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Feeding combination of *Lactobacillus casei* and extracts of dahlia tuber or garlic on intestinal bacteria, nutrients digestibility and performance of broiler chickens

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ABSTRACT: This study aimed to evaluate the effect of dietary inclusion of 2% synbiotic (*Lactobacillus casei* + dahlia tuber extract/LEDT or *Lactobacillus casei* + garlic extract/LEGT) on intestinal bacteria, nutrient digestibility, and growth of broiler chickens. A total of 150 birds of one-day-old male Cobb broiler were randomly assigned into three groups of treatment with five replications (10 birds each). Dietary treatments applied were T0 = basal ration, T1 = basal ration + 2% LEDT, T2 = basal ration + 2% LEGT. Synbiotics were given to starter chicken (from 2 to 3 weeks old). Parameters observed were intestinal bacteria (total lactic acid bacteria/LAB and coliform counts), nutrients digestibility, and body weight. Data were subjected to analysis of variance and followed by Duncan test. The results showed that the population of intestinal LAB in synbiotic-treated birds significantly ($P<0.05$) increased while coliform decreased as compared to control group. Protein and crude fiber digestibilities, and growth of broiler chickens given dietary inclusion of synbiotic either LEDT or LEGT were also significantly ($P<0.05$) higher than those fed control diet. In conclusion, dietary inclusion of 2% synbiotic LEDT increase body weight of broiler chickens through the improved balance of intestinal microbiota and nutrients digestibility.

Keywords: Synbiotic; Intestinal microbiota; Nutrient digestibility; Growth; Broiler chicken

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INTRODUCTION

Poultry meat demand is estimated to continuously increase due to the population as the consumers of animal protein is also growing fast. The high growth rate with high meat production of broiler is considerably hope to contribute to the fulfillment of meat demand. To support such high productivity, broilers need rations with high nutrients content and balance to fulfill the requirement. The availability of nutrients for growth, especially protein, can be identified from the rate of digestibility because the higher digestibility the more nutrients that can be possibly supplied for body tissue synthesis, muscle protein in particular. The enhancement of nutrients digestibility can be achieved when supported by the healthy gastrointestinal tract (GIT). Microbiota population plays a significant role in the health of the digestive tract, digestive processes, productivity, and the possibility of disease susceptibility. The improved composition and balance of intestinal microbiota are closely related to the condition dominated by beneficial bacteria, but not pathogens. Commensal bacteria play an important role in the gut health and metabolic processes of the host, while pathogenic bacteria cause harmful effects either directly or indirectly. The condition of intestinal microbiota, either composition or balance, is influenced by kinds of feed provided (Yadav and Jha, 2019).

Therefore, the feed given to animals must support the condition of the gut and maintain the balance of intestinal microbes. The results of previous studies indicated that administration of synbiotics can improve intestinal microbiota and increase growth in chickens (Sugiharto, 2016; Fair and Magray, 2012; Abdeel-Raheem, 2012). Synbiotic is defined as a combination of probiotics and prebiotics (Huyghebaert *et al*., 2011). Previous studies have shown the beneficial effects of synbiotics on gut microbial ecosystems and immune function of chickens. Feeding diet added with a combination of 1.2% dahlia tuber inulin and 1.2 mL *Lactobacillus* sp. increased jejunal villi height, protein digestibility, final body weight, and decreased jejunal coliform counts in highly selected pure native chickens (Purbarani *et al*., 2019). Microorganisms in Bacteria derived from synbiotic in the intestine produce substances that have bactericidal or bacteriostatic properties, which can suppress the colonization of undesirable microorganisms. This can control the balance of host intestinal microbiota from pathogenic bacteria. Synbiotic administration has been proven to be effective in increasing the growth of broilers (Abdel-Raheem et al., 2012). Prebiotics combined with probiotics cause the better growth of beneficial bacteria, which effect is due to the availability of substrate, in term of prebiotic as "source of feed," to support its growth. Bacteria, especially LAB, can ferment low molecule weight carbohydrates such as lactose, to produce short-chain fatty acids such as acetic, propionic, and butyric acids, causing the decrease in intestinal pH of the host. Some species also produced hydrogen peroxide that could inhibit the growth of gram-negative bacteria (Yirga, 2015; Bajagai et al., 2016). Bactericidal agent produced by LAB can penetrate the outer membrane of gram-negative bacteria and deactivate them together with others, creating an antimicrobial environment, such as low temperature, organic acid production and detergents (Alakomi *et al*., 2000). This condition caused a healthy digestive tract, improved intestinal morphology so that it could improve the digestive process (Awad *et al*., 2009). Finally, it increased the availability of nutrients for body tissue synthesis and could improve the growth performance of broiler chickens.

