Performances and haematological profile of broilers fed fermented dried cassava (Manihot esculenta Crantz)

by Sugiharto Sugiharto

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REGULAR ARTICLES

Performances and haematological profile of broilers fed fermented dried cassava (*Manihot esculenta* Crantz)

Sugiharto Sugiharto[1] **· Turrini Yudiarti**[1] **· Isroli Isroli**[1]

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Abstract The effect of feeding fermented dried cassava (*gathot*) on the performances and haematological profile of broilers was investigated. There were four dietary treatments arranged in a completely randomized design, i.e. control diet and diets containing 25, 50 or 100 g/kg *gathot*. The birds were provided with the treatment diets ad libitum from 8 to 35 days of age. Body weight, feed intake and feed conversion ratio (FCR) were determined weekly. At day 32, the birds were blood sampled, sacrificed and immediately the internal organs and abdominal fat were removed and weighed. Feeding *gathot* at various levels did not affect ($P > 0.05$) the growth and FCR, but tended ($P = 0.09$) to reduce the feed cost per kilogramme live weight gain of broilers. The dietary treatments did not cause toxicological effect on broilers, indicated by the values of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) that were not different from those in the control diet. Dietary inclusion of *gathot* lowered heterophils to lymphocytes ratio (H/L ratio) ($P < 0.05$) and albumin to globulin ratio (A/G ratio) ($P = 0.14$) of broilers as compared to the control diet. Total triglyceride was lower ($P < 0.05$) in the serum of broilers fed diets containing 5 and 10 % of *gathot*, when compared with that in the control diet. The treatments resulted in reduced abdominal fat deposition in broilers. In conclusion, dietary inclusion of *gathot* at up to 10 % had no negative impact on the growth performance of broilers. Feeding *gathot* has potential to improve the health and physiological stress responses as well as reduce body fat deposition in broilers.

✉ Sugiharto Sugiharto
sgh_undip@yahoo.co.id

[1] Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java 50275, Indonesia

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Introduction

In the modern poultry industry, feed is considered the most important factor and plays a major role in total production cost. Due to competition between food, feed and agro-fuels, the price of feed ingredients for broilers has increased steadily in the past few years. For this reason, the use of unconventional feed ingredients in broiler rations is a necessity. Indonesia is one of the five biggest countries producing cassava (*Manihot esculenta* Crantz) in the world. Due to its high content of starch, cassava root meal can be a promising alternative to maize and other cereal grains. The utilization of cassava root meal in broiler rations, however, is limited by, e.g. the content of cyanogenetic glycosides compounds (Chauynarong et al. 2009). Akapo et al. (2014) reported that although inclusion of 10 % peeled cassava root meal in the diet had some economic advantages, this practice might impair the growth and health of broiler chickens due to reduced protein content and the presence of cyanogenetic glycosides residues leading to incidence of toxicity. Earlier study has confirmed that fermentation could be a simple means to detoxify cyanogenetic glycosides in cassava roots (Kobawila et al. 2005). Hence, there is a possibility to include cassava root meal in broiler diet at a higher level after fermentation.

Gathot is a traditional fermented dried cassava that originated from East and Central Java, Indonesia. This cassava product is characterized by the black colour of the inside and outside part of the tuber as a result of the spontaneous fermentation. Our previous study showed that the black colour in *gathot* is ascribed by the filamentous fungi (*Acremonium charticola* and *Rhizopus oryzae*) growing in the tuber during

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the fermentation process (Yudiarti and Sugiharto 2016). Indeed, the fungi isolated from *gathot* exhibited probiotic potential and antioxidant activity (Sugiharto et al. 2015, 2016). Therefore, the use of *gathot* in broiler rations was expected not only to substitute maize, but also to improve the health and physiological condition of broiler chickens as feeding probiotic and antioxidant may improve the immune competences of broilers (Sugiharto 2014). The aim of the present study was to investigate the effect of feeding *gathot* on the performances and haematological profile of broilers.

