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FEEDING EFFECT OF INULIN DERIVED FROM DAHLIA TUBER COMBINED WITH *Lactobacillus* sp. ON MEAT PROTEIN MASS OF CROSSBRED KAMPONG CHICKEN

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ABSTRAK

Penelitian bertujuan untuk mengevaluasi pengaruh pemberian tepung umbi dahlia yang dikombinasikan dengan *Lactobacillus* species (*Lactobacillus* sp.) terhadap aktifitas antioksidan, massa kalsium, dan massa protein pada daging ayam kampung persilangan. Ternak penelitian adalah ayam kampung persilangan umur satu hari unsex sebanyak 168 ekor diberi perlakuan mulai umur 3 minggu. Penelitian menggunakan rancangan acak lengkap pola faktorial 2x3 dengan 4 ulangan. Faktor pertama adalah pemberian dua level tepung umbi dahlia yaitu 0,8% (A1) dan 1,2% (A2), dan faktor kedua adalah pemberian tiga level *Lactobacillus* sp. yaitu tanpa penambahan (B0), 1,2 mL (10^8 cfu/mL) (B1) dan 2,4 mL (10^8 cfu/mL) (B2). Parameter yang diamati adalah aktifitas antioksidan, massa kalsium, dan massa protein daging. Data dianalisis ragam dan jika terdapat pengaruh nyata (P<0,05) dilanjutkan dengan Uji wilayah ganda Duncan. Hasil penelitian menunjukkan bahwa pemberian tepung umbi dahlia sebagai sumber inulin dikombinasikan dengan *Lactobacillus* sp. nyata (P<0,05) meningkatkan nilai aktifitas antioksidan dan massa protein daging, tetapi tidak terhadap massa kalsium daging. Pemberian tepung umbi dahlia 1,2% dan *Lactobacillus* sp. 1,2 mL (10^8 cfu/mL) merupakan kombinasi terbaik dilihat dari peningkatan aktifitas antioksidan dan massa protein daging.

Kata Kunci: inulin umbi dahlia, Lactobacillus sp., daging ayam kampung persilangan

ABSTRACT

The objective of the study was to determine the effects of feeding *Lactobacillus* species (*Lactobacillus* sp.) and inulin derived from dahlia tuber powder on antioxidant activity, calcium mass, and protein mass of crossbred kampong chicken meat. A total of 168 birds of 21 days old crossbred kampong chickens were randomly allocated into 6 treatments with four replications per treatment. The present experiment was assigned in a completely randomized design with 2 x 3 factorial scheme. The first factor was levels of dahlia tuber powder, namely 0.8% (A1) and 1.2% (A2), and the second factor was levels of *Lactobacillus* sp., namely none (B0), 1.2 mL (10^8 cfu/mL/B1) and 2.4 mL (10^8 cfu/mL/B2). The parameters measured were antioxidant activity, meat calcium and protein mass. Data were subjected to analysis of variance and followed by Duncan multiple range test (P<0.05) when the treatment indicated significant effect. The supplementation of dahlia tuber powder and *Lactobacillus* sp. significantly (P<0.05) increased antioxidant activity and protein mass of meat. However, calcium mass of meat was not significantly affected by treatments. In conclusion, feeding dahlia tuber powder at the level of 1.2% combined with *Lactobacillus* sp. at 1.2 mL (10^8 cfu/mL), can be categorized as the best combination based on the increase in antioxidant activity and meat protein mass.

Keywords: inulin of dahlia tuber, Lactobacillus sp., crossbred kampong chicken meat

INTRODUCTION

Poultry farming plays a major role in the fulfillment of food demand derived from animal product, especially poultry meat. Kampong chicken (*Gallus gallus*) is one poultry breed that can be a potential source of meat producer. This local chicken has been known since ancient times and has an important role same as other poultry. Kampong chicken is still being the interesting preference of poultry commodity for the Indonesian community (Pramono, 2006). Considering the low productivity and slow growth, it is important to find the way to improve the productivity of kampong chicken by crossbreeding system (Rahayu *et al*., 2010). The fillial produced through cross-mating model is known as crossbred kampong chicken. Although the productivity of crossbred kampung chicken is claimed to be slightly better than their uncester, the gaining is still lower than that of modern poultry. Therefore, the effort to improve the productivity of crossbred kampong chicken is still required. In the previous decade antibiotic in poultry production system was very common substance to be used as growth promoter. However, nowadays, antibiotic is no longer recommended as the growth promoter and it has been baned in the world-wide, because it might cause residue in poultry product and bacterial resistance which harmful for the consumer. Natural substance as an alternative for antibiotic has been the popular compound that can be able to improve productivity of poultry and also consumer health friendly. As it has been reported previously by Verdonk *et al*. (2005) that the natural substance of feed supplement can ensure to be beneficial for the host animals. Feeding the promising substance of natural compound, probiotic combined with prebiotic, was evaluated for crossbred kampong chicken in the present study.

