

FEEDING EFFECT OF INULIN DERIVED FROM *Dahlia variabilis* TUBER ON INTESTINAL MICROBES IN STARTER PERIOD OF CROSSBRED NATIVE CHICKENS

L. Krismiyanto, N. Suthama and H. I. Wahyuni

Faculty of Animal and Agricultural Sciences, Diponegoro University,
Tembalang Campus, Semarang 50275 - Indonesia
Corresponding E-mail: nsuthama@gmail.com

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ABSTRAK

Penelitian ini bertujuan untuk mengkaji efek pemberian inulin dari umbi *Dahlia variabilis*, baik dalam bentuk tepung maupun ekstrak, terhadap keberadaan mikroba usus halus pada ayam kampung persilangan periode starter. Materi penelitian adalah 280 ekor ayam kampung persilangan *unsex*, dan tepung maupun ekstrak umbi dahlia sebagai sumber inulin yang mulai diberikan pada umur 22 hari. Penelitian menggunakan rancangan acak lengkap dengan 7 perlakuan dan 4 ulangan (masing-masing 10 ekor ayam). Perlakuan terdiri dari : T0 (ransum basal/RB), T1 (RB+0,4% tepung umbi dahlia), T2 (RB +0,8% tepung umbi dahlia), T3 (RB+1,2% tepung umbi dahlia), T4 (RB+0,39% ekstrak umbi dahlia), T5 (RB+0,78% ekstrak umbi dahlia), dan T6 (RB+1,17% ekstrak umbi dahlia). Parameter yang diamati adalah bakteri asam laktat (BAL), *Escherichia coli*, pH digesta usus halus (duodenum, jejunum dan ileum), laju digesta, dan pertambahan bobot badan harian (PBBH). Data dianalisis ragam dan dilanjutkan dengan uji wilayah ganda Duncan, serta polinomial ortogonal. Hasil penelitian menunjukkan bahwa pemberian inulin dalam bentuk tepung maupun ekstrak umbi dahlia nyata ($P<0,05$) meningkatkan populasi BAL dan PBBH, tetapi menurunkan jumlah *Escherichia coli*, pH, dan memperlambat laju digesta. Simpulan penelitian adalah pemberian inulin dari umbi dahlia dalam bentuk tepung optimum pada taraf 0,9% dan dalam bentuk ekstrak masih linier sampai taraf 1,17%.

Kata kunci : ayam kampung persilangan, inulin umbi dahlia, mikroba usus halus, pertambahan bobot badan harian

ABSTRACT

The purpose of the research was to study the effects of feeding inulin derived from *Dahlia variabilis* tuber powder and extract on the existence of intestinal microbes in crossbred native chicken starter. Experimental animals were 280 unsex crossbred native chickens and powder and extract of dahlia tuber as source of inulin, which were started to be fed on day 22. The present experiment was assigned in a completely randomized design with 7 treatments and 4 replications (10 birds each). The treatments were: T0 (basal diet/BD), T1 (BD+0.4% powder form), T2 (BD+0.8% powder form), T3 (BD+1.2% powder form), T4 (BD+0.39% extract form), T5 (BD+0.78% extract form), and T6 (BD+1.17% extract form). Parameters measured were the number of Lactic acid bacteria (LAB), *Escherichia coli*, intestinal potential hydrogen (pH) (duodenal, jejunal and ileal), rate of passage and daily body weight gain (DBWG). Data were subjected to ANOVA and followed by Duncan and polynomial orthogonal test. The results indicated that feeding inulin derived from dahlia tuber powder and extract form significantly ($P<0.05$) increased LAB population and DBWG, but decreased *Escherichia coli* number and intestinal pH, and slow the rate of passage. In conclusion, feeding inulin of dahlia tuber powder was optimum at 0.9% but that of extract is linier until 1.17%.

Keywords : crossbred native chicken, inulin of dahlia tuber, intestinal microbes, daily gain

INTRODUCTION

The potency of Indonesian local chicken is very perspective to be developed as a poultry farm diversification in the future. Local chickens are known to have better adaptability to various fluctuated environment condition due to their higher endurance as compared to modern poultry, such as broiler chicken. Products of native chicken, both egg and meat, have an important contribution to animal protein demand at the regional as well as national levels. It is stated that native chicken meat contributes to the national need by 7.46% (Direktorat Jenderal Peternakan dan Kesehatan Hewan, 2013). In order to balance between supply and demand on local chicken meat, it is important consideration to establish crossbred local chicken with higher growth rate by cross meeting of local male and broiler hen. Crossbred between distantly related-blood species lead to positive heterocyst effect (Zainal *et al.*, 2012).

