Jejunal ecosystem of broiler fed glucomannan extract of porang (Ammorphopallus onchophyllus) tuber

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Abstract. The research aimed to examine the ecological changes of gastrointestinal digesta in broiler fed ration with added glucomannan exctract of porang tuber (GEPT). Forty eight birds of day old broiler were used with average body weight of 42.08±0.86 g. The basal ration was added with 0.1% prebiotic of GEPT and fed from one day old until 35 days of age. Parameters observed were populations of lactic acid bacteria (LAB) and *Coliform* in jejunum and ileum, pH of duodenum, jejunum and ileum, and production of short chain fatty acid (SCFA) in jejunum. All parameters were observed weekly starting from day 14, and the data trend were evaluated as age increased. Feeding GEPT significantly ($P<0.05$) depressed *coliform* population strating at week 3 and reached the lowest population at week 5 either in jejunum (0.44 cfu/g) or ileum (0.77 cfu/g). Population of LAB were significantly ($P<0.05$) increased and reached its peak at week 5 (225.67 cfu/g) in both jejunum and ileum (148.17 cfu/g). The duodenal, jejunal and ileal pH were not significantly ($P>0,05$) different among ages and ranging from 5.25 to 6.63. Production of jejunal SCFA, such as acetate, propionate and butyrate, were significantly different ($P<0.05$) among ages and acetate was the highest concentration especially at 3 weeks of age. The conclusion is that the addition of 0.1% GEPT for 5 weeks enhances LAB population and depresses *Coliform* counts without any change in pH profile along the small intestine. Short chain fatty acids (acetate, propionate and butyrate) is detected at the jejunal segment.

1. Introduction

Broiler chickens have a short life span, during which period they are also encouraged to have maximum growth. This would take consequencies because broiler chickens are very susceptible to diseases. Anticipation can be carried out to overcome the unfavourable condition through the provision of prebiotics in the ration as an alternative to antibiotic that have been banned, because of the residues in the meat and has a negative impact on consumers.

Prebiotic is a spesific carbohydrate compound that can stimulate the health of gastrointestinal tract but it cannot be digested by digestive enzyme of the host [1]. Gastrointetinal health would be achieved through the increase in population of dominant beneficial microbiota. Improving the balance of microbiota population in the digestive tract could be overcome through the provision of prebiotic [1, 2]. The use of prebiotics in poultry ration would help in making the digestive tract healthier, because prebiotics can be fermented by the beneficial endogenous bacteria and lead to the increase in their population, but on the other hand, decrease the number of pathogenic bacteria via the competitive

exclusion [2]. Therefore, the presence of prebiotic could suppressed the development of pathogenic bacteria resulting the healthier digestive tract and producing higher health status of poultry [2, 3].

The used of prebiotic such as oligosaccharide in broiler ration stimulated beneficial microbe population of the small intestine [4]. Similar results have been reported concerning the feeding effect of inulin as prebiotic in layer [5] and in crossbred native chicken [6]. The enhancement of beneficial digestive microbe population was due to dietary inclusion of prebiotic that can be fermented by endogenous related microbe. Prebiotics in the digestive tract play as a "food source" that would be fermented by the beneficial bacteria producing lactic acid and SCFA, therefore its presence at the digestive tract could be modified through the diet [2].

Short chain fatty acids, such as acetate, propionate and butyrate, as fermentation product has acidic properties, which is in contrast to the condition of the digestive tract, especially the small intestine. Digesta in the small intestine should be neutral for optimal digestive processes. However, low pH would be in accordance with the depressed growth of pathogenic bacteria such as Salmonella, and LAB could generally live effectively under acidic condition at pH 2.5 [8]. Another research showed that acetate was the only SCFA indicated higher concentration at the jejunum in broiler fed inulin as compared to that in control group [9].