Probiotic is a live microbial feed supplement in adequate quantities that beneficially influences the host animal by improving the intestinal microflora ecosystem. Source of probiotic both *Lactobacili* and *Bifidobacteria* can easily and quickly use prebiotic, such as inulin, as a source of “feed ingredient” for fermentation to produce lactate, and short chain fatty acids (SCFAs), including acetate, propionate, and butyrate (Kelly, 2008). The products of fermentation process as described above bring about the change of the intestinal pH to be acidic condition which in turn favorable for the growth of the beneficial bacteria and lead to the improvement of gut health.

One compound can functions as a “partner” of probiotic is known as prebiotic. Prebiotic is food ingredient that can’t be digested by the enzymes and providing a beneficial effect to the host by stimulating the growth or the activity of selective microbes in the intestine (Fuller, 1989, and Choudhari *et al*., 2008). Prebiotic is also a residual-free natural compound and no harmful for consumer’s health. The increase in the number and activity of *Bifidobacteria* and lactic acid bacteria (*Lactobacillus*) which provided the impact on nutrients utilization and lead to the improved productivity is the beneficial effect of prebiotic for the host. A source of natural prebiotic, known as inulin, derived from the tuber of Indonesian local plant, called dahlia (*Dahlia variabilis*), was evaluated in the present study. The total inulin content of dahlia tuber was about 65-75% from the total carbohydrate (Haryani *et al*., 2013). Dahlia is also specified as the highest inulin-producing plant containing fructose and active compounds such as phytic and benzoic acids (Shivayogeppa *et al*., 2009). Among other sources, inulin was the best prebiotic (Azhar, 2009) and water soluble which can’t be digested by host digestive enzymes, but can be selectively fermented by intestinal microbes. Roberfroid (2007) reported that inulin function as “feed ingredient” can be almost completely fermented by *Bifidobacteria* and *Lactobacilli*.

The utilization of local resources such as dahlia tuber as a source of prebiotic inulin combined with *Lactobacillus* sp. is expected to be a synbiotic and have an impact on the increase in nutrients utilization and productivity of crossbred kampong chicken. As a comparisson, feeding phytobiotic contained a combination of probiotic and prebiotic substances has been reported to function as natural growth promoter (NGPs) (Panda *et al*., 2009). Therefore, the present study was conducted to evaluate the effect of inulin derived from dahlia tuber combined with *Lactobacillus* sp. on antioxidant activity and meat production quality based on calcium and meat protein mass, and to determine the beneficially appropriate combining levels of both substances in crossbred kampong chicken.

MATERIALS AND METHODS

The experimental animals used in the present study were 168 birds of day old unsex crossbred kampong chicken. The basal diet was composed with protein and energy contents of 19% and 2,800 kcal/kg, rescpectively, for starter period, and 18% and 2,800 kcal/kg, recpectively, for finisher period (Table 1).

Day old chicks were kept until 15 days in the brooder cage and fed a standard commercial poultry feed (AD11). The birds were adapted to experimental diet during the period of 15 to 20 days, before starting the main experiment. The test diets were provided on day 21 until 2.5 months of age. Twenty one day old chickens were then randomly allocated into 168 units of battery cage of 25 x 35 x 30 cm in size each. Seven birds were housed in each unit of battery. The experimental chickens were given *ad libitum* to diet and drinking water. Chicken were fed twice a day at 06.00 am and 16.00 pm. A small portion of diets (about 20 g) were mixed with dahlia tuber powder and *Lactobacillus* sp., according to the level of respective treatment, and were given at 16.00 pm every afternoon to ensure both substances can be completely consumed before feeding other portion of diet.

Experimental design of a 2 x 3 factorial scheme in a completely randomized design with 4 replications was assigned in the present study. The first factor was 2 levels of dahlia tuber powder, namely 0.8% (A1) and 1.2% (A2), and the second factor was 3 additional levels of *Lactobacillus* sp., namely none (B0), 1.2 mL (10^8cfu/mL/B1), and 2.4 mL (10^8cfu/mL/B2). Therefore, the combination of the experimental treatment were as follows: A1B0, A1B1, A1B2, A2B0, A2B1, and A2B2. Parameters observed were antioxidant activity, and calcium and meat protein mass.

