Feeding of combination inulin and *Lactobacillus acidophilus* in feed containing microparticle protein on jejunal condition of broiler chicken

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Abstract

This study was conducted to evaluate the effect of combination inulin and *Lactobacillus acidophilus* in feed contains microparticle protein on jejunal condition of broiler chicken. The inulin used were sourced from dahlia tuber extract and *Lactobacillus acidophilus* as probiotic. The experimental animal using broiler strain *Cobb* with treatment started at the age of 8 days with average body weight 172,95 ± 2,96 g. Feed stuff as protein micropartikel from fish meal and soybean meal. The research design using completely randomized design of factorial 3x3 with 3 replicates. First factor is inulin level 0% (A1), 0.58% (A2) dan 1.16% (A3), while second factor were level *Lactobacillus acidophilus* 0% (B1), 0.6% (B2) dan 1.2% (B3). The parameter measure were length, relative weight and jejunal villi length. The data test with analysis of variance and Duncan test at 5% level. The results that effect of combination inulin and *Lactobacillus acidophilus* in feed contains microparticle protein have interactions (p<0,05) on length, relative weight and jejunal villi length. The conclusion that effect of combination inulin 1.16% (A3) and *Lactobacillus acidophilus* 1.2% (B3) in feed contains microparticle protein can increase length, relative weight and jejunal villi length.

Keywords: broiler chicken, inulin, jejunal condition, *Lactobacillus acidophilus*, protein microparticle

Introduction

Along with the increasing population of Indonesia, the need for animal protein, one of which is from broiler chickens, is also increasing. The advantage of broilers is that they have fast growth within 35 days and can be marketed with high meat stock. The growth of broiler chickens is influenced by the nation, gender, age, ration quality, environment, and health. The feed has an important role in the success of broiler chicken maintenance because it is related to basic life and production. Feed with nutritional content as needed and balanced can support maximum growth [1].

A good quality feed does not necessarily produce as expected, if the digestive system is not supportive. The productivity of broilers must be supported by a healthy digestive tract condition, so that the absorption of nutrients is maximized. The addition of prebiotics and probiotics is expected to help improve the balance of good bacteria and pathogens that have an important role in creating digestive tract health in relation to nutrient absorption. The combination of probiotics and prebiotics is beneficial to stimulate the growth of beneficial bacteria, so that the digestive tract becomes healthy and absorption of nutrients is maximized [2]. Feeding with microparticle protein is strongly supported by a healthy digestive tract due to the addition of probiotic and prebiotic additives, so that the use of nutrients, especially protein, is more efficient.

The prebiotic inulin in this study was derived from dahlia tubers which are commonly used as feed additives for livestock, especially poultry. The inulin content in dahlia tubers ranges from 65 to 75% [3]. Prebiotic inulin is a source of "food" that can be fermented by lactic acid bacteria (LAB) in the intestine to produce short chain fatty acids (SCFA). Short chain fatty acids products can further lower the intestinal pH, so that the growth and development of bacteria that cannot tolerate acid conditions (pathogenic bacteria) are inhibited. Short chain fatty acids can lower intestinal pH and inhibit the growth of pathogenic microorganisms, as well as stimulate the production of mucin by goblet cells which can increase colonization of LAB [4]. Increased LAB colonization means being able to compete with pathogenic bacteria.

The number of LAB increases with the production of SCFA can affect the development of villi through the role of butyric acid as an energy source for the multiplication of intestinal cells. The digestive tract with good villi development strongly supports the provision of feeds using protein microparticles which lead to maximum digestion and absorption of protein. The use of microparticle protein sources, proteins are more easily absorbed due to the hydrolysis process by enzymes into simple molecules. The reduced protein particle size (microparticles) means making the accessibility of digestive enzymes more intensive to the ration substrate, especially protein [5]. Increased protein absorption is a positive feedback for the development of the digestive tract and has an impact on increasing body weight of broiler chickens [6]. On the other hand, the addition of Lactobacillus acidophilus as a probiotic in collaboration with endogenous LAB can strengthen the physiological phenomena of a healthy digestive tract. A better balance of intestinal microflora can be created because LAB has an inhibitory power against pathogenic bacteria through fermentation products in the form of SCFA as previously discussed.