Materials and Methods

Gathot was prepared by spontaneous fermentation according to Yudiarti and Sugiharto (2016). In brief, cassava root tubers were peeled, washed thoroughly with fresh water and sun-dried. The tubers were let to stand on the roof and exposed to rainwater and sunshine for approximately 1 month, until the tubers were grown by black fungus and give a patchy greyish appearance in the exterior and interior of the tuber. *Gathot* was then collected, milled and stored in a dry space until used. Samples of *gathot* meal were pooled, and enumeration and identification of fungi in *gathot* were then performed (Yudiarti and Sugiharto 2016). Due to the need for rainwater, the process of making *gathot* is done mostly on the rainy season. The nutritional composition of *gathot* is presented in Table 1.

Four treatment diets (one control, and three *gathot* meal 25, 50 and 100 g/kg) were formulated to equal levels of calculated metabolizable energy (ME) and crude protein (CP) (Table 2), at which *gathot* substituted maize in the control. The diets were prepared to meet or exceed the minimum NRC (1994) requirements for broilers.

A total of 200 Lohman (MB-202) day old chicks (body weight = 45.39 ± 3.28 g; mean ± SD) purchased from a local hatchery were placed in an open-sided naturally ventilated broiler house. The house had concrete floor pens (with rice husk as bedding material) and was equipped with a tray feeder and manual drinker. Birds were provided with commercial starter feed (CP 22 % and ME 2900 kcal/kg) for the first week of rearing. At 8 days of age, the birds were weighed, and 160 birds with uniform weight were randomly allotted into 4 groups of 40 chicks each and 5 replicates of 8 chicks. The birds received treatment diets from 8 to 35 days of age in mash form. Diets and water were provided ad libitum for the entire period of the experiment. Body weight, feed intake and feed conversion ratio (FCR) were determined weekly for each pen.

For evaluation of haematological profile, blood was obtained from the birds' wing veins and collected in EDTA-containing vacutainers at day 32. The rest of the blood was collected in vacutainers containing no anticoagulant and allowed to clot for 2 h at room temperature. After centrifugation at 2000 rpm for 15 min, the serum was obtained and stored at −20 °C until serum biochemistry analysis. The same birds that were blood sampled were sacrificed after being weighed and, immediately

Table 1 Nutritional composition of *gathot*

Items (%, unless otherwise noted)	Amount
Dry matter	86.8
Crude protein	1.95
Crude fat	1.51
Crude fibre	2.81
Ash	1.43
Metabolizable energy[a] (kcal/kg)	3569

[a] Value was obtained based on the formula according to Bolton (1967)

Table 2 Composition of the experimental diets

Items	*Gathot*			
	Control	2.5 %	5 %	10 %
Ingredients (%)				
Maize	57.0	53.5	49.0	41.0
Gathot	0.00	2.50	5.00	10.0
Rice bran	11.2	11.3	10.4	12.6
Soybean meal	7.00	9.30	9.00	12.1
Fish meal	9.70	10.0	9.80	8.00
Poultry meat meal	5.10	4.00	4.20	4.50
Pollard	9.00	8.40	11.6	10.8
Top mix[a]	1.00	1.00	1.00	1.00
Calculated composition (%, unless otherwise noted)				
Metabolizable energy (kcal/kg)	2944	2947	2949	2954
Crude protein	20.1	20.4	20.3	20.4
Crude fibre	5.86	5.81	6.03	6.21
Crude fat	3.94	3.77	3.74	3.57
Ca	1.10	1.05	1.05	0.94
P	0.83	0.81	0.82	0.77
Analyzed composition (%, unless otherwise noted)				
Metabolizable energy[b] (kcal/kg)	2915	2982	3000	2996
Dry matter	89.7	89.6	89.9	89.8
Crude protein	16.5	17.4	17.8	17.9
Crude fibre	6.38	6.99	6.49	6.20
Crude fat	3.74	4.54	4.22	3.55
Ash	8.71	7.18	7.10	6.52

[a] Each kilogramme top mix contained vitamin A (1,200,000 IU), vitamin D_3 (200,000 IU), vitamin E (800 IU), vitamin B1 (200 mg), vitamin B2 (500 mg), vitamin B6 (50 mg), vitamin B12 (1200 μg), vitamin K (200 mg), vitamin C (2500 mg), Ca D-pantothenate (600 mg), niacin (4000 mg), manganese (1200 mg), iron (2000 mg), iodine (20 mg), zinc (10,000 mg), cobalt (20 mg), copper (400 mg), santoquin (1000 mg), zinc bacitracin (21,000 mg), choline chloride (1000 mg), methionine (3000) and lysine (3000 mg)

[b] Values were obtained based on the formula according to Bolton (1967)

the internal organs and abdominal fat were removed and weighed.

