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Effects of feeding cassava pulp fermented with *Acremonium charticola* on growth performance, nutrient digestibility and meat quality of broiler chicks

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Abstract

Cassava pulp is an energy-rich by-product of the tapioca industry, and is known as a good media for growing filamentous fungi. It may therefore be not only an alternative to maize in poultry diets, but also a carrier for beneficial fungi. This study aimed to investigate the effects of dietary inclusion of the fungus *Acremonium charticola* (grown in *A. charticola*-fermented cassava pulp) (AC-FCP), with or without antibiotic growth promoters (AGPs), on growth performance, nutrient digestibility, and meat quality of broiler chicks. A total of 192 broiler chicks were assigned to one of four dietary treatments, including a control diet (maize-soybean-meal-based diet), control diet + AGPs (neomycin) (0.0003% of diet), AC-FCP diet (containing 16% of AC-FCP), and AC-FCP + AGPs. There was a tendency towards lower feed costs per kilogram live bodyweight (BW) gain in AC-FCP and AC-FCP + AGPs than in the control and control + AGPs birds. The birds fed the AC-FCP diet had greater spleen relative weight than the control and AC-FCP + AGPs birds. The birds fed diets containing AC-FCP and AC-FCP + AGPs had heavier ileum and caecum, and tended to have smaller livers than the control and control + AGPs birds. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) percentage inhibition values were lowest and highest in the AC-FCP and control birds, respectively. The breast meat of the control birds had lower crude protein content than that of other experimental groups. In conclusion, dietary inclusion of AC-FCP reduced the feed cost per kilogram live weight gain of broiler chicks. The fungus *A. charticola* (grown in AC-FCP) seems to play an important role in increasing the relative weight of spleen, ileum and caecum, alleviating oxidative stress, and increasing the protein content of breast muscle of broiler chicks.

Keywords: *Acremonium charticola*-fermented cassava pulp, broilers, digestibility, growth, meat characteristics, oxidative stress

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Introduction

The broiler industry is currently facing fluctuations in feed costs as the result of the rising prices of conventional feedstuffs. This has encouraged poultry nutritionists to search for alternative feed ingredients. Cassava pulp, which is a by-product of the tapioca industry, has recently been used as an energy source in broiler diets to partly replace maize. However, its utilization is limited by its high fibre and low protein contents (Khempaka *et al.*, 2009). To increase the inclusion level of cassava pulp in chicken diets, fermentation by yeast has been applied (Khempaka *et al.*, 2014). In addition to yeast, fungi are microorganisms that are commonly used for fermenting unconventional feedstuffs. In a previous study, the authors showed that fermenting cassava waste pulp with *A. charticola*, a filamentous fungus isolated from Indonesian fermented dried cassava, and urea could decrease the fibre and increase the protein contents of cassava pulp (Sugiharto *et al.*, 2015). Indeed, the inclusion of 16% AC-FCP in the diets did not impair the physiological and health conditions of broiler chicks (Sugiharto *et al.*, 2016a).

Aside from their health-promoting purpose, AGPs have long been used to improve nutrient digestibility and growth performance of poultry (Dibner & Richards, 2005). In addition to AGPs, certain feed additives and functional feedstuffs have commonly been included in poultry diets to enhance the growth rate of broiler chicks, including probiotics, antioxidants, and fermented feed (Lokaewmanee *et al.*, 2012; Sugiharto, 2016). Besides being used separately, AGPs and other feed additives may be used simultaneously to improve the weight gain and feed efficiency of animals. A study demonstrated that the inclusion of AGPs in probiotic-supplemented diets improved the performance of weaning pigs (Choi *et al.*, 2011). But the use of AGPs in

poultry diets is no longer permitted in some countries (especially European Union countries) because of controversy about the phenomena of antibiotic resistance in animals and human beings as consumers (Sugiharto, 2016). Hence, the role of feed additives other than AGPs has become more substantial. Earlier data showed that *A. charticola* exhibited probiotic and antioxidant properties (Sugiharto *et al.*, 2015; 2016b), which may be beneficial for chickens. The authors' previous work showed that cassava pulp was a good media for growing the fungus (Sugiharto *et al.*, 2015). This feedstuff, therefore, may not only be an alternative to maize in poultry diets, but also be a carrier for *A. charticola*.