Productive capability of local crossbred chicken can be improved near to that of broiler through the improved dietary quality followed by intensive rearing management. However, the nutrient provided to the crossbred native chicken still the same to that of standard diet given to the modern poultry in which it is not suitable for native chicken in general. Nutrient for crossbred native chicken has to be improved but also considers to the efficiency production. In relation to nutrient requirement for crossbred native chicken, it is based on an assumption that dietary formulation has been improved, but the effort to increase productive efficiency is still needed. Diet manipulation is one way to improve feed efficiency in order to increase productive capability (Hidayat, 2012). Starter period of chickens, crossbred native chicken in particular, is an important period for the physiological development of the digestive tract. Development of digestive organ at early growing period requires a suitable diet containing nutrients match with requirement (Iskandar, 2004).

Diet manipulation for local crossbred chicken can be created by adding natural additives. Additive usually used by the farmers is antibiotic or medicinal substance. However, prolonged and continuous providing antibiotic has a negative impact on the lives of microbes (resistant microbes) and rising problem of residue in poultry product (meat) which is human health hazard. Therefore, a natural additive such as

probiotic is more recommended. An additive can be specifically qualified as probiotic if it is resistant to gastric acid and not hydrolyzed by digestive enzymes and not absorbed in the small intestine (Gibson *et al.*, 2004). Besides, it can be selectively fermented by beneficial microbes in the intestines and stimulates the chicken growth. One of eligible substance can be classified as probiotic is known as inulin which has been found by Roberfroid (2007). Inulin is a prebiotic derived from plants which have the most significant influence on the chicken growth among other types of prebiotic. One species of plants in Indonesia which can be used as the source of inulin (prebiotic) is *Dahlia variabilis* tuber. Dahlia tuber powder contains inulin with the range of 69.50-75.48% (Fajrih *et al.*, 2014). Inulin functions as a probiotic because it becomes a food substrate component for beneficial microbes in the small intestine. *Dahlia variabilis* tubers content high inulin, it has a great chance to be developed and explored as a natural feed additive. *Dahlia variabilis* yields tubers up to 2 kg/plant and 1400 m² of land can yield 400-500 quintals (Prihatman, 2000).

The study of Park and Park (2011) showed that feeding dietary microencapsulated inulin of 250 g/ton to 35 days broiler chickens could increase the number of *Bifidobacteria* and *Lactobacillus*, decreased the number of *Escherichia coli* and significantly improved feed conversion and weight gain. *Bifidobacteria* and *Lactobacillus* produced short chain fatty acids (SCFA) and lactic acid which inhibited the growth of *Escherichia coli* in the small intestine (Rinttila and Apajalahti, 2013). Inulin can be fermented by beneficial microbes in the intestine and produced SCFA (acetate, propionate and butyrate) which have important function physiologically. Butyric acid in the digestive organ can affect directly as well as indirectly tissue growth and repair (Nabizadeh, 2012). Based on the above description, the present research was conducted to study the effect of feeding inulin derived from *Dahlia variabilis* tuber, either in the form of powder or extract, on the existence of microbes in the starter period of crossbred native chicken's small intestine.

MATERIALS AND METHODS

A total of 280 birds of crossbred native chicken (local male chicken with laying hen) of 22 days old with an average body weight of

180.46 ± 1.21 g. The basal diet was composed with 19.46% protein and 2843.50 kcal/kg metabolizable energy (Table 1).

Feeding trial was began at the age of 22 days after all the chickens were provided adaptation period to basal diet and rearing model using battery cages. A completely randomized design with 7 treatments and 4 replications (10 birds each) was arranged in the present study. The treatments applied were as follows : T0 (basal diet/BD), T1 (BD +0.4% powder of dahlia tuber), T2 (BD +0.8% powder of dahlia tuber), T3 (BD +1.2% powder of dahlia tuber), T4 (BD +0.39% extract of dahlia tuber), T5 (BD +0.78% extract of dahlia tuber), T6 (BD +1.17% extract of dahlia tuber).

Basal diet was given in the morning and evening with an equal amount of a ratio of 50:50. In order to ensure that powder and extract of *Dahlia variabilis* tuber can be totally consumed

according to the treatment levels, it was provided every morning by mixing with a small amount of feed (approximately 20 g). The remaining portion of basal diet without either powder or extract of *Dahlia variabilis* tuber was fed thereafter to fulfill daily intake requirement, and given free access of drinking water.