The capacity and ability of the digestive tract in broiler chicken developes as the age increased, and the development involving microbial population growth as well. Bacteria was not found in newly hatched birds, whereas feacal *Streptococci* and *E. coli* detected in all segments of digestive tract at 3 days after hatching, while microbial stability in the small intestine was reached after 2 weeks [2]. The digestive condition would be surely changed due to the presence of prebiotics with the increasing age. Observation on the microbial fermentation activity in the presence of prebiotic was usually conducted at the distal part of digestive tract namely, caecum [9], and colon [10]. Observation on proximal part such as jejunum [9] was rarely conducted. In fact, microbial fermentation process might be occurred along the digestive tract, but it mostly appeared in caecal and colon digesta since these two segments are the last part for undigested carbohydrate passed away and could be fermented by the beneficial microbes [2].

There were several kinds of prebiotics have been studied either synthetic or extracted form derived from natural resources of plants. Glucomannan ecxtracted from porang tuber [11] could be used as prebiotic since it contain D-glucose and D- manose monomers having β-1,4 lingkage of around 8% branch chain [12]. Carbohydrate of non-digestible oligosaccharides polimers containing monomers like fructose, xylose, galactose, glucose and mannose [2] function as a potential prebiotic compound. Therefore, this study was conducted with the aim to observe the small intestinal ecosystem changes in broiler chickens given glucomannan prebiotic from porang tuber extract.

2. Materials and Methods

The study used 48 birds of one day old broilers with an average body weight of 42.08±0.86 g, and glucomannan extract as prebiotic. Glucomannan extract of porang tuber (GEPT) was prepared according to the method previously developed [11]. The rations were formulated containing 3000 kcal/kg metabolisable energy (ME) and 21% of crude protein (CP). The formula and nutritional content of the basal ration is presented in Table 1. The basal ration was than added with 0.1% GEPT, and fed *ad libitum* to the chicks from day one until 35 days of age. Drinking water were also provided *ad libitum*.

Data were recorded every week throughout the observation started from 14 days of age. Twelve birds of broiler chicken were randomly taken to be used in each observation. Digesta from each segment of the small intestine (duodenum, jejunum, and ileum) were expelled immediately and put into the tightly sealed sterile bottles, and then temporarily stored in a cooling box before directly sending to the laboratory for analysis. Populations of LAB and *Coliform* at the jejunum and ileum were measured based on the total plate count method [13]. The acidity of duodenal, jejunal, and ileal digesta was determined with pH meter (EC-PHTEST30, TQC Sheen B.V.-HQ, Netherlands) by emerging the glass electrode probe to the digesta and read the result from the panel. The pH data of

each digesta sample was an average value of three times measurement. The SCFA (acetate, propionate and butyrate) concentration was measured in the jejunal digesta using the slightly modified method as previously described [11]. The data trend was evaluated as the age increased.

Table 1. Composition and nutritional content of basal ration.

Feedstuff	Composition (%)
Yellow corn	54.00
Rice bran	14.20
Soy bean meal	18.00
Meat bone meal	5.75
Poultry meat meal	6.75
Dicalcium phosphate	0.50
L-Lysine	0.10
DL-Methionine	0.20
Calcium Carbonate	0.25
Premix	0.25
Total	100.00
Nutritional content[1](%)	
Metabolisable energy (Kkal/kg)[2]	2965.69
Crude protein	21.33
Ether extract	4.68
Crude fiber	4.45
Methionine[2]	0.55
Lysine[2]	1.16
Ca	1.03
P	0.71

[1]Proximate analisis value at the Laboratory of Nutrition and Feed Science, Faculty of Animal and Agricultural Sciences, Diponegoro University; [2]Calculated value based on the table of nutrient content of feestuff [14].