The purpose of this study was to examine the combination of dahlia tuber extract (*Dahlia variabillis*) and Lactobacillus acidophilus added to the diet using microparticle protein on the length and relative weight of the jejunum and the length of the jejunum villi in broiler chickens. The benefit of the research is that the combination of dahlia tuber extract (*Dahlia variabillis*) and *Lactobacillus acidophilus* is an additive mixture that is effective in increasing the utility of protein microparticles supported by better digestive tract development in broiler chickens.

Materials and Methods

Animal, Feed and Equipment

The research livestock used were 216 DOC broiler chickens strain Cobb with an average body weight of 42.62 ± 0.20 g. Combination of dahlia tuber extract and *Lactobacillus acidophilus* as treatment material. The equipment used for data collection are scissors, analytical scales, measuring instruments and tapes. Basal feed with nutritional content is listed in Table 1.

Table 1. Feed Formulation and Nutritional Content

Feed Stuff	Composition (%)
Yellow Corn	54.48
Wheat Bran	15.25
Soybean Meal of Microparticle	20.41
Fish Meal of Microparticle	9.00
$CaCO_3$	0.30
Premix	0.21
Lysine	0.10
Methionine	0.25
Nutritional Content *(%)	
Metabolizable Energy** (Kkal/kg)	3,093.69
Crude Protein	18.30
Crude Fiber	5.88
Ether Extract	4.01
Calcium	1.14
Phosphor	0.60
Methionine	0.46
Lysine***	1.03
Arginine***	1.30

* Result of feed analysis in Laboratory of Animal Science, Faculty of Animal and Agriculture Sciences, Universitas Diponegoro, Semarang, 2020
** Calculation results using Bolton's formula, [2]
*** Result of feed analysis in Laboratory of BPMSP, Bekasi, 2020

Dahlia Tuber Extract Procedure and Microparticle Protein

Prebiotic preparation refers to research [7], done by dissolving dahlia tuber flour with a ratio of dahlia tuber flour compared to distilled water and 70% alcohol, which is 1:5:5. Dissolution is carried out for 30 minutes after boiling. The results of the dissolution are then filtered, which is separated between the dregs and the water. The filter results are cooled in the freezer and allowed to form a precipitate. The precipitate is then poured into a tray and dried in the sun to dry, then mashed again. Microparticle protein ingredients consisting of vegetable protein sources (soybean meal) and animal protein (fish meal) are first mashed. The feed ingredients were then mixed with distilled water in a ratio of 1:4 (w/v) plus 2 ml of virgin coconut oil for every 500 ml of solution, then exposed to ultrasonic waves using an ultrasound transducer for 60 minutes [8]. The solid components are first dried in an oven at 40°C to avoid protein damage, then dried.

The Treatment and Experimental Design

The experiment was carried out in a completely randomized design (CRD) with a 3 x 3 factorial pattern with 3 replications. Factor A is dahlia tuber extract (3 levels) namely A1 (0%), A2 (0.58%) and A3 (1.16%), and factor B is Lactobacillus acidophilus (3 levels) with code B1 (0%). The form of the treatment combination is described as follows:

A1B1 = without dahlia tuber extract and *Lactobacillus acidophilus*
A1B2 = without dahlia tuber extract + *Lactobacillus acidophilus* 0.6 %
A1B3 = without dahlia tuber extract + *Lactobacillus acidophilus* 1.2 %
A2B1 = dahlia tuber extract 0.58% without *Lactobacillus acidophilus*
A2B2 = dahlia tuber extract 0.58% + *Lactobacillus acidophilus* 0.6 %
A2B3 = dahlia tuber extract 0.58% + *Lactobacillus acidophilus* 1.2%
A3B1 = dahlia tuber extract 1.16% without *Lactobacillus acidophilus*
A3B2 = dahlia tuber extract 1.16% + *Lactobacillus acidophilus* 0.6 %
A3B3 = dahlia tuber extract 1.16% + *Lactobacillus acidophilus* 1.2 %