The numbers of erythrocytes and leukocytes were determined using the dilution flask method, and a Bürker chamber was used to count corpuscles. The erythrocyte sedimentation rate (ESR) was determined using the automated erythrocyte sedimentation rate analyzer (Streck ESR-Auto Plus, Streck, Omaha, NE). The differential leukocytes of chickens were determined using a light microscope with an immersion lens. Coverslip technique was applied when preparing blood smears. Heterophils to lymphocytes ratio (H/L ratio) was calculated by dividing the numbers of heterophils and lymphocytes. Total triglyceride in the serum was determined based on enzymatic colorimetric method using glycerol-3-phosphate-oxidase (GPO) (DiaSys Diagnostic System GmbH, Holzheim, Germany). To determine the toxicology of liver in chickens, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were measured spectrophotometrically using a Reflotron system (Roche Diagnostics Corporation, Indianapolis, IN, USA). A total serum protein was estimated by photometric test according to the biuret method using the kit (total protein kit DiaSys Diagnostic System GmbH, Holzheim, Germany) according to the manufacturer's instructions. Albumin was determined by photometric test using bromocresol green (DiaSys Diagnostic System GmbH, Holzheim, Germany). Globulin was obtained by subtracting albumin values from total serum protein. Albumin to globulin ratio (A/G ratio) was calculated by dividing albumin values and globulin values.

The data were analyzed based on a completely randomized design by ANOVA using the General Linear Models Procedure in SAS (SAS Inst. Inc., Cary, NC, USA). Pen was treated as the experimental unit and the results are presented as least squares means (LSMEANS) and standard error of the mean (SEM). Significant differences among treatment groups were further analyzed with Duncan's multiple-range test. A significant level of $P < 0.05$ was applied.

Results

Total fungal counts in *gathot* were 3×10^8 colony forming units (cfu)/g, and based on the macroscopic and microscopic identification the fungi were *A. charticola* and *R. oryzae*. Data of broiler performances are presented in Table 3. Inclusion of *gathot* into broiler rations at various levels did not affect ($P > 0.05$) the growth and FCR, but tended ($P = 0.09$) to reduce the feed cost per kilogramme live weight gain of broilers. No morbidity and mortality were observed in chickens throughout the study. The haematological and serum biochemical parameters of broilers fed *gathot* are shown in Table 4. Feeding *gathot* lowered ($P < 0.05$) the H/L ratio of broilers as compared to the control diet. The rest of haematological parameters did not differ ($P > 0.05$) among the treatment diets. Total triglyceride was lower ($P < 0.05$) in the serum of broilers fed diets containing 5 and 10 % of *gathot*, when compared with the control diet. There was no significant difference with regard to AST, ALT, total protein, albumin and globulin in the serum of broilers. Although significant difference was not observed, the A/G ratio in serum seemed to decrease ($P = 0.14$) after feeding *gathot* to broiler chickens. The data of giblets, liver, eviscerated carcass and abdominal fat of broilers are presented in Table 5. No significant difference ($P > 0.05$) was found with respect to the observed parameters. However, abdominal fat was numerically lower in broilers fed *gathot* as compared to those fed the control diet.

