed that a\* values were substantially lower in FBP-10 than other birds. It was difficult to relate the lower a\* values of breast meat with the inclusion of banana peel meals in diets, as the a\* values of breast meat in FBP-15 broilers did not differ from those of CONT birds.

In conclusion, dietary inclusion of FBP at the levels of up to 15% had no detrimental effect on growth and health performances of broiler chickens. Such fermented by-product may be used as feed ingredient in broiler rations to reduce the use of maize.

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**Compliance with ethical standards** All applicable international, national and/or institutional guidelines for treating and handling of animals were followed in the current study.

**Conflict of interest** The authors declare that they have no conflict of interest.

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