Probiotic administration is associated with improved chemical, nutritional, and sensorial properties of broiler meat (Liu *et al.*, 2012). Likewise, dietary antioxidant is effective in improving the meat quality of broilers, especially under oxidative stress (Salami *et al.*, 2015). If one considers the improving effect of AGPs on the nutritional composition of broiler meat, the administration of AGPs simultaneously with probiotics (and/or antioxidants) may be expected to improve the meat quality of broiler chickens (Abdulla *et al.*, 2017). Irrespective of the use of AC-FCP as a feedstuff (an alternative to maize), the present study aimed to investigate the effects of dietary inclusion of *A. charticola* (grown in AC-FCP) with or without AGPs on growth performance, nutrient digestibility and meat quality of broiler chickens.

Materials and methods

To prepare the inoculum of *A. charticola*, the fungal stock culture, which was maintained on potato dextrose agar (PDA) (Merck KGaA, Darmstadt, Germany) and stored at 4 °C, was retrieved, streaked on PDA medium and incubated aerobically at 38 °C for two days. The fungal mycelia were dislodged from the PDA and diluted in 200 mL sterilized distilled water. The suspension was then used to inoculate 200 g sterilized dry cassava pulp (87.5% dry matter) (DM). After aerobic incubation at room temperature for four days, the fungal colony in the inoculation starter was enumerated, based on the colony counting method. The starter was then used to ferment the cassava pulp for an in vivo trial.

Fermented cassava pulp was prepared according to Sugiharto *et al.* (2015) with certain modifications. Briefly, 10 kg steamed cassava pulp (87.5% DM) was soaked with sterile water (1 : 1). The cassava pulp was inoculated with 110 g/kg fungal starter (containing fungal colonies 3.6×10^{10} cfu/g) and 41 g/kg urea and then thoroughly mixed. The mixture was incubated for four days and turned every two days. The AC-FCP was sundried for two days before use in the in vivo experiment. Proximate analysis (AOAC, 1995) showed that AC-FCP contained (as-dry basis) 2,887 kcal/kg gross energy, 8.5% crude protein, 1.18% crude fat, 20.8% crude fibre, and 2.20% total ash.

A total of 192 male Lohman MB-202 day-old-chicks that had been purchased from a local hatchery were placed in an open-sided naturally ventilated broiler house. The chicks were raised on a commercial starter diet and randomly allotted to 24 wire-netting floor pens (1.20 × 1 m), equipped with round bottom feeders and manual drinkers, at 11 days old. Six pens per treatment were assigned to each of four experimental diets, including the control diet (maize-soybean-meal-based diet), control diet + AGPs (0.0003% of diet), AC-FCP diet (containing 16% AC-FCP) and AC-FCP + AGPs. The diets were formulated to be isonitrogenous and isocaloric and met the Indonesian National Standards for Broiler Feed (SNI, 2006) (Table 1). The dietary level of AC-FCP used in the present study was based on Sugiharto *et al.* (2016a), which reported no detrimental effects of AC-FCP on physiological and health conditions when included up to 16% in broiler diets. Neomycin was used in this trial, because this antibiotic is commonly used in poultry feed as AGPs in Indonesia. The dose of neomycin in this study was in accordance with that used in commercial practice in Indonesia. The diets were fed ad libitum in mash form until the end of experiment (day 34). The birds were given free access to water throughout the trial. The birds were vaccinated with commercial Newcastle disease virus (NDV) vaccine on day 4 through eyedrops and on day 21 through drinking water. Bodyweight (BW), feed intake and feed conversion ratio (FCR) were recorded at days 21, 28 and 34. The present study was approved by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Diponegoro University.