One of the potential bacteria that can function as probiotics is *Lactobacillus casei*. Probiotic from *Lactobacillus casei* could produce anti-microbial substances and suppressed the growth of pathogenic bacteria (Sunaryanto *et al*., 2014). The existence of this probiotic bacteria would be successfully improved when fed together with prebiotic inulin as a "source of nutrient," called as a synbiotic or just a status of combination. Inulin can be obtained from dahlia tuber and garlic bulb in the forms of either powder or extract. Dahlia tuber in the form of powder contains inulin with the range of 69.50 to 75.48%. However, inulin in garlic is far lower than that in dahlia tuber namely, around 9 to 16% of fresh weight. Extraction can be carried out to obtain inulin from dahlia tuber and garlic bulb. Inulin extraction principle was to utilize the solubility of inulin in water and ethanol (Sundari *et al*., 2014).

The solubility of inulin that recrystallized with ethanol was greater than that recrystallized with water. At a temperature of 60º, the solubility of inulin that recrystallized with ethanol was 47.0 g / 100 g, while that with water was 1.57 g / 100 g (Azhar, 2009). Previous studies have shown that dahlia tuber in the forms of either powder at 1.2% or extract at 1.17% as source of prebiotic inulin reduced meat fat and cholesterol and increased body weight gain of crossbred local chickens (Fajrih *et al*., 2014). Similar levels and forms of dahlia inulin was also found to increase protein digestibility, SCFA production, and growth of crossbred local chickens (Fanani *et al*., 2016). Furthermore, Faradila *et al*. (2016) confirmed that feeding a combination of prebiotic inulin of dahlia tubers at 1.2% and *Lactobacillus* sp. at 1.2 ml (108 cfu / ml) decreased *Escherichia coli* population and intestinal pH but increased the amount of LAB and weight gain in crossbred local chicken.

There are many studies have been previously conducted using prebiotic alone or in combination with probiotic. Feeding diet added with *Lactobacillus* sp. alone as probiotic successfully improved chickens health and produced higher meat quality in broiler (Cholis *et al*., 2018; Wulandari *et al*., 2018). The improvement of health status and production quality and performances were also reported in crossbred local chicken when probiotic fed in combination with prebiotic (Abdurrahman *et al*., 2016a; 2016b). A combination of *Lactobacillus acidophilus* and 10,000 ppm red onion inulin were able to inhibit growth of *Escherichia coli* (Hartono *et al*., 2012).

Research on synbiotics have been previously conducted many elsewhere, but that concerning a combination of *Lactobacillus casei* and extracts of dahlia tuber or garlic as a synbiotic given in broiler of starter period has never been done. Therefore, the present study was conducted to evaluate the effect of dietary inclusion of synbiotics. Namely, *Lactobacillus casei* plus dahlia tuber extract (LEDT) compared to *Lactobacillus casei* plus garlic extract (LEGT) on intestinal bacterial populations, nutrients digestibility, and growth of starter broiler.

MATERIALS AND METHODS

Experimental Animal and Diet

One hundred and fifty birds of 7-day-old broilers with initial body weight of 184,62 ± 10,32 g were randomly placed in 15 experimental units, and given experimental diet for three weeks. The experimental diet, containing 3000 kcal/kg ME and 21.5% crude protein, were composed of corn, rice bran, soybean meal, meat bone meal (MBM), and premix (Table 1).