Antioxidant activity was analyzed according to procedure of Brand-Williams *et al*. (1995) using the 2.2-diphenyl-1-picrylhydrazyl (DPPH), and calculated with the formula as follow:

Table 1. Ingredients and Nutrient Compositions of the Experimental Basal Diet

Ingredient	Starter	Finisher
	.... (%)	....
Yellow corn	53.30	54.50
Rice brand	16.00	20.00
Soybean meal	19.50	15.00
Fish meal	10.00	9.30
$CaCO_3$	0.70	0.70
Vitamin and mineral	0.50	0.50
Total	100.00	100.00
Nutrient Composition (%)		
Metabolizable energy (kcal/kg)*	2,879.55	2,879.16
Crude protein**	19.67	17.73
Ether extract **	6.42	6.35
Crude fiber**	6.38	6.60
Methionine*	0.42	0.40
Lysine*	1.95	1.06
Calcium***	1.17	1.10
Phosporus***	0.68	0.68

*Based on Table of Badan Standarisasi Nasional (2006) and National Research Council (1994).

**Result of chemical analysis at the Laboratory of Feed Science, Faculty of Animal Science, Hasanuddin University.

***Result of chemical analysis at the Laboratory of Nutrition and Feed Science, Faculty of Animal and Agricultural Sciences, Diponegoro University (2013).

[(Absorbance of control - absorbance of the sample) / absorbance of control] x 100%. Meat protein mass was measured starting with the determination of protein content using method of micro Kjeldahl Nitrogen (Apriyantono *et al*., 1989), and calculated with the formula of Suthama (2003) as follow: [mass of meat protein = % meat protein content x meat mass (g)]. Analogue to protein mass determination, calcium mass was initiated by measuring calcium content of meat using the method of atomic absorption spectrophotometer according to AOAC (1995) and continued to calculate calcium meat mass with the formula of Suthama (2003) as follow: [meat calcium mass = % meat calcium content x meat mass (g)]. Data were subjected to analysis of variance and followed by Duncan multiple range test at 5% probability when the treatment indicated significant effect.

RESULTS AND DISCUSSION

Feeding dahlia tuber powder as a source of inulin combined with *Lactobacillus* sp. showed significant effect (P<0.05) on antioxidant activity and meat protein mass, but not on meat calcium mass of crossbred kampong chicken. The highest value of antioxidant activity were achieved by the combination of dahlia tuber powder at 1.2% and *Lactobacillus* sp. At 2.4 mL (10^8 cfu/mL) (A2B2) and significantly (P<0.05) different as compared to all other treatment combinations. On the contrary, the combination of 0.8% dahlia tuber powder with 2.4 mL *Lactobacillus* sp. (A1B2) resulted significantly (P<0.05) the lowest value of antioxidant activity same to the treatment without addition of *Lactobacillus* sp. (A1B0). However, the combination of dahlia tuber powder at the levels of 0.8% and 1.2 % with 1.2 mL of *Lactobacillus* sp. (A1B1 and A2B1) were the same and indicated medium values (Table 2).

The present results suggested that additional *Lactobacillus* sp. can coorporate with endogenous lactic acid bacteria supported by the inclusion of inulin from dahlia tuber powder, and decreased pathogenic bacteria, such as *E. coli*. The decrease in pathogenic bacteria population was an indication of the improved health status of the gastrointestinal tract that bring about the increase in crossbred kampong chicken body resistance. This evidence supported by previous results (Krismiyanto *et al*., 2014) that *E.coli* count reduced with feeding inulin in starter period of crossbred native chicken. The improvement of health status of gastrointestinal tract implicated to the improved antioxidant activity in body tissue. The reduced pathogenic bacteria population can be assumed to have impact on the lightening body defence via phagocytocis load (Abbas and Litshman, 2009; Arief *et al*., 2010), and this mechanism in the present study was indicated by the higher value of antioxidant activity (Table 2). As it have been reported by Susanti *et al*. (2012) and Chen *et al*. (2013) that during the process of phagocytosis, phagocytic receptors that bind to bacterial pathogens sends a signal to activate the reactive oxygen species (ROS) such as superoxide anion-free radicals and H_2O_2 which can damage cells of pathogenic bacteria.