3. Results and Discussion

3.1. Bacterial population changes

Figure 1 showed that the first 2 weeks of feeding GEPT could not stimulated the development of LAB in the jejunum or ileum considering the *Coliform* population showed the highest number during the second week of age. This was in accordance with the previous reports that feeding with the increasing levels of inulin prebiotic (0.2-0.6%) for the first 2 weeks had no effect on the populations of colon's LAB and *Coliform* [10], because microbial community at the small intestine established wihtin 2 weeks of age [2]. Early feeding prebiotic would be beneficial for the small intestine in relation to its readyness in stimulating the population development of LAB. Jejunal and ileal digesta in this study was still a favorable medium for the growth of *Coliform* rather than LAB even though it has been given GEPT prebiotic for 2 weeks. It presumably need another 3 weeks for LAB development to reach the highest population while depressed *Coliform* counts to the lowest number. This was an indication that it needed 5 weeks for the beneficial role of feeding ration with added prebiotic at the level of 0.1% GEPT.

The number of LAB increased along with the increased age both in the jejunum (R^2= 0.88) and ileum (R^2=0.92), whereas the number of *Coliform* in these two intestinal segments decreased

significantly (R^2 = 0.92 for both jejunum and ileum, Figure 1). *Coliform* population sharply declined during week 3 while LAB number drastically increased after week 4. The present results indicated that 0.1% GEPT could be used by LAB as a substrate for fermentation activity, on the other hand, it depressed *Coliform* development which was started at 3 weeks of age. Microbiota of the small intestine could be controlled and modified through the diet, especially continously feeding diet.

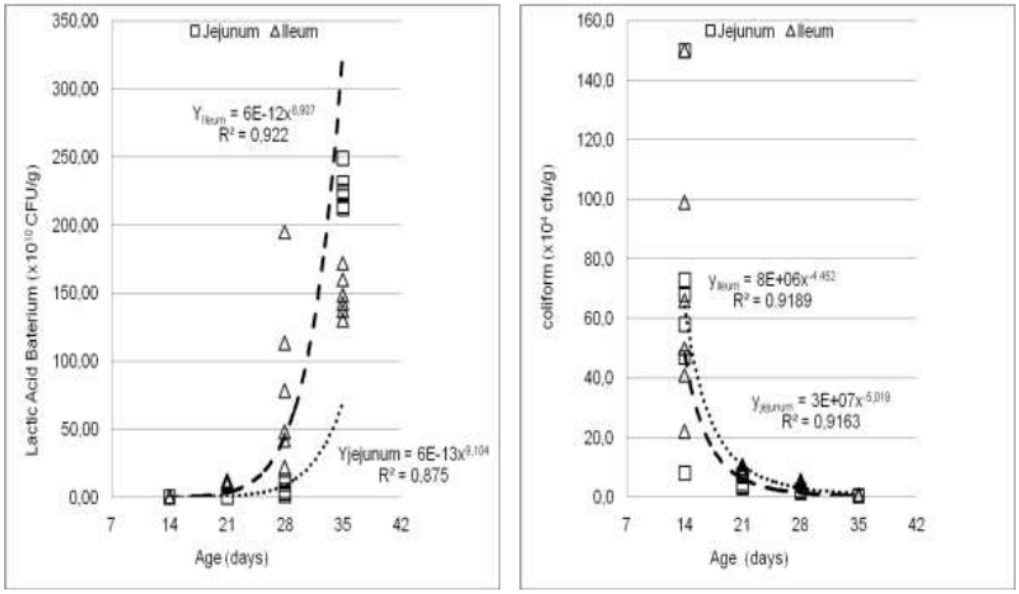

Figure 1. Average population of lactic acid bacteria (left) and *Coliform* (right) in the jejunal and ileal digesta in different age of broilers fed ration with added 0.1% glucomannan extracted from porang tuber.

containing prebiotic [2]. Therefore, feeding GEPT could modified and maintained beneficial bacteria population in the small intestine, especially at the jejunum and ileum.

3.2. Small intestine acidity

Jejunal digesta had the lowest pH, while ileal digesta had the highest pH at all ages measured (Figure 2). There was a slightly linear decreased in digesta pH at duodenum, jejunum and ileum with the range of 5.25- 6.63 (Figure 2). The pH value of duodenum, jejunum and ileum at 14 up to 42 days of ages in broiler chicken fed ration without prebiotic ranged from 5.94-7.14 [7] which value was higher as compared to the present results. Therefore, feeding ration with added 0.1% of GEPT for 5 weeks tended to slightly reduce the pH of those 3 small intestinal segments and the lowest linear decreased was found in the jejunal digesta.