Synbiotics tested were combination of *Lactobacillus casei* and extract of dahlia tuber (LEDT) and *Lactobacillus casei* and extract of ginger bulb (LEGT). Synbiotic preparation was started with the extraction of dahlia tuber and garlic bulb using maceration method, with 70% alcohol, and followed by the rejuvenation of *Lactobacillus casei* bacteria. One dose of *Lactobacillus casei* isolate was inoculated in 50 ml of 10% skim milk (10 g in 100 ml of sterile distilled water), and than it was incubated for 2 x 24 hours.

Liquid culture obtained from the results of incubation were then calculated for bacterial population by the total plate count (TPC) method. 50 ml of liquid culture of incubation result was re-inoculated in 450 ml of skim milk (liquid mixture was 500 ml), and then counted again with TPC. Furthermore, 500 ml of liquid mixture was added with prebiotic sources (2%) of sterilized dahlia tuber or garlic extracts, at a concentration of 2% and was re-incubated for 2 x 24 hours to produce synbiotic. The bacterial population was finally counted with TPC method (Ngatirah and Ulfah, 2013).

Table 1. Composition and nutrient contents of experimental ration

Ingredient	Composition (%)
Maize	44.00
Rice bran	19.00
Soybean meal	27.20
MBM	8.5
$CaCO_3$	0.3
premix[1]	1.00
Total	100
Nutrient contents:	
Metabolizable energy (kcal/kg)[2]	3001.11
Crude protein (%)[3]	21.6
Crude Fiber (%)[3]	5.69
Ether extract (%)[3]	3.84
Calcium (%)[3]	1.12
Phosphorus (%)[3]	0.63
Methionine (%)[4]	0.42
Lysine (%)[4]	1.31

[2]Metabolizable energy was calculated based on formula of Bolton (1967) as follows: 40.81 [0.87 (Crude Protein + 2.25 crude fat + nitrogen-free extract) + 2.5]
[3]Analisis results of the Laboratory of Nutrition and Feed Science, Faculty of Animal and Agricultural Sciences, Diponegoro University
[4]The values were calculated based on table of National Research Council (1994)

Experimental Design for *In vivo* synbiotic test

In vivo trial was arranged in a completely randomized design with three dietary treatments and five replications. Dietary treatments tested were as follows :

T0 = basal ration
T1 = basal ration + 2% synbiotic of LEDT
T2 = basal ration + 2% synbiotic of LEGT

Parameters Measured

a. Populations of lactic acid bacteria and *Escherichia coli* in the small intestine

One bird of 21-day old was randomly taken from each replication. The birds were then slaughtered and dissected to obtain part of the small intestine for digesta collection. Intestinal digesta samples were put into sterile bottles before laboratory analysis for total lactic acid bacteria (LAB) and *Escherichia coli* (*E. coli*) counts. Calculation for total LAB was performed based on pour plate method using MRSA media, while that for *E. coli* was processed with TBX media. One ml diluted digesta sample was put into petri dish for preparation of either LAB or *E. coli* calculation. The media was poured into a 15 ml petri dish, then covered and placed on the shaking table to form number eight and left frozen. Solid samples were incubated in a reverse incubator for 48 hours at 44^oC for LAB and 37^oC for *E. coli*. After the incubation period was complete, the number of colonies formed was calculated by using the Quebec colony counters.

b. Nutrient Digestibility and Body Weight

Digestibility trial was performed using total collection method, and excreta was collected from day 21 to day 24. Excreta was then dried and analyzed for crude protein and crude fiber. Feed intake was recorded

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during excreta collection to calculate nutrients consumption subtracted by fecal nutrients excretion. Nutrient, protein and crude fiber, digestibility ~~is~~ was measured according to Meluzi *et al*. (2001), and Kong and Adeola (2014), respectively, as follows:

$$\text{Nutrient digestibility} = \left[\frac{\text{(nutrient consumed - nutrient in excreta)}}{\text{nutrient consumed}}\right] \times 100\%$$

Nutrients digestibility (protein and crude fiber) was estimated to support the performance; therefore, body weight was also determined at the end of the experiment (end of starter period) on 21-day old.