Lactic acid bacteria (LAB), *Escherichia coli*, potential hydrogen (pH) of the small intestine, rate of passage, and daily body weight gain (DBWG) were the parameters measured on week 7 (42-49 days). Population of LAB and *Escherichia coli* were measured in each segment of small intestine (duodenal, jejunal and ileal) using media deman Rogosa Sharpe (MRS) and eosin methylene blue agar (EMBA). Planting process was proceeded with total plate count method according to Fardiaz (1993) with the following formula:

Total colony (cfu/g) = total colony x 1/dilution factor per plate

Potential hydrogen (pH) was measured from the digesta of each segment of small intestine (duodenal, jejunal and ileal) using a pH meter. Rate of passage measurement was recorded based on the total collection method using indicator according to Indreswari *et al.* (2009). Data were statistically analyzed using analysis of variance and continued to Duncan's multiple test at the probability of 5%, and orthogonal polynomials test was also performed to determine the optimal level of dietary inclusion of powder or extract of dahlia tuber.

RESULTS AND DISCUSSION

Feeding inulin in the form of either powder or extract of *Dahlia variabilis* tuber significantly affected populations of LAB and *Escherichia coli*, intestinal pH, rate of passage, and daily body weight gain/DBWG (Table 2). Duodenal LAB population due to the feeding inulin in the form of extract at the level of 1.17% (T6) was significantly highest among other treatments. However, those in the jejunal and ileal significantly increased with similar pattern, especially when feeding higher levels of dahlia tuber in the form of powder of 0.8% (T2) and 1.2% (T3), as well as that of extract of 0.78% (T5) and 1.17% (T6). On the contrary, intestinal pH of all segments were the lower with the higher levels of feeding powder and extract of *Dahlia variabilis* tuber. Similarly, population of *Escherichia coli* in the ileal was decreased by

Table 1. Basal Diets Research and Nutrient Content

Feedstuff	Composition
	----- % -----
Yellow Corn	51.30
Rice Bran	15.00
Soybean Meal	22.50
Fish Meal	10.00
CaCO ₃	0.70
Vitamin and Mineral	0.50
Total	100.00
Nutritional Content (%) :	
Metabolic Energy (kcal/kg)*	2,843.50
Crude Protein	19.46
Crude Fat	4.99
Crude Fiber	4.81
Methionine**	0.44
Lysine**	1.26
Arginine**	1.39
Calsium	1.02
Posphor	0.66

*Based on Hartadi *et al.* (2005)

**Based on NRC (1994)

Table 2. Populations of lactic acid bacteria (LAB) and *Escherichia coli*, Intestinal pH, Rate of Passage, and Daily Body Weight Gain (DBWG) in Crossbred Native Chicken

Parameters	Treatment						
	T0	T1	T2	T3	T4	T5	T6
LAB (10^4 cfu/g)							
Duodenal	0.15 ^b	0.25 ^b	0.35 ^b	0.23 ^b	0.23 ^b	2.35 ^b	5.78 ^a
Jejunal	0.40 ^c	4.00 ^{bc}	8.75 ^a	6.50 ^{ab}	5.25 ^{ab}	6.50 ^{ab}	9.50 ^a
Ileal	1.30 ^c	2.50 ^c	9.75 ^{ab}	6.50 ^b	2.93 ^c	7.25 ^b	11.75 ^a
<i>Escherichia coli</i> (10^2 cfu/g)							
Duodenal	1.25	1.00	1.00	1.00	1.00	1.00	1.00
Jejunal	6.25	5.25	4.50	5.00	5.00	3.50	3.25
Ileal	19.00 ^a	9.75 ^b	9.00 ^b	8.50 ^b	12.25 ^b	8.75 ^b	8.50 ^b
Intestinal pH							
Duodenal	6.20 ^a	5.53 ^b	5.70 ^{ab}	5.83 ^{ab}	5.80 ^{ab}	5.50 ^b	5.63 ^b
Jejunal	6.58 ^a	6.38 ^{ab}	6.20 ^b	6.30 ^{ab}	6.25 ^{ab}	6.18 ^b	6.15 ^b
Ileal	7.50 ^a	7.00 ^b	6.68 ^{bc}	6.70 ^{bc}	6.48 ^c	6.58 ^c	6.55 ^c
Rate of passage (min)	176.55 ^c	205.20 ^b	226.35 ^{ab}	233.10 ^a	216.90 ^{ab}	221.10 ^{ab}	231.00 ^{ab}
DBWG (g/bird/day)	14.63 ^b	16.45 ^a	16.65 ^a	15.94 ^{ab}	16.56 ^a	16.56 ^a	16.79 ^a