The lowest pH of jejunal digesta during week 5 could be attributed to the highest LAB population (Figure 1) due to the positive feed back of stimulating effect of fermentation activity of LAB with 0.1% GEPT addition. Fermentation activity increased with the increased LAB population that could produced lactic acid [2] and influenced the digesta pH. Continuously feeding ration with prebiotic triggers the development of LAB which slightly provided an impact on pH reduction (Figure 2). The declined pH value might be also supported by SCFA production (Figure 3).

3.3. *Short chain fatty acid production*

Concentration of acetate in the jejunal digesta showed the highest value as compared to that of propionate and butyrate at all ages measured (Figure 3). Concentration of acetate was linearly declined but the slight linierly increased was found for that of propionate and butyrate (Figure 3). The result indicated that addition of 0.1% GEPT prebiotic in broiler ration for 5 weeks showed the jejunal microbial fermentation activity by producing SCFA components such as acetate, propionate and butyrate. The jejunal SCFA production in the present study had similar trend with that in 21 and 42 days of broilers fed corn-soy diet [15]. It had similar trend also with the digesta of cecum and colon in broiler fed inulin [10] and with that fed xylo-oligosaccharide [3]. On the other hand, the propionate and butyrate could not be detected in the jejunal digesta of broiler chicken fed ration without prebiotic which was determined weekly from day 28 to 42 days of age [7]. However, feeding ration to the broiler chicken with additional synthetic inulin after 42 days was not only jejunal acetate can be detected, but it was also found in the caecal digesta [9].

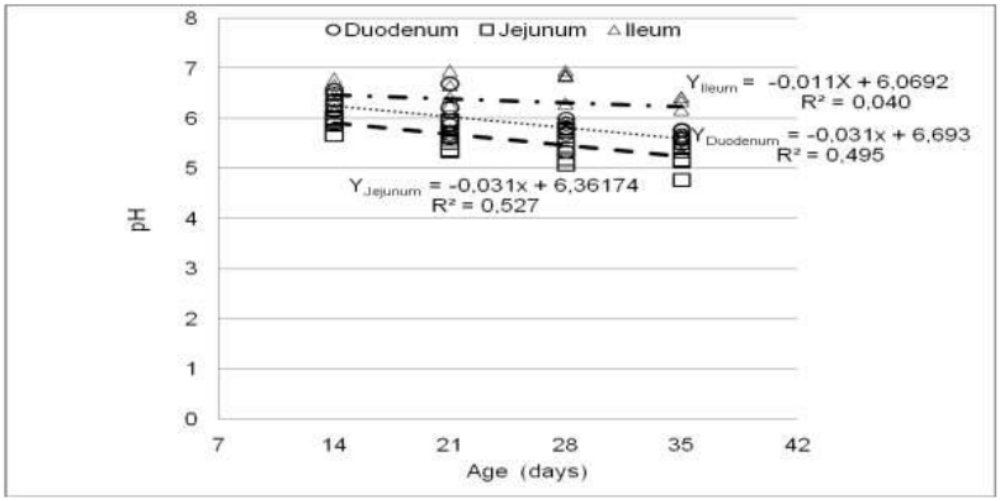

Figure 2. Average pH of duodenal, jejunal and ileal digesta- in different age of broilers fed ration with added 0.1% glucomannan extracted from porang tuber.