The mechanism of why antioxidant work causing the fluctuated value of activity can be explained by the existence of free radical superoxide anion neutralized by the endogenous antioxidant, such as superoxide dismutase (SOD). Sarker *et al*. (2010) described that the antioxidant activity was the ability to maintain the quality of the meat from the oxidation process. Based on the above mechanism, the increase in the number of lactic acid bacteria in the gut due to the feeding effect of inulin derived from dahlia tuber (Faradilla, 2015) was relevance with the increasing antioxidant activity as found in the present study. Therefore, the phenomenone of the increased antioxidant activity in the tissue can be associated with the decrease in gastrointestinal pathogenic bacteria number due to the supplementation of inulin from dahlia tuber powder in combination with *Lactobacillus* sp. Although the enzyme was not examined in the present study, it can be assumed that probiotic can release antioxidant, such as GSH-Px enzyme, that prevents oxidative damage by pathogenic bacteria in the gut. This enzymatic theory is consistent with Peran *et al*. (2006); Peran *et al*. (2007), and Rajput *et al*. (2013) that probiotic, by releasing GSH-Px enzyme as an antioxidant, can prevent oxidative damage due to irritation of the small intestine by pathogenic bacteria.

Naturally, the animal body produces GSH-Px enzyme that its formation was influenced by the intake level of methionine (Wang *et al*., 1997). The result of the present experiment, especially the treatments of A2B1 and A2B2, showed the increased meat antioxidant activity, so it can be assumed that methionine intake level was still at the normal range. The influence of methionine intake on the increase of antioxidant activity in this study (GSH-Px) was found in the treatment of

Table 2. The Mean Value of Protein Massa, Massa Calcium and Antioxidant Activity

Level of Dahlia Tuber Powder	Level of *Lactobacillus* sp.			Mean
	B0	B1	B2	
Antioxidant Activity (%)				
A1	5.612^{cd}	8.992^{b}	4.368^{d}	6.324^{b}
A2	6.488^{c}	10.651^{b}	17.937^{a}	11.692^{a}
Mean	6.0498^{b}	9.8214^{a}	11.1526^{a}	9.008
Calcium Mass (g/bird)				
A1	7.687	7.388	9.863	8.313
A2	9.587	9.551	9.116	9.418
Mean	8.637	8.470	9.489	8.865
Protein Mass (g/bird)				
A1	63.105^{b}	64.013^{b}	79.714^{a}	68.944
A2	62.731^{b}	74.483^{ab}	65.134^{ab}	67.450
Mean	62.918	69.248	72.424	68.197

$^{a\text{-}b}$Mean values within row with different superscript are significantly different (P<0.05).

$^{a\text{-}b}$Mean values within column with different superscript are significantly different (P<0.05).

$^{a\text{-}d}$Mean values among combinations within row and column with different superscript are significantly different (P<0.05).

A2B1 with the highest value of methionine intake (0.104 g/bird/day), while that of A2B2 (0.097 g/bird/day) was relatively same as those of A1B0 (0.099 g/bird/day) and A2B0 (0.100 g/bird/day). This result indicated that dietary inclusion of high levels of both dahlia tuber powder as a source of inulin and *Lactobacillus* sp. (A2B2) through their modifying effect on methionine utilization have a positive contribution to the increase of antioxidant activity in meat. However, the increase of meat antioxidant activity was not always followed by the improvement of meat mass protein because of methionine intake-dependent possibility, for this case, it can be compared between treatments A1B2 and A2B2. The difference biochemical phenomenone can be connected with the different level of methionine utilization for GSH-Px synthesis and protein synthesis. This finding is consistent with Wang *et al*. (1997), that there was a difference in the minimal level of methionine requirement for synthesis of GSH than that needed for protein synthesis.