Statistical Analysis

Data were statistically analyzed using analysis of variance and continued to Duncan's multiple range test when the treatment indicated significant effect.

RESULTS AND DISCUSSION

Dietary synbiotic supplementation of both *L. casei* and inulin of dahlia tuber extract (LEDT, T1), and *L. casei* and garlic extract (LEGT, T2) significantly increased intestinal LAB population and reduced the amount of coliform of broiler chickens compared to control (Table 2). The increased LAB population can be correlated with the increase in the production of organic acids or short-chain fatty acids due to the bacterial fermentation effect on either dahlia tuber or garlic extracts containing prebiotic, which made the intestine to be acidic condition (lower pH). This microenvironmental condition of the intestine causes the decrease in pathogenic bacteria (coliform) population in T1 and T2 treatments.

The results of this study were following Mountzouris *et al.* (2007) who stated that probiotic such as *Lactobacillus* sp. culture reduce the availability of nutrients or food for pathogenic bacteria and increase colonization of lactic acid bacteria. Rebole *et al*. (2010) reported that the addition of inulin from chicory root powder at a level of 10 g / kg and 20 g / kg in chicken rations were able to increase intestinal amount of *Lactobacillus* sp.

The increasing lactic acid bacteria (LAB) counts, in addition, to produce higher amount of lactic acid and short-chain fatty acids (SCFA); this beneficial bacteria was also known to produce harmful metabolites. Hydrogen peroxide, carbon dioxide (CO_2), and antimicrobial (bacteriocin) are the harmful metabolites that are antagonistic to the growth of pathogenic bacteria, and on the other side, improves growth of beneficial bacteria in the small intestine (Azhar, 2009). Other possible factor is that the decrease in the number of pathogenic bacterial colonization is caused by the lost ability of pathogenic bacteria in competing to use nutrients in the small intestine. Result of this study showed a significant increase in the number of colonization of LAB due to dietary addition of synbiotic of either *L. casei* + dahlia tuber extract/LEDT or *L. casei* garlic extract/LEGT (Table 2).

This result proved that both synbiotics were able to change intestinal microbes population and balance. *L. casei* function as a probiotic bacteria had a capability in modifying the digestive tract micro-environment of broilers and lead to the increased growth of nonpathogenic microbes, facultative anaerobic and gram-positive bacteria that can produce lactic acid and hydrogen peroxide (Yang *et al*., 2009). Those toxic or harmful compounds produced by LAB suppressed the growth of intestinal pathogens as indicated by the decreased coliform counts found in the present study, as a response to dietary treatment with synbiotics supplementation. The present result was in accordance with the previous study (Mountzouris *et al*., 2007), that dietary addition of *Lactobacillus* sp. significantly reduced cecal coliform and *Salmonella enteritidis* populations, and also *Clostridium perfringen* number (Kizerwetter-Swida, 2009).

The increased intestinal LAB and the decreased *E. coli* counts was observed in starter period of crossbred native chickens given prebiotic inulin of dahlia tuber (Krismiyanto *et al*., 2014), and in broilers fed glucomannan extract of porang tuber (Perdinan *et al*., 2019), without any additional probiotic. The same result was also reported previously (Suthama *et al*., 2018) that the increased LAB population was found in soybean meal extract-fed broiler.

Probiotic, however, had been reported and well documented to be powerful and beneficial bacteria in supporting poultry production in the tropical country, such as Indonesia. For example, Cholis *et al*. (2018) reported that *Lactobacillus* sp., when added alone to the low protein diet without combination with prebiotic dramatically increased LAB and reduced coliform population and improved body weight. It can be postulated that the improved bacterial balance is in concomitant with the increased short-chain fatty acids, a fermentation product due to the biological activity of beneficial effects of LAB. In addition, *Lactobacillus casei* produced anti-microbial substances that can suppressed the growth of pathogenic bacteria (Sunaryanto *et al*., 2014).