Those above described conditions indicated that feeding prebiotic regardless its origin, stimulated the endogenous microbial fermentation resulting in the production of SCFA that could be detected at the jejunal digesta, but the concentration seems to depend on the duration of feeding and the kind of prebiotic used. Feeding broiler with 0.1% GEPT for 35 days resulting in better condition of the small intestine as it was indicated by the jejunal segment condition with regard to SCFA (acetate, propionate and butyrate) production. The result of this study was supported by the finding previously reported of broiler chicken feeding without prebiotic [7] which shown that acetate was found in the ileum and cecum from 14 to 42 days of age. Low level of acetate was also detected in the jejunum and the concentration increased with age, but propionate was found in different segments of the gastrointestinal tract only at 14 days of age. It is clearly known that SCFA was the major end-product of microbial fermentation of the prebiotic or non-digested oligosacharide either in the cecum [2,9] or ileum [2]. Lactate, acetate, propionate and butyrate were found at a considerable amount in the caecal digesta of broiler chicken fed ration without prebiotic [7]. However, caecal propionate and butyrate were higher in 21 and 42 days of broiler fed xylo-oligosacharide [3], but in case of the present study, acetate, propionate and butyrate could be found at the jejunal segment. The condition could be due to the fermentation activity at the jejunal segment or there might be a reflux of SCFA from the distal part of the digestive tract. However, these fenomenon of conditions still need to clarify in the next

research, since microbial profile of the caecum reflected the efficiency of feed digestion and absorption in the proximal digestive tract [16].

The very low concentration of jejunal propionate and butyrate might be related to the used of these two component as an energy substrates for intestinal epithelial cells. There were important finding supported this result that butyrate could be used as the preferred energy source for the enterocytes which is known to regulate cellular differentiation and proliferation of the intestine [16]. These conditons would be beneficial for the increase in the intestinal health status related to the absorptive surface area of the digestive tract that lead to the better nutrient digestibility. This phenomenon could be connected with the cumulative body weight gain (CBWG) of the broilers observed in this experiment. The highest CBWG (99.52 g/bird/day) was obtained at 4 weeks of age, and it can be compared to other stages of age namely, week 2 (22.93 g/bird/day), week 3 (31.35 g/bird/day), and week 5 (45.90 g/bird/day).

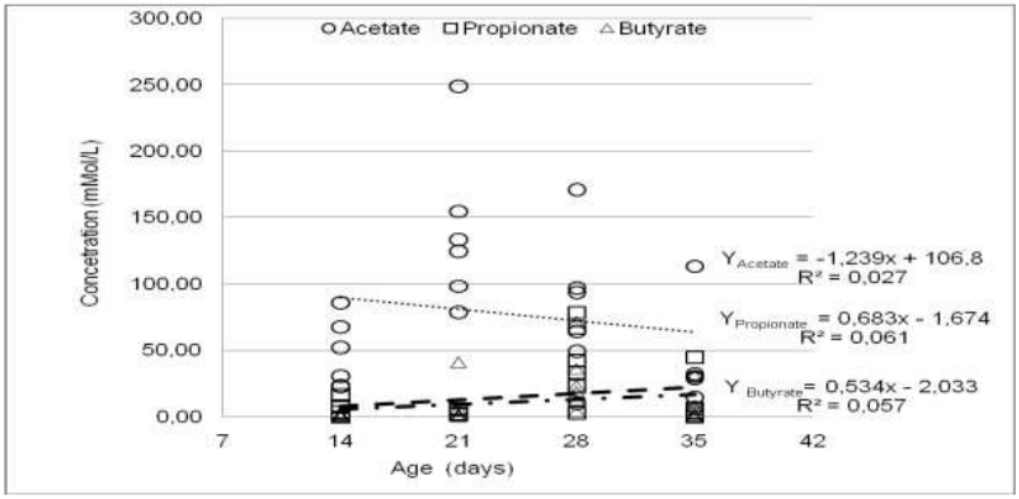

Figure 3. Average short chain fatty acid production of jejunal digesta in different age of broilers fed ration with added 0.1% glucomannan extracted from porang tuber.

4. Conclusion

It can be concluded that addition of 0.1% GEPT for 5 weeks strongly enhances LAB population and depresses *Coliform* counts without changing the pH profile along the small intestine of broiler. Short chain fatty acids (acetate, propionate and butyrate) is detected at the jejunal segment.

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