At 28 days old, 24 broiler chicks with relatively uniform weight (981 ± 5.94 g) (mean \pm SD) were placed in individual cages (20 × 35 × 45 cm). Chromic oxide (Cr₂O₃) was added (0.3% of diet) to the diets as an indigestible marker for measuring total tract digestibility. Excreta were collected for two days, sprayed with 5% hydrochloric acid, and sundried for two days. Proximate analysis was conducted to determine the DM, organic matter, and total nitrogen in diets and excreta, according to the standard methods (AOAC, 1995).

At day 34, one bird from each pen was randomly selected, from which blood was obtained from the bird's wing veins (before water and feed were offered) and collected in vacutainers, which did not contain anticoagulant. The blood was allowed to clot for two hours at room temperature. After centrifugation at 2,000 rpm for 15 min, the serum was obtained and stored at -20 °C until serum antibody titer and antioxidant activity were determined. After being weighed, the same birds were slaughtered, de-feathered, and

Table 1 Ingredients and nutrient composition (as-dry basis) of experimental diets

Items (% unless otherwise noted)	Dietary treatments			
	Control diet	Control diet + AGPs	AC-FCP	AC-FCP + AGPs
Maize	59.0	59.0	45.5	45.5
Soybean meal	29.0	29.0	23.5	23.5
AC-FCP	-	-	16.0	16.0
Fish meal	9.00	9.00	12.0	12.0
Broken rice	0.75	0.75	1.23	1.23
DL-methionine	0.23	0.23	0.23	0.23
L-lysine	0.06	0.06	0.06	0.06
Limestone	1.01	1.01	0.53	0.53
Dicalcium phosphate	0.20	0.20	0.20	0.20
Premix ¹	0.50	0.50	0.50	0.50
NaCl	0.25	0.25	0.25	0.25
Neomycin	-	0.0003	-	0.0003
Calculated composition:				
Metabolizable energy (kcal/kg) ²	2,896	2,896	2,877	2,877
Crude protein	22.5	22.5	22.2	22.2
Crude fat	3.70	3.70	3.52	3.52
Crude fibre	2.69	2.69	5.51	5.51
Calcium	1.04	1.04	1.00	1.00
Total phosphorus ³	0.54	0.54	0.58	0.58
Methionine	0.67	0.67	0.66	0.66
Lysine	1.46	1.46	1.42	1.42
Analysed composition:				
Metabolizable energy (kcal/kg) ²	3,216	3,264	3,220	3,237
Dry matter	86.5	87.2	82.6	83.4
Crude protein	20.2	20.1	19.8	19.7
Crude fat	3.71	4.21	3.92	3.87
Crude fibre	6.83	6.21	6.59	6.81
Ash	10.1	10.0	10.5	9.74

¹Mineral-vitamin premix provided (per kg of diet): 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, vit A 375 IU, vit D 150 IU, vit E 0.080 mg

²Values were obtained based on the formula according to Bolton (1967), in which metabolizable energy = 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen-free extract] + 2.5}

³The available data on phosphorus content in AC-FCP were total phosphorus (not available phosphorus); therefore, the phosphorus contents in other feed ingredients were presented in total phosphorus during calculation

Control: control diet (maize-soybean-meal-based diet); control + AGPs: control diet with AGPs (neomycin) (0.0003% of diet); AC-FCP: diet containing 16% of AC-FCP; AC-FCP + AGPs: diet containing AC-FCP and AGPs; AGPs: antibiotic growth promoters; AC-FCP: cassava waste pulp fermented with *A. charticola*.

Key: AGPs

eviscerated. Immediately, the internal organs, carcass, breast and thigh muscles were weighed. The breast muscles were skinned and deboned to measure pH, drip loss and chemical composition of meat. The trial was terminated at day 34, which was in accordance with the average slaughter age of broiler chicks in Indonesia (5 weeks old).