The Chicken Rearing and Parameter

Chicken maintenance begins with cage preparation including cage cleaning, liming, making litter system cages, making electrical circuits and installing feed and drinking places. Chickens were reared for 35 days, namely at the age of 1 to 21 days using 27 units of litter cages (8 birds per unit), while at the age of 21 to 35 days using battery cages. The feeds used consisted of commercial feeds and treatment feeds. Commercial feeds are given during the 1st week, while the treatment feeds are in the 2nd to 5th week. Chickens were given an adaptation period starting at the age of 8 days by being given a mixture of starter feeds and treatment feeds with a ratio of 75: 25%, 9 days old 50: 50%, 10 days old 25: 75%, and 11 days old 100% treatment feeds.

The length of the small intestine was measured using a measuring tape, while the weight was measured using an analytical balance. The relative weight of the jejunum is calculated by the following formula:

$$\text{Relative weight} = \frac{\text{organ weight}}{\text{live weight}} \times 100\%$$

The sample of the jejunal intestine is then placed in a tube containing formalin with a concentration of 10% which has been diluted. The length of the villi was measured from the sample which had been separated and immersed in 10% formalin [9].

Statistic Analysis

The research data were analyzed of variance with the F test at the 5% level to determine the effect of treatment. The effect of the real treatment was then continued with Duncans multiple difference test [10].

Results and Discussion

Jejunal Lenght

Based on analysis of variance, the combination of dahlia tuber extract and Lactobacillus acidophilus had a significant effect ($P<0.05$) on jejunum length (Table 3). Duncan's test showed that treatment A1B1 was significantly lower ($P<0.05$), compared to other treatments (A1B2, A1B3, A2B1, A2B2, A2B3, A3B1, A3B2 and A3B3). However, among the eight treatment combinations, except A1B1, there was no significant difference.

Table 3. Length of Jejunum Broiler Chickens Aged 35 Days Which Received Combination Treatment of Dahlia Tuber Extract and Lactobacillus acidophilus.

Level of Dahlia Tuber Extract	Level of *Lactobacillus acidophilus*			Average
	B1	B2	B3	
	---------------------------------- cm ----------------------------------			
A1	54.17^{b}	64.83^{a}	65.87^{a}	61.62
A2	64.40^{a}	66.17^{a}	66.17^{a}	65.58
A3	66.77^{a}	67.17^{a}	67.33^{a}	67.09
Average	61.78	66.06	66.46	

$^{a-b}$ Different superscripts in the values between treatment combinations showed significant differences ($P<0.05$)

The combination of A1B1 is a treatment without dahlia tuber extract and Lactobacillus acidophilus, so there is no inulin as a "food source" for Lactobacillus acidophilus and endogenous LAB, so there is no improvement in the balance of intestinal microflora. Lactic acid bacteria in A1B1 treatment did not develop well because the intestinal pH was high (data not yet published) and on the other hand pathogenic bacteria (coliform) increased (Krismiyanto, data unpublished). The presence of low LAB and high coliform bacteria creates an unhealthy digestive tract condition, so it is not conducive to nutrient digestion. Jamilah et al. [11] reported that a high number of coliform bacteria can cause depletion in the development of the digestive tract, such as inhibition of villi growth, resulting in low nutrient digestibility. This condition is evidenced by protein digestibility data (Rifai, data unpublished) in A1B1 treatment of 66.17%, and significantly lower than other treatments (A1B2 – A3B3), which is 78.09 to 85.63%. A1B1 treatment even though using rations with protein microparticles, but because without added protein digestibility additives it does not support as a positive feedback on jejunal growth.