Discussion

In the present study, *gathot* was included in broiler rations to partially substitute the expensive maize, given that *gathot* contained ME comparable to maize (ME of maize 3283 kcal/kg and *gathot* 3569 kcal/kg). Results showed that dietary inclusion of *gathot* at up to 10 % did not adversely affect the final BW and FCR of broilers. Indeed, feeding *gathot* tended to reduce the feed cost per kilogramme live weight gain of broiler. This present finding was different from that of reported by Akapo et al. (2014) when feeding 10 % peeled cassava root meal, resulting in reduced final BW and increased feed-to-gain ratio of broilers. The increased dietary crude fibre and HCN and the reduced CP contents with increasing the levels of cassava root had been suggested to predispose the cassava-fed birds to gain less weight as compared to control (Akapo et al. 2014). This seemed, however, not the case in our present study as we balanced the energy, CP and fibre contents in our experimental diets. Indeed, the fibre content of *gathot* was not our major concern when formulating the broiler rations, as it is somewhat comparable to that of maize (crude fibre of *gathot* 2.81 % and maize 2.55 %). One thing that should be taken into account when using *gathot* as substitute for maize is the CP content of *gathot* that is far lower than that of maize (1.95 against 9.56 %). Note that spontaneous fermentation (process of making *gathot*) could not significantly increase the CP content of cassava root meal (from 1.70 to 1.95 %). In this study, no differences in the activities of AST and ALT were observed among the treatment diets. The AST and ALT ranged between 234.22 to 257.12 and 20.22 to 21.95 U/L, respectively, which were in the normal range (Coles 2007). In accordance with AST and ALT, the difference in liver weight (relative to live BW) was not observed in the current study. These indicated that feeding *gathot* did not induce toxicological effect (caused by HCN) on broiler chickens. Taken together, our data further confirms that fermentation is a good method to process the unconventional

Table 3 Growth performance of broilers fed *gathot*

Items	*Gathot*				SEM	*P* value
	Control	2.5 %	5 %	10 %		
Initial BW (g/bird)[a]	176	173	173	171	6.24	0.95
Final BW (g/bird)[b]	1177	1175	1220	1126	33.0	0.29
BWG (g/bird)[c]	1000	1002	1047	956	28.8	0.21
FCR[d]	3.14	3.08	3.00	3.20	0.08	0.39
Feed cost per kg live BWG[e]	20,941	20,036	18,960	19,154	524	0.09

BW body weight, *BWG* body weight gain, *FCR* feed conversion ratio

[a] BW determined at day 8 of the experiment

[b] BW determined at day 35 of the experiment

[c] BWG was calculated as final BW minus initial BW

[d] FCR was calculated as the feed intake per unit gain (from day 8 to 35)

[e] Values are presented in IDR (Indonesian currency) as at the time of study, and was calculated by the cost of feed consumed to attain a kilogramme live weight gain

feedstuffs before being included into the diets of broilers. Just for comparison, inclusion of 10 % unfermented peeled cassava root meal in the diets posed a threat on growth and health status of broiler chickens (Akapo et al. 2014), but such threat was not observed when 10 % *gathot* meal was included in broiler diets in the current study.

In this study, feeding *gathot* was associated with the lowered H/L ratio in chickens as compared to the control diet, indicating that chickens less suffered from infections, inflammation or stress (Davis et al. 2008; Sugiharto et al. 2014). In accordance with Yudiarti and Sugiharto (2016), *gathot* used in this study contained the filamentous fungi *A. charticola* and *R. oryzae*. It was therefore reasonable to infer that the probiotic and antioxidant properties in *A. charticola* and *R. oryzae* growing in *gathot* perhaps play important roles in alleviating the infections, inflammation or stress (oxidative stress) in chickens (Sugiharto 2014) during the present experiment, irrespective of other feed ingredients that may also exhibited probiotic and antioxidant capacities to some extent. Concomitant with the H/L ratio, the A/G ratio also tended to be lower in the chickens fed *gathot* in the present study. Note that lower A/G ratio may implicate in a better disease resistance and immune response of chickens (Abdel-Fattah et al. 2008). Taken together, dietary inclusion of *gathot* is worthy to improve the health and physiological stress responses of commercial broiler chickens.