Serum antibody titers to NDV vaccine were measured using haemagglutination inhibition (HI) (Villegas, 1987). The titers were expressed as geometric mean titer (\log_2). The antioxidant activities in serum were measured by a DPPH free radical scavenging assay (Sohaib *et al.*, 2012). Serum (0.5 mL) was

dissolved in 3 mL diluted DPPH solution. The absorbances of the resulting solution and the blank were recorded after 30 min at room temperature. The conversion of the colour of DPPH was read spectrophotometrically at a 517 nm. Inhibition of free radicals by DPPH in percentage (%) was calculated using the following equation: $100 \times (A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}$. Carcass characteristics were measured according to Wang *et al.* (2015) with few modifications. Around 45 min after slaughter, the breast muscles were weighed and the pH was measured. The muscles were then placed in a Whirl-pak bag, stored in a refrigerator (5 °C) and reweighed for the final pH measurement. The muscle drip loss was determined based on the weight loss and expressed as percentage. The samples of breast muscles were analysed for chemical composition according to the standard methods mentioned above.

The experiment was arranged according to a completely randomized design, and the data were analysed using the general linear model procedure of SAS (SAS Institute, 1985). The quantile-quantile plot (Q-Q plot) was employed to check the normality of the data. Pen was considered the experimental unit, and the results are presented as means and standard errors (SE). Duncan's multiple-range test was used to compare treatment means. A significant level of $P \leq 0.05$ was applied, and tendencies were noted at $P < 0.10$.

Results

The data of growth performance of broiler chicks are presented in Table 2. Broiler chicks in the control and control + AGPs groups had greater ($P < 0.01$) final BW and BW gain than those in AC-FCP + AGPs group. Final BW and BW gain of broiler chicks were not different ($P > 0.05$) among the control, control + AGPs, and AC-FCP groups. Feed conversion ratio was lower ($P < 0.05$) in the control and control + AGPs than in AC-FCP and AC-FCP + AGPs birds. There was a tendency ($P = 0.07$) towards the lower feed cost per kilogram live BW gain in AC-FCP and AC-FCP + AGPs than in control and control + AGPs birds.

Table 2 Effect of dietary inclusion of *A. charticola*-fermented cassava pulp on growth performance of broiler chicks (means and SE)

Items	Treatments				SE	P value
	Control	Control + AGPs	AC-FCP	AC-FCP + AGPs		
Initial BW (g/bird) ¹	298	298	291	298	4.00	0.57
Final BW (g/bird) ²	1,423 ^a	1,426 ^a	1,350 ^{ab}	1,301 ^b	25.1	<0.01
BW gain (g/bird) ³	1,125 ^a	1,129 ^a	1,059 ^{ab}	1,004 ^b	24.9	<0.01
FCR ⁴	1.61 ^a	1.60 ^a	1.73 ^b	1.75 ^b	0.04	0.02
Feed cost per kg live BW gain ⁵	0.893	0.890	0.830	0.840	0.020	0.07

^{a,b} Means with different superscripts in each row are significantly different

¹ BW determined at day 11 of the experiment

² BW determined at day 34 of the experiment

³ BW gain was calculated as final BW minus initial BW

⁴ FCR was calculated as the feed intake per unit gain (from day 11 to 34)

⁵ Values are presented in USD currency as at the time of study, and were calculated by the cost of feed consumed to attain a kilogram live weight gain

Control: control diet (maize-soybean meal-based diet); control + AGPs: control diet with AGPs (neomycin) (0.0003% of diet); AC-FCP: diet containing 16% AC-FCP; AC-FCP + AGPs: diet containing AC-FCP and AGPs; AGPs: antibiotic growth promoters; AC-FCP: cassava waste pulp fermented with *A. charticola*; BW: bodyweight; FCR: feed conversion ratio

The data on digestibility and retention of broilers are presented in Table 3. In general, the digestibilities of DM and organic matter, as well as nitrogen retention, were not different ($P > 0.05$) among the experimental groups.

The data of internal organs of broiler are presented in Table 4. Broilers in the AC-FCP group had greater ($P < 0.05$) spleen weight than those in the control and AC-FCP + AGPs groups. The AC-FCP and AC-FCP + AGPs birds had greater ($P < 0.05$) weights of ileum and caecum and tended ($P = 0.07$) to have lower weight of liver than the control and control + AGPs birds.