^{abc} Different superscript on the same row indicate significant differences ($P < 0.05$). T0 (basal diet/BD), T1 (BD +0.4% powder of dahlia tuber), T2 (BD +0.8% powder of dahlia tuber), T3 (BD +1.2% powder of dahlia tuber), T4 (BD +0.39% extract of dahlia tuber), T5 (BD +0.78% extract of dahlia tuber), T6 (BD +1.17% extract of dahlia tuber).

whatever levels of powder as well as extract compared to control (T0), but that in the duodenal and jejunal were not affected. The present results were parallel with the finding of Jozefiak *et al.* (2008) that feeding inulin by 0.2% was able to increase the population of small intestinal LAB and inhibit the growth of pathogenic bacteria. Dietary inclusion of *Dahlia variabilis* tuber both in forms of powder and extract at all levels was observed to slowing the rate of passage down. According to Schneeman (1999), the increase LAB population and decrease intestinal pH and slow the rate of passage down. However, DBWG due to feeding effect of 0.4% and 0.8% powder form (T1 and T2) and also that of 0.39%, 0.78% and 1.17% extract form (T4, T5 and T6) was higher than control (T0), while other treatments was the same.

Feeding inulin in the form of extract at

1.17% (T6) could increase the population of LAB in the duodenal to be the highest value compared to other feeding levels of both powder and extract (Table 2). The increasing level of feeding extract, the higher inulin as "food substrate" availability used by the LAB and lead to the increase in the population, although the number of LAB in the duodenal was lower than that in the jejunal and ileal. The more effective inulin utilized or fermented by the LAB is supported by the reduced anti-nutritional content of tannin from 2.28% in powder form to 1.18% in extract form. Normal population of LAB in the jejunal was 10^1 - 10^5 cfu/g and that in ileal was 10^3 - 10^9 cfu/g (Qi *et al.*, 2006). Based on orthogonal polynomial test on LAB population, inulin given in the form of powder was optimum at 0.90%, and this value was closer to the level of 0.80% (T2). Lactic acid bacteria population test in the jejunal can be

explained by the equation of $y = -91406x^2 + 16731x - 75$ ($R^2 = 0.702$) and that in the ileal with the equation of $y = -43099x^3 + 70625x^2 - 18354x + 13000$ ($R^2 = 0.777$) while feeding the form of extract until 1.17% (T6) indicated linear equation of $y = 89188x + 4550$ ($R^2 = 0.84$). The absorption of nutrients occurs in the jejunal, while in the ileal tend to occur in a fewer amount but more fermentation process by LAB. Increased LAB population due to feeding effects of powder form at 0.8% (T2) and 1.2% (T3), and of extract form of 0.78% (T5) and 1.17% (T6) provides an indication that LAB was able to compete with *Escherichia coli* (Table 2). The present results were supported by the finding of Xu *et al.* (2003) that feeding inulin or fruktooligosakarida by 8 g/kg was able to increase the population of small intestinal LAB. Similarly, Park and Park (2011) states that the number of *Bifidobacteria* and *Lactobacillus* was increased, while *Escherichia coli* was decreased by dietary inclusion of 250 g/ton of microencapsulated inulin in 35 days broiler chickens. Since inulin contains the β (2 \rightarrow 1) glycosidic bond, it was resistant to digestive enzymes (Gibson *et al.*, 2004), and enhanced the growth of beneficial bacteria, improved the health and suppressed the growth of pathogenic bacteria (Zentek *et al.*, 2003).

The characteristic of LAB in the digestive tract was homofermentative and able to utilize available substrate (inulin) with the final products are lactic acid, organic acid and CO₂. The existence of lactic acid as a metabolite product may function as the one growth inhibitor of *Escherichia coli*. According to Lunggani (2007), antimicrobial mechanism of lactic acid is based on pH homeostasis and chemiosmotic theory. When lactic acid is secreted into the surrounding, some of the molecules dissociated into H⁺ and anions, while nothing happen on the others. One of the factors that plays a role on dissociation or the absence of a particular molecule is pH. This biochemical process leads to an increase in the transmembrane proton which is finally bring about proton gradient. The gradient differences lead to faster protons entering the cell, thus increasing energy demand to maintain an alkaline pH in the cell.