Treatments A2B1 and A3B1 only added dahlia tuber extract, while treatments A1B2 and A1B3 were only given Lactobacillus acidophilus, resulting in the same jejunum length as other treatments that received both (dahlia tuber extract and Lactobacillus acidophilus). These results indicate that the administration of only one additive or both has the same effect on the health of the digestive tract because it is strengthened by the use of microparticle protein sources. The addition of either dahlia tuber extract or Lactobacillus acidophilus or a combination of both is able to produce SCFA which can lower the pH of the digestive tract, so that the LAB population increases and the development of pathogenic bacteria is inhibited. Improved intestinal microflora balance supports better digestive tract health, increases protein digestibility and has an impact on the formation of epithelial cells. Ketaren [12] stated that the development of epithelial cells that are getting better and more numerous is an indication of increased jejunal growth.

Relative Weight of Jejunal

Analysis of variance showed that there was no significant interaction between the addition of dahlia tuber extract and Lactobacillus acidophilus on the relative weight of the jejunal in broiler chickens (Table 4). However, factors A and B partially each had a significant effect on jejunum weight, Duncan's test showed that the relative weight of the jejunum on A3 was significantly ($P<0.05$) higher than A1 and A2, which were not different between the two. Similarly, factor B showed that B2 and B3 significantly ($P<0.05$) increased jejunum weight when compared to B1, while there was no difference between B2 and B3 (Table 4).

Table 4. Jejunal Relative Weight of Broiler Chickens Aged 35 Days Obtained Combination Treatment of Dahlia Bulb Extract and Lactobacillus acidophilus

Level of Dahlia Tuber Extract	Level of *Lactobacillus acidophilus*			Average
	B1	B2	B3	
	---------- g ----------			
A1	0.85	1.07	1.13	1.02^{b}
A2	0.89	1.10	1.21	1.07^{b}
A3	1.08	1.22	1.29	1.20^{a}
Average	0.94^{b}	1.13^{a}	1.21^{a}	

$^{a-b}$ Different superscripts in the values between treatment combinations showed significant differences ($P<0.05$)

The factor without the addition of Lactobacillus acidophilus (B1) resulted in the lowest jejunal relative weight and was significantly different from other treatments (Table 4). Treatment without the addition of Lactobacillus acidophilus (B1) resulted in high coliform counts, as previously discussed in the jejunal length parameter. According to Halimatunisroh et al. [13] that the number of coliforms in the small intestine of finisher broiler chickens is 6.04×10^6 cfu/g. If the number of coliforms is too high, it can interfere with the health of the digestive tract. On the other hand, the addition of Lactobacillus acidophilus (B2 and B3) gave positive feedback for jejunal growth. Lactobacillus cooperates with endogenous LAB in inhibiting pathogenic bacteria, balancing intestinal microflora and improving nutrient absorption. According to Lokapirnasari et al. [14] that the addition of lactic acid bacteria can dominate the microbes in the intestine, resulting in a balance of the population of microorganisms that help the digestive process.

The decrease in the number of pathogenic bacteria and the increase in the LAB population can create a healthier digestive tract condition, so that the source of microparticle protein in the ration can be utilized effectively. Digestion of nutrients, especially protein (data unpublished), can support general gut development, including jejunal weight, as a positive feedback aspect. The result of the digestive process and the small intestine is a relationship like a "circle" back and forth. Protein is useful for the formation of intestinal epithelial cells, the higher the digestibility of protein causes the digestive tract to develop, or vice versa, the development of the digestive tract is good, the higher the digestibility of protein. According to Winarti et al. [15] that probiotics and their populations can have a health effect on the intestines with well-developed villi having an impact on increasing the weight and length of the digestive organs.

A similar phenomenon occurred in partial factor A with the administration of dahlia tuber extract at the level of 1.16% (A3) resulting in the highest real jejunal weight (Table 4). These results indicate that higher inulin from dahlia tuber extract can effectively be fermented by LAB to produce SCFA and lower intestinal pH, so that coliform growth is suppressed (Krismiyanto, data not yet published). Krismiyanto et al. [16] stated that inulin is fermented by LAB in the small intestine and the depolymerization process of inulin compounds produces hydrogen molecules which contribute to a decrease in intestinal pH, so that the growth of pathogenic bacteria is inhibited. The low number of pathogenic bacteria does not interfere with intestinal performance, so that it has an impact on improving digestion, especially protein, and can support the development of higher jejunal weights. According to Adriani et al. [17] that the decrease in pathogenic bacteria can increase enzyme activity and expand the surface of the intestinal villi which can affect the increase in digestibility, absorption of nutrients, and form and increase the size of new tissues.