The data on NDV antibody titer and antioxidant activity of broilers are presented in Table 5. The DPPH percentage inhibition values of the serum were lowest and highest ($P < 0.01$) in AC-FCP and control birds, respectively. There was no difference ($P > 0.05$) in NDV antibody titer among the experimental groups.

The data on carcass traits of broilers are presented in Table 6. The relative weight of abdominal fat was lower ($P < 0.05$) in birds receiving AGPs than birds that did not receive AGPs. No differences ($P > 0.05$)

were observed in eviscerated carcasses, giblets, and commercial cuts (breast, thigh, drumstick, and wing) of broiler chickens.

Table 3 Effect of dietary inclusion of *A. charticola*-fermented cassava pulp on nutrient digestibility in broiler chicks (means and SE)

Items	Treatments				SE	P value
	Control	Control + AGPs	AC-FCP	AC-FCP + AGPs		
Digestibility (%)						
Dry matter	66.6	61.2	52.0	59.7	6.36	0.46
Organic matter	67.9	62.4	51.9	59.9	6.32	0.39
N retention (%)	76.3	73.9	70.8	74.3	4.08	0.82

Control: control diet (maize-soybean meal-based diet); control + AGPs: control diet with AGPs (neomycin, 0.0003% of diet); AC-FCP: diet containing 16% of AC-FCP; AC-FCP + AGPs: diet containing AC-FCP and AGPs; AGPs: antibiotic growth promoters; AC-FCP: cassava waste pulp fermented with *A. charticola*; N: nitrogen

Table 4 Effect of dietary inclusion of *A. charticola*-fermented cassava pulp on internal and digestive organs of broiler chicks (means and SE)

Items (% live body weight)	Treatments				SE	P value
	Control	Control + AGPs	AC-FCP	AC-FCP + AGPs		
Heart	0.81	0.95	0.83	0.85	0.10	0.79
Liver	3.06	3.18	2.80	2.70	0.14	0.07
Spleen	0.09 ^a	0.11 ^{ab}	0.13 ^b	0.09 ^a	0.01	0.02
Thymus	0.19	0.19	0.25	0.22	0.03	0.40
Bursa of Fabricius	0.15	0.14	0.18	0.17	0.02	0.52
Proventriculus	0.59	0.54	0.51	0.54	0.03	0.36
Gizzard	1.44	1.42	1.42	1.56	0.06	0.28
Duodenum	0.43	0.49	0.55	0.53	0.04	0.16
Jejunum	0.88	0.92	1.05	1.02	0.06	0.13
Ileum	0.69 ^a	0.66 ^a	0.82 ^b	0.84 ^b	0.04	0.01
Caecum	0.05 ^a	0.04 ^a	0.06 ^b	0.07 ^b	0.00	<0.01

^{a,b} Means with different superscripts in each row are significantly different

Control: control diet (maize-soybean meal-based diet); control + AGPs: control diet with AGPs (neomycin, 0.0003% of diet); AC-FCP: diet containing 16% of AC-FCP; AC-FCP + AGPs: diet containing AC-FCP and AGPs; AGPs: antibiotic growth promoters; AC-FCP: cassava waste pulp fermented with *A. charticola*

Table 5 Effect of dietary inclusion of *A. charticola*-fermented cassava pulp on serum antibody titers to Newcastle disease virus vaccine and antioxidant activity of broiler chicks (means and SE)

Items	Treatments				SE	P value
	Control	Control + AGPs	AC-FCP	AC-FCP + AGPs		
Antibody titer (Log ₂ GMT)	3.20	3.33	4.00	3.83	0.75	0.83
Antioxidant activity (DPPH, % inhibition)	27.3 ^a	19.4 ^b	10.1 ^c	19.1 ^b	2.38	<0.01

^{a,b,c} Means with different superscripts in each row are significantly different