Table 2. Effect of synbiotic on intestinal bacteria, nutrient digestibility and body weight of broiler chicken

Parameter	Treatment		
	Control (T0)	LEDT (T1)	LEGT (T2)
LAB (10^9 cfu/g)	5.02^b	5.21^a	5.20^a
Coliform (10^6 cfu/g)	4.24^a	3.54^b	3.65^b
Protein Digestibility (%)	78.46^b	81.03^a	78.50^b
Crude fiber digestibility (%)	24.41^b	27.68^a	27.01^a
Body weight (g)	602.39^b	623.5^a	633.46^a

$^{a–b}$Different superscript within the same row indicate significant difference (P<0.05)

Nutrient Digestibility

Synbiotics composed of *L. casei* and dahlia tuber extract, as well as *L. casei* and garlic extract improved digestion process, based on protein and crude fiber digestibility. The increased nutrients digestibility was closely related to the improvement of intestinal microbes balance as indicated by higher LAB and lower *E. coli* populations (Table 2) which supports the healthy intestinal tract.

It was reported elsewhere that healthy intestinal tract facilitates the maximal digestive enzyme activity and bring about digestibility improvement. The present result was supported by Afriyati *et al*. (2019) that dietary addition of *Lactobacillus* sp. as a probiotic at the level of 1.2 ml in broiler chicken–increased crude fiber digestibility by 24.35% as compared to control group (24.99 vs. 20.97%). The increased digestibility of crude fiber in T1 and T2 (Table 2) provided a positive impact on the improved protein digestibility. It is well known that crude fiber act a negative effect on nutrient digestibility and availability, in this case, protein, when it could not be digested more due to its nutritional binding characteristic properties.

Dietary addition of synbiotics LEDT (T1) and LEGT (T2) were able to change the condition of the digestive tract leading to the improved digestion process. Phenomenon of the present study can be described that inulin from dahlia tuber and garlic extracts provided a substrate and function as “feed source” for the development of *L. casei* in particular, and LAB in general.

Bodyweight

Increased bodyweight of chickens in T1 and T2 treatments was thought to be due to the role of the synbiotic in improving the intestinal microbes balance that has been discussed in the previous parameter. The improved bacterial balance can be correlated with the higher intestinal healthy and thus better nutrients digestibility, especially protein, can be resulted (Table 2). The higher protein digestibility serve as the important substrate for body protein synthesis that contributes to the increased body weight. The livability of probiotic bacteria increase when combined with prebiotic, called as synbiotic, because specific substrates as its “feed/nutrition source” is available for fermentation, and in turn, animal gets more advantages from the feeding a combination of probiotics and prebiotics. It has been previously described that some species also produce hydrogen peroxide, which inhibits the growth of gram-negative bacteria.

Healthy digestive tract condition impacts on better digestion process and increased nutrients availability, especially protein, for meat protein synthesis, and lead to the increase in body weight. Synbiotic administration has been proven to be effective in increasing the growth of broilers (Abdel-Raheem *et al*., 2012). The results of the present study were supported by Awad *et al*. (2009).

Broilers fed synbiotic with bacterial concentration of 5 × 108 cfu / ml was better than those given probiotics alone. It was reported that synbiotic increased the percentage of carcass weight and body weight gain by 66.77% and 51.61 g/day, respectively, and were higher than those observed with probiotic alone namely, 59.54% and 49.28 g/day, respectively. It has been previously described that the addition of symbiotic either LEDT (T1) or LEGT (T2) indirectly increased nutrients utilization efficiency, protein in particular, indicated by protein digestibility (Table 2) due to the improved intestinal health condition.

The increase in protein availability is important factor for body tissue synthesis which is finally improve body weight. Improvement of intestinal morphology indicated by better villi growth in native chickens given *Lactobacillus* sp. combined with prebiotic inulin increased protein digestibility and provided a positive impact in promoting body weight (Purbarani *et al*. 2019).

CONCLUSION

The conclusion is that dietary addition of 2% synbiotic LEDT (T1), a combination of *Lactobacillus casei* and 2% extract of dahlia tuber, results the best performance of broiler chickens through the improved intestinal microbiota and protein digestibility.

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