Jejunal Villi Length

Based on the results of the analysis of variance, it can be seen that there was a significant interaction ($P<0.05$) between dahlia tuber extract and Lactobacillus acidophilus on the length of the villi of the jejunal in broiler chickens (Table 5). Duncan's test showed that A3B3 treatment was the highest ($P<0.05$) and significantly different from other treatments. On the other hand, A1B1 treatment resulted in the lowest real jejunal villi length ($P<0.05$) followed by A1B2 and A2B1 with the second lowest value (Table 5).

The combination of dahlia tuber extract 1.16% and Lactobacillus acidophilus 1.2% (A3B3) produced the highest jejunal villi and significantly different from other treatments. Bacterial balance in the A3B3 treatment was assumed to be optimal because inulin as a "food source" could be fermented effectively by lactic acid bacteria, especially Lactobacillus acidophilus. The result of fermentation is short chain fatty acids (SCFA) consisting of acetate, butyrate and propionate. SCFA products, especially butyrate, are used as an energy source for the growth of small intestinal villi. Satimah [18] stated that the SCFA product in the form of butyric acid is beneficial in the proliferation of epithelial cells and expands the absorption area of the intestinal lumen. In addition, SCFA can improve the performance of goblet cells in producing mucin which acts as a barrier to the attachment of pathogenic bacteria in the intestinal villi. Goblet cells produce mucin which contains glycoproteins, which function to help the process of absorption of nutrients, blocking pathogenic microbes [19].

Table 5. The length of the villi of the Jejunal section of 35-day-old Broiler Chickens that received a combination of Dahlia tuber extract and Lactobacillus acidophilus treatment.

Level of Dahlia Tuber Extract	Level of *Lactobacillus acidophillus*			Average
	B1	B2	B3	
	---------------------------------- µm	--------------------------------		
A1	1048.51^{f}	1185.29^{e}	1268.21^{d}	1167.34
A2	1154.38^{e}	1217.54^{de}	1681.10^{b}	1351.01
A3	1524.68^{c}	1654.71^{b}	1781.76^{a}	1653.72
Average	1242.52	1352.51	1577.02	

$^{a\text{-}f}$ Different superscripts in the values between treatment combinations showed significant differences (P<0.05).

The use of microparticle protein sources, on the other hand, can lead to more effective enzyme penetration, resulting in faster production of easily absorbed amino acids and increased protein intake. Protein digestibility of 85.65% (Rifai, unpublished data) is evidence of the highest protein intake in A3B3 which can support the formation of intestinal epithelial cells. The increasing number of epithelial cells is evidence that the villi can develop properly [12].

The shortest villi length was produced by the combination treatment A1B1 (Table 5). The physiological process of digestion that occurs in A1B1, without the addition of the two additives, is the opposite of the A3B3 treatment. The combination of A1B1 did not improve the balance of intestinal microflora. Lactic acid bacteria in A1B1 treatment did not develop properly because SCFA products were not produced in contrast to A3B3 treatment, so that the intestinal pH was high (data not yet published) and on the other hand, pathogenic bacteria increased (Krismiyanto, unpublished data). The presence of high coliform bacteria can interfere with intestinal performance and nutrient absorption, so that the growth of villi is inhibited. [11] that a high population of pathogenic bacteria such as coliforms can lead to depletion of digestive tract development and reduced growth rate of intestinal villi.

Conclusion

Based on the results of the study, it can be concluded that the A3B3 treatment, namely a combination of 1.16% dahlia tuber extract and 1.2% Lactobacillus acidophilus was able to increase the length and weight of the jejunal and the length of the jejunal villi of broiler chickens.

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