Control: control diet (maize-soybean meal-based diet); control + AGPs: control diet with AGPs (neomycin, 0.0003% of diet); AC-FCP: diet containing 16% of AC-FCP; AC-FCP + AGPs: diet containing AC-FCP and AGPs; AGPs: antibiotic growth promoters; AC-FCP: cassava waste pulp fermented with *A. charticola*; DPPH: 2,2-diphenyl-1-picrylhydrazyl; GMT: geometric mean titer

The data on breast meat characteristics of broiler chickens are presented in Table 7. Breast meat from control birds had lower ($P < 0.05$) crude protein content than meat from other birds. The AC-FCP birds had lower ($P < 0.05$) crude ash content in their breast meat than other experimental groups. There was no difference ($P > 0.05$) in pH and drip loss of breast meat among the birds.

Table 6 Effect of dietary inclusion of *A. charticola*-fermented cassava pulp on carcass traits of broiler chicks (means and SE)

Items	Treatments				SE	P value
	Control	Control + AGPs	AC-FCP	AC-FCP + AGPs		
% Live weight						
Eviscerated carcass	66.5	64.2	66.2	66.2	1.29	0.47
Giblets ¹	5.31	5.54	5.05	4.75	0.29	0.27
% Eviscerated carcass						
Breast	34.2	33.9	32.5	32.1	0.70	0.11
Thigh	16.1	16.4	15.3	15.9	0.35	0.17
Drumstick	14.4	15.0	15.2	14.8	0.42	0.58
Wing	11.9	12.7	13.0	13.0	0.39	0.20
Abdominal fat	1.88 ^{ab}	1.12 ^c	2.15 ^a	1.40 ^{bc}	0.22	0.02

^{a,b,c} Means with different superscripts in each row are significantly different

¹ Giblets: heart, gizzard and liver

Control: control diet (maize-soybean meal-based diet); control + AGPs: control diet with AGPs (neomycin, 0.0003% of diet); AC-FCP: diet containing 16% of AC-FCP; AC-FCP + AGPs: diet containing AC-FCP and AGPs; AGPs: antibiotic growth promoters; AC-FCP: cassava waste pulp fermented with *A. charticola*

Table 7 Effect of dietary inclusion of *A. charticola*-fermented cassava pulp on breast meat traits of broiler chicks (means and SE)

Items	Treatments				SE	P value
	Control	Control + AGPs	AC-FCP	AC-FCP + AGPs		
pH _{45 min}	5.83	5.67	5.67	5.67	0.11	0.61
pH _{24 h}	5.52	5.48	5.53	5.55	0.03	0.36
Drip loss (%)	1.59	1.89	0.71	0.74	0.50	0.26
Moisture (%)	74.1	74.1	74.3	73.9	0.18	0.58
Crude protein (%)	22.6 ^a	23.1 ^b	23.6 ^b	23.3 ^b	0.18	0.01
Crude fat (%)	0.76	0.79	0.72	0.81	0.03	0.22
Ash (%)	0.79 ^a	0.73 ^a	0.64 ^b	0.76 ^a	0.03	<0.01

^{a,b} Means with different superscripts in each row are significantly different

Control: control diet (maize-soybean meal-based diet); control + AGPs: control diet with AGPs (neomycin, 0.0003% of diet); AC-FCP: diet containing 16% of AC-FCP; AC-FCP + AGPs: diet containing AC-FCP and AGPs; AGPs: antibiotic growth promoters; AC-FCP: cassava waste pulp fermented with *A. charticola*

Discussion

In the present study, *A. charticola* was used to ferment cassava pulp, not only to improve the nutritional quality of cassava pulp, but also to take advantage of the functional properties (probiotic and antioxidant) of the fungus on broiler performances. The current data showed that broiler chicks that received AC-FCP gained less weight than those that received the control diet or the control diet with AGPs, though the difference was not significant. Indeed, diets containing AC-FCP tended to reduce the feed cost per kilogram live weight gain of broiler chicks. In an earlier work, Khempaka *et al.* (2014) reported that inclusion of *Aspergillus oryzae*-fermented cassava pulp up to 16% of diets did not significantly affect the digestibilities of DM and organic matter or nitrogen retention in broiler chicks. Accordingly, the present data showed no significant effect of dietary AC-FCP and/or AGPs inclusion on the digestibilities of DM and organic matter and nitrogen retention in broiler chicks. This finding may suggest that AC-FCP could be included in broiler rations up to 16% to partially replace the maize. However, caution should be taken as dietary inclusion of 16% of AC-FCP resulted in higher FCR values in the present study.