Feeding inulin can stimulate the growth and population stability of the LAB. The increasing LAB population is followed by the higher production of SCFA, lactic acid and antimicrobial substances. This is in accordance with the report of Fanani (unpublished data) that feeding 1.17%

extract *Dahlia variabilis* tuber as inulin source brought about the highest production of acetic, propionate, and butyric acids with the value was 24.38, 6.37, and 6.45 mMol/L, respectively. Acetic acid is the major substrate can be used for the synthesis of cholesterol and produce lactic acid and acetoacetat, which are absorbed in the intestine and are utilized by peripheral tissues and microbial growth (Hijova and Chmelarova, 2007).

The increase in SCFA and lactic acid as discussed previously, was followed by the decrease in the intestinal pH. The decreased pH in the duodenal, jejunal and ileal was due to the increasing level of feeding inulin source either in the form of powder or extract. Fermentation activity of LAB determines the value of intestinal pH (Dzirkova *et al.*, 2005), and the larger population of LAB that can ferment the substrate produces the lower pH value. The ability of LAB to lowering pH is associated with the production of large amount enzymes that can degrade polysaccharide compounds into simpler monomers form. The level of feeding inulin source in the form of powder to decrease pH in the ileal was optimum at 0.90% and this value was very close to the real level at 0.80% (T2) with the equation of $y = 0.820x^2 - 1.665x + 7.508$ ($R^2 = 0.747$), but when the extract form was fed the value indicated linier until 1.17% level (T6) with the equation of $y = -4.693x + 7.46$ ($R^2 = 0.61$). The approximate optimum pH for LAB growth was ranging from 3.0 to 5.0 (Dzirkova *et al.*, 2005), whereas, normal pH in the duodenal, jejunal, and ileal was ranging between 5.0 – 6.0, 6.5 – 7.0, and 7.0 – 7.5, respectively (Emma *et al.*, 2013).

In connection with the slow rate of passage down due to both feeding powder and extract was consistence with the increased LAB and the decreased ileal *Escherichia coli* populations, and the reduced intestinal pH, although it was linear until the leves of 1.2% powder (T3) and of 1.17% extract (T6). *Escherichia coli* population in the ileal decreased if fed optimum level of inulin source at 0.89% and this value was very close to the real level at 0.8% (T2) with the equation of $y = 1367x^2 - 2446x + 1858$ ($R^2 = 0.757$), whereas, when extract form was fed it indicated linier ($y = -850x + 1697$) until the level of 1.17% (T6). However, the duodenal and jejunal *Escherichia coli* had no effect because of the succesfully competition of LAB due to its high population which brought about the failure of *Escherichia coli* attached into the duodenal and jejunal

mucosa (Nabizadeh, 2012). This phenomenon can be further explained by the similar the population growth of *Escherichia coli* (Table 2).

Feeding source of inulin in the form of powder as well as extract could increase BWDG (Table 2), this can be correlated with the existence of LAB in the intestine (duodenal, jejunal, and ileal) that able to compete with *Escherichia coli*. The growth of *Escherichia coli* was depressed due to the reduced intestinal pH, so that it implicates to the improved health status of the digestive tract. Heterophyl and lymphocyte (H/L) ratio can be used as an indicator of health or body resistance status which was caused by feeding inulin source in the form of either powder or extract form. The value of H/L ratio was categorized normal (0.5 in average) and lower when compared to control/T0 (0.69) (Fajrih *et al.*, 2014). According to Kusnadi (2008), H/L ratio was the indication of discomfort condition of the poultry, the higher the ratio, the higher the level of discomfort. Therefore, improvement of digestive tract condition through dietary inclusion of inulin source provided an impact on body health status which is further affected nutrients utilization or absorption and finally improved BWDG. Nutrients absorption, especially protein, can be increased by the higher inclusion of inulin in the form of extract (T6) by 10.15 g of absorbed protein, and it was significantly higher than control/T0 (Fanani, unpublished data). It can be briefly stated that the higher existence of intestinal LAB, the increased health status or body resistance, and high level of protein supply due to the feeding inulin source were the important factors contributed to the DBWG.

CONCLUSION

Feeding inulin source in the form of powder as well as extract of *Dahlia variabilis* tuber can increase lactic acid bacteria (LAB) population, lowering the intestinal pH (duodenum, jejunum and ileum), decrease ileal *Escherichia coli* number, and slow the rate of passage down. Feeding inulin derived from dahlia tuber is optimum at level of 0.9% powder form and at 1.17% extract form although it is still linear.

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