Combination of 0.8% dahlia tuber powder without *Lactobacillus* sp. (A1B0) shows the lowest value of antioxidant activity and not significantly different with the combination of 0.8% dahlia tuber powder and 2.4 mL of *Lactobacillus* sp. (10^8 cfu/mL) (A1B2). This phenomenon showed that supplementation of low level of dahlia tuber powder combined with higher level of *Lactobacillus* sp. (A1B2) did not provide a good balance, so that the value of antioxidant activity is not higher than that of supplemental low level of dahlia tuber powder without addition of *Lactobacillus* sp. (A1B0). Combination of 0.8% dahlia tuber powder with 1.2 mL of *Lactobacillus* sp. (10^8 cfu/mL) (A1B1) indicated higher antioxidant activity than combination of 0.8% dahlia tuber powder without *Lactobacillus* sp. (A1B0) because the treatment achieved the better balance state. Combination of 1.2% dahlia tuber powder with 1.2 mL and 2.4 mL of *Lactobacillus* sp. (10^8 cfu/mL), respectively (A2B1 and A2B2) proven that the combination was assumed to be optimal synbiotic because *Lactobacillus* sp. could evolved since it received the adequate supply of substrate ("food"). Therefore, high level of supplemental dahlia tuber powder combined with high level of *Lactobacillus* sp. (A2B2) was an efficient combination. Prebiotic compound when combined with probiotic (synbiotic) sustained the growth of the probiotic, and thus, increased stabilization and improvement of the probiotic effect (Awad *et al*., 2008; and Awad *et al*., 2009). The balance combination of dahlia tuber powder as a source of inulin and *Lactobacillus* sp. can be categorized to be the most positive effect as a synbiotic to increase antioxidant activity in meat as it has been discussed previously. On the other hand, the combination of high level of *Lactobacillus* sp. with low level of dahlia tuber powder did not show a positive effect as a synbiotic.

The decreasing intestinal pH due to the increasing number of beneficial bacteria as the part of this study results (Faradilla, 2015) may be associated with the higher absorption of calcium and protein which can contributes to the deposition into the meat. Lopez *et al*. (2000) and Scholz-Ahrens *et al*. (2007) reported that the substance of metabolite produced by non-pathogenic microbes in the gut can decreased the pH value so that causes the increased absorption of calcium. The lower intestinal pH has also an impact on the increase in protein absorption and further increasing protein metabolism for meat protein deposition (Saputri, 2012). However, feeding dahlia tuber powder as a source of inulin with additional *Lactobacillus* sp. did not affect meat calcium mass, because it can be assumed that the calcium absorption was mostly deposited into the bone since the birds were still at the growing period, thus, deposition in the meat did not show any differences. This result is consistent with the reports of Haryati and Supriyati (2010), and Chen and Chen (2004) that bone calcium content increased in connection with the decrease in the intestinal pH due to the fermentation of fructooligosaccharide which produced short chain fatty acids (butyrate, propionate and acetate) so that dissolved the insoluble calcium salts components.

Although the status of meat calcium did not specifically determined, calcium in the meat is assumed to be the form of salt and low calcium ion content. Calcium ion is known as an activator for muscle protease known as calcium-activated neutral protease (CANP), an enzyme is able to degrade muscle protein. When the level of calcium ion was low, it did not intentsely excert it activator function on CANP enzyme activity, and resulted the increase in meat protein mass (Table 2). This condition is in agreement with previous reports (Suthama, 2006; Jamilah *et al*., 2013) that protein mass of meat was influenced by meat calcium content in the free ion form as the activators of CANP enzyme. The present study showed the tendency that when the combination of inulin from dahlia tuber and *Lactobacillus* sp. was provided at whatever levels, most calcium was appeared to bound in salt form and the CANP activity was similarly low with the same consumption of calcium. Calcium in the form of ions can increased the degradative activity of CANP which brought about the decreased meat protein deposition (Suthama, 2006; Suthama *et al*., 2014), However, when the meat calcium mass in the form of calcium ions is low, although the total calcium is high, the mass of protein meat unchanged and still high. This phenomenon as described above is presumably occurred in the treatments of A1B2, A2B1, and A2B2. The capacity of protein deposited into the meat defined as meat protein mass as it has been descused previously depending on antioxidant activity and the condition of meat calcium mass based on the presence of calcium ion as an activator for muscle protease known as calcium-activated neutral protease (CANP). The highest meat protein mass was resulted by the combination of 0.8% dahlia tuber powder and 2.4 mL (10^8 cfu/mL) *Lactobacillus* sp. (A1B2) but statistically similar to those of 1.2% dahlia tuber powder combined with 1.2 mL and 2.4 mL *Lactobacillus* sp., respectively (A2B1 and A2B2). When comparing among the three treatments of A1B2, A2B1, and A2B2, the treatment of A2B1 can be considered as the approriate level of combination since it produced the high meat quality indicated by the high antioxidant activity and protein mass with slightly high calcium content.

CONCLUSION

Dietary inclusion of 1.2% dahlia tuber powder as a source of inulin and 1.2 mL *Lactobacillus* sp. (10^8 cfu/mL) can be an appropriate combination for the higher meat quality yield based on high antioxidant activity, meat calcium mass, and meat protein mass in crossbred kampong chicken.

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