In the present study, there was a tendency toward a lower relative weight of the liver in AC-FCP and AC-FCP + AGPs than in the control and control + AGPs birds. The rationale for this condition was not fully known. However, probiotic activity of *A. charticola* grown in AC-FCP seemed to decrease the weight of liver. Mehr *et al.* (2007) and Bozkurt *et al.* (2009) reported a decreased relative liver weight in broilers after feeding probiotic Protexin and probiotic mixture Primalac®, respectively. In such cases, probiotics may decrease the population of pathogenic microorganisms in the intestine. As a consequence, less toxin will be produced and liver will have less work in detoxifying these toxin (Mehr *et al.*, 2007). In terms of the antioxidant property of

the fungus, it might have a minimal effect on the liver weight, as Jang *et al.* (2014) reported no effect of antioxidants (vitamins C and E) on the relative weight of liver in broiler chicks. Eventually, the lower liver weight in the present study may confirm that the toxicological effect of urea used as a nitrogen source in the fermentation process did not appear in broiler chicks (Khempaka *et al.*, 2014; Sugiharto *et al.*, 2016a). Birds in AC-FCP group had greater weight of spleen than birds in other experimental groups. The probiotic activity of the fungus grown in AC-FCP seems to play an important role in enhancing the weight of spleen in the present study. In agreement, Awad *et al.* (2009) showed a greater spleen in the probiotic-supplemented birds than in control. Antioxidant properties of the fungus might also affect the weight of spleen in broiler chicks fed AC-FCP diet. This was supported by Hosseini-Vashan *et al.* (2016), who found an increased weight of bursa of Fabricius and spleen in broiler chicks fed tomato pomace (as source of natural antioxidants). In this study, the relative weights of ileum and caecum were higher in AC-FCP and AC-FCP + AGPs compared with the control and control + AGPs birds. In such conditions, both probiotic and antioxidant activities of the fungus grown in AC-FCP diets seem to be responsible. This inference was supported because the administration of probiotic *Lactobacillus* spp. (Olnood *et al.*, 2015) or processed apple peel waste (as a natural antioxidant) (Heidarisafer *et al.*, 2016) increased the small intestinal weight (jejunum and ileum) of broiler chicks.

Rapid growth in broiler chickens has been attributed to a high metabolic rate and thus high production of free radicals (pro-oxidants). Naturally, the bodies of broiler chicks produce antioxidants to prevent the oxidative damage induced by free radicals. Compared with other experimental groups, birds that received an AC-FCP diet exhibited lower antioxidant activity in the serum, as indicated by the lower value of DPPH percentage inhibition. Sohail *et al.* (2011) suggested that antioxidant production was augmented following the increase of free radical production in broiler chicks. Taken together, the antioxidant capacity of *A. charticola* grown in AC-FCP diets (Sugiharto *et al.*, 2015; 2016b) was perhaps capable of alleviating oxidative stress in broiler chicks and thus the increased production of antioxidants as a natural protective response to oxidative stress did not appear in AC-FCP birds. This inference was supported by Sugiharto *et al.* (2016c), who reported that feeding *gathot* (fermented dried cassava from which *A. charticola* was isolated) reduced the heterophil to lymphocyte (H/L) ratio (indicator of oxidative stress) in broiler chicks compared with the control birds. Owing to the limited number of repetitions, however, the interpretation of the current findings should be taken with caution.