Table 4 Haematological and biochemical parameters of broilers fed *gathot*

Items	*Gathot*				SEM	*P* value
	Control	2.5 %	5 %	10 %		
Haematological parameters (10^9/L, unless otherwise noted)						
Erythrocytes (10^{12}/L)	2.42	2.37	2.31	2.46	0.09	0.74
ESR (after 1 h, mm)	1.80	2.00	1.80	2.00	0.14	0.58
ESR (after 2 h, mm)	4.00	5.60	4.60	4.20	0.56	0.23
Leukocytes	9.73	12.8	11.7	11.3	2.09	0.74
Heterophils	3.46	4.30	3.28	3.08	0.74	0.60
Lymphocytes	5.84	7.61	7.63	7.36	1.22	0.68
Eosinophils	0.19	0.53	0.50	0.71	0.24	0.46
Basophils	0.03	ND	ND	ND		
Monocytes	0.21	0.37	0.25	0.14	0.08	0.19
H/L ratio	0.63^{a}	0.55^{ab}	0.42^{b}	0.40^{b}	0.06	0.03
Serum biochemical parameters (g/dL, unless otherwise noted)						
AST, U/L	257	248	248	234	7.69	0.25
ALT, U/L	20.2	21.9	21.9	20.4	1.50	0.73
Total triglyceride	0.07^{a}	0.06^{ab}	0.03^{c}	0.04^{bc}	0.01	0.02
Total protein	2.84	4.42	2.66	2.66	0.74	0.28
Albumin	0.86	0.76	0.76	0.68	0.06	0.25
Globulin	1.98	3.66	1.90	1.99	0.74	0.28
A/G ratio	0.43	0.31	0.40	0.34	0.04	0.14

Means on the same row with different letters (a, b, c) are significantly ($P < 0.05$) different

ESR erythrocyte sedimentation rate, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *H/L ratio* heterophils to lymphocytes ratio, *A/G ratio* albumin to globulin ratio, *ND* not detected

Table 5 Giblets, liver, eviscerated carcass and abdominal fat of broilers fed *gathot*

Items (% live BW)	*Gathot*				SEM	*P* value
	Control	2.5 %	5 %	10 %		
Giblets[a]	5.59	5.09	5.43	5.09	0.20	0.22
Liver	2.24	2.23	2.58	2.19	0.14	0.21
Eviscerated carcass	56.2	51.5	50.3	52.3	1.99	0.21
Abdominal fat	1.87	1.32	1.36	1.39	0.31	0.49

[a] *Giblets* heart, gizzard and liver

Result in the present study showed that feeding *gathot* decreased serum total triglyceride and abdominal fat content of broilers. This finding was slightly different from that reported by Bhuiyan and Iji (2015), in which inclusion of cassava pellet or cassava chip in broiler diets at up to 50 % (with enzyme supplementation) did not affect the abdominal fat weight of broilers. It has been reported that dietary fibre was responsible for the lowered serum triglyceride and abdominal fat content of broilers (Rama Rao et al. 2005; Khempaka et al. 2014). In this study, the crude fibre contents were balanced across the experimental diets, and therefore the difference in the dietary crude fibre content was not likely to influence the serum total triglyceride and abdominal fat of broilers. In addition to the dietary fibre, feeding probiotic microorganisms (Kalavathy et al. 2003) and antioxidants (Fouad and El-Senousey 2014) have been reported to reduce the accumulation of fat in broiler chickens. With regard to the probiotic and antioxidant potentials of the fungi living in *gathot*, we therefore considerably suggested that feeding *gathot* may lower the serum triglyceride and abdominal fat traits in broiler chickens.

It could be concluded from the present study that inclusion of *gathot* into broiler diets at up to 10 % had no negative impact on the growth performances of broilers. Feeding *gathot* has potential to improve the health and physiological stress responses as well as lower body fat deposition in broiler chickens. Moreover, dietary inclusion of *gathot* reduced the feed cost per kilogramme live weight gain and therefore makes economic sense in broiler nutrition. Further broiler study using a higher level of *gathot* in the diets is necessary.

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Compliance with ethical standards

Statement of animal rights All applicable international, national and/or institutional guidelines for the care and use of animals were followed in the present study.

Conflict of interest The authors declare that they have no competing interests.

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