The current data showed that birds that received AGPs had lower abdominal fat than the AGPs-fed birds. This result was in line with Mokhtari *et al.* (2010), who reported decreased abdominal fat weight in broiler chicks fed avilamycin as an AGP. In this study, feeding AC-FCP did not significantly affect the abdominal fat content of chicks compared with the control. This finding was different from those of Adeyemi *et al.* (2008), who reported a decrease in abdominal fat content in broiler fed 25% cassava root meal fermented with rumen filtrate. In contrast, Zhang *et al.* (2016) reported that dietary inclusion of fermented feed at a level of 6% significantly increased the abdominal fat percentage of 56-day-old broiler chickens. The nature and level of inclusion of fermented feed and the conditions of study may explain these contradictory results.

Although the number of repetitions was limited, inclusion of AC-FCP and/or AGPs in the diets increased the protein content of broiler meat in the present study. Concomitant results were reported by Hossain *et al.* (2012) and Nie *et al.* (2015), in which dietary supplementation with fermented *Alisma canaliculatum* and *Candida tropicalis*-fermented cotton seed, respectively, increased the crude protein content of breast meat of broiler chicks. The mechanism by which AC-FCP increased the protein content of breast meat is unclear, but the enhancing effect of probiotic fungus grown in AC-FCP on the protein efficiency ratio and thus production of protein in the musculature of broilers may be the reason (Khaksefidi & Rahimi, 2005; Hossain *et al.*, 2012). In terms of the effect of antioxidants on the crude protein content of breast meat, the data in the literature are inconclusive. Rahman & Kim (2016) reported that dietary supplementation of *Nigella sativa* seed (source of antioxidant) increased the protein content of broiler meat, whereas Marzoni *et al.* (2014) showed no effect of dietary natural antioxidants (dry extracts of tomato skin, orange peel, and green tea leaves) on the crude protein content of breast and thigh meat of broiler chickens and Muscovy ducks. With regard to the effect of AGPs on crude protein content of meat, the present findings were different from those reported by other workers, which showed no effect of various AGPs, for example 0.05% chlortetracycline and HCL + cyanocobalamin (Yang *et al.*, 2003), 0.5 g/kg flavomycin (Attia *et al.*, 2011), 0.005% oxytetracycline (Hossain *et al.*, 2012), and 100 ppm combination of oxytetracycline and neomycin (Abdulla *et al.*, 2017). The rationale for this condition is not fully known, but the various types and doses of AGPs and the conditions of the study may partly explain the conflicting results.

In the current study, crude ash was lower in the meat of AC-FCP birds than in the other experimental groups. This may indicate that meat from AC-FCP broiler chicks had lower retention of minerals. The present finding was different from that of Nie *et al.* (2015), who showed no effect of fermented cottonseed meal on crude ash of breast muscle of broiler chicks. Several studies have reported enhancing effects (Khaksefidi &

Rahimi, 2005) and absent effects (Abdulla *et al.*, 2017) of probiotics on the crude ash content of broiler meat. With regard to the effect of antioxidants on the ash content of broiler meat, Kim *et al.* (2015) demonstrated that dietary supplementation with 1% sea urchin shell powder (rich in antioxidants) significantly reduced the ash content of broiler meat, whereas Marzoni *et al.* (2014) and Rahman & Kim (2016) reported no effect of dietary natural antioxidant on ash content of meat from broiler chicks. The explanation for the lower ash content in the meat of AC-FCP broiler chicks in the current study therefore remains unelucidated.

Conclusion

Dietary inclusion of AC-FCP reduced the feed cost per kilogram live weight gain of broiler chicks. The fungus *A. charticola* (grown in AC-FCP) seems to play an important role in increasing the relative weight of spleen, ileum and caecum, alleviating oxidative stress, and increasing protein content of breast muscle of broiler chicks. Further studies with more number of repetitions and longer trial periods are needed to verify the present findings.

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Authors' Contributions

SS designed the study, carried out the animal experiment, data analysis and manuscript writing. II and EW carried out the animal experiment. TY and FDP conducted the laboratory analysis.

Conflict of Interest Declaration

The authors declare that they have no competing interests.

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