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Effect of Fermented Lime Waste Flour on the pH of the Small Intestine and Microbes of Broiler Chicken Ileum

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ABSTRACT

The balancing of digestive tract microbe can improve the digestive health of broiler chickens. Fermented lime waste flour (FLWF) contains citric acid, which can decrease digestive tract pH value to suppress pathogenic bacteria development and improve lactic acid bacteria growth in the small intestine of the broiler. The purpose of the study was to evaluate the effect of using FLWF on pH value and small intestine microbial of broiler chickens. This study used 200 female broiler chickens. The research used a completely randomized design with four treatment levels of FLWF by 0%, 1%, 2%, and 3% in every ration, with each treatment replicated five times. The parameters observed are the pH value of the small intestine, lactic acid bacteria ileum, and *Coliform* ileum. Data were calculated using the analysis of variance and difference test with Duncan's Multiple Range Test using the SPSS 19.0 program. The result indicates that using FLWF decreased ($p<0.05$) *Coliform* in the ileum, while pH value of small intestine and ileum lactic acid bacteria among treatments were not influenced ($p>0.05$). It concludes that adding FLWF at a 1% level could decrease ileum *Coliform.* Still, it could not decrease the pH value of the small intestine and increase the total lactic bacteria ileum.

Key words*:* citric acid, broiler, ileum microbial, small intestine's pH value, and fermented lime waste powder

INTRODUCTION

Today, the utilization of lime (*Citrus aurantifolia*) waste from the beverage industry and MSMEs (Micro, Small, and Medium Enterprises) is not optimal. The content of organic acids in the form of citric acid in lime peel has the potential as an acidifier for poultry feed. The high pectin fiber content hampers lime waste as a poultry feed additive, so the benefits of citric acid in lime waste cannot be optimal. Kermanshahi et al. (2018) reported that the provision of 3% pectin fiber in the diet can reduce the health of the digestive tract and disrupt the balance of the ileal microflora. One way to degrade pectin fiber is to use the enzyme pectinase. The fungus Aspergillus niger is known to produce pectinase enzymes that can help degrade pectin fibers (Ahmed et al., 2016).

Fermentation treatment with the help of *Aspergillus niger* is expected to help break down pectin fibers and optimize the use of bioactive substances in the form of citric acid in lime waste. An organic acid in the form of citric acid can help decrease the pH of the digestive tract of broiler chickens. The development of pathogenic bacteria will be inhibited, while the development of lactic acid bacteria will increase (Jamilah et al., 2013; Jamilah 2016). The purpose of this study was to examine the effect of using lime (*Citrus aurantifolia*) waste flour fermented with *Aspergillus niger* on the pH value and microbial ileum of broiler chickens. The benefit of this research is to provide scientific information about the effectiveness of using lime waste flour (*Citrus aurantifolia*) fermented with *Aspergillus niger* on the pH value of the small intestine and ileal microbes of broiler chickens. This research hypothesizes that fermented lime (*Citrus aurantifolia*) waste flour can reduce the pH of the small intestine and ileal coliforms and increase the total lactic acid bacteria in the ileum of broiler chickens.

MATERIALS AND METHODS

The research was conducted in September-December 2020 at the Poultry Livestock Production Laboratory, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang. The material in this study was 200 days old female chick strain Lohman MB 202, which was reared for 35 days. The treatment was given to broiler chickens at 14 days with an average body weight of 379.73 ± 18.72 g, CV 4.93%. The use of fermented lime waste flour in the ration was 0%, 1%, 2%, and 3%. The experimental design used was completely randomized.

Preparation of Fermented Lime Waste

Making fermented lime waste is that lime waste is collected, cleaned, and washed in running water and then drained. Lime waste is sliced thinly, air-dried for two days, and ground into flour. The lime waste flour was then sterilized using an autoclave at 121^0C for 15 minutes, then cooled at room temperature. Lime waste flour was fermented using *Aspergillus niger* as 3%, then added with distilled water until the water content reached 65%. Lime waste flour was fermented aerobically at room temperature for five days. The fermentation results are air-dried for approximately 24 hours before being given to the chickens.

The citric acid content was analyzed using the HPLC method (Baziramakenga et al., 1995). The analysis results showed that the citric acid of fermented lime waste was 0.94 mg/kg higher than that of unfermented lime waste was 0.10 mg/kg. The extraction method also measured Pectin levels (Lazim et al., 2013). The pectin content of fermented lime waste was 2.73% wb, lower than that of non-fermented lime waste 7.80% wb.

Ration Formulation

The following table presents the ration formulations (Tables 1 and 2) used during the study.

Data Collection

The carcass process was carried out on day 36 by taking one broiler in each experimental unit. The next stage is to slaughter the chicken in the jugular vein and clean the feathers on the abdomen. The following process is dividing the abdomen and taking the small intestine. The ileal digest was collected and inserted into the tube for the bacterial count. The remainder of the digest in each segment, both the duodenum, jejunum, and the duodenum, jejunum, and ileum, were measured with a pH meter (Emma et al., 2013; Mabelebele et al., 2014).

Total Lactic Acid Bacteria (LAB) and Coliform were analyzed by making MRS Agar medium as a lactic acid bacteria culture medium with a dose of 68.2 g MRS Agar per 1 liter of distilled water and MacConkey's medium for coliform bacteria culture medium at a dose of 50 g MacConkey per 1 liter aqua dest. The next step is to sterilize the culture medium into an autoclave, pour it into a petri dish as much as ± 15 ml, and relax—calculation of total lactic acid bacteria and Coliform using the retail method. Sample dilutions were started from 10-1 to 10-10. A sampling of lactic acid bacteria culture at the dilution level of 10-5 and 10-6, while coliform bacteria at the dilution level of 10-3 and 10-4.

Table 1. Formulation and nutrient composition of broiler chicken feed aged 15-21 days

Feed ingredients	FLWF (%)			
	0	1	2	3
Yellow Corn	56.40	56.40	55.40	54.40
Soybean Meal	29.80	29.80	29.80	29.80
Fish flour	7.70	7.70	7.70	7.70
bran	2.50	2.50	2.50	2.50
Fermented Lime Waste Flour (FLWF)	0.00	1.00	2.00	3.00
Soybean Oil	2.00	1.00	1.00	1.00
Premix	0.50	0.50	0.50	0.50
$CaCO_3$	0.50	0.50	0.50	0.50
D –L Methionine	0.40	0.40	0.40	0.40
L – Lysine	0.20	0.20	0.20	0.20
Nutrient content				
Crude Protein (%)*	21.01	21.13	21.18	21.23
Crude Fiber (%)*	4.49	4.71	4.90	5.09
Ether Extract (%)*	3.21	3.23	3.22	3.21
Ash (%)*	6.06	6.16	6.64	6.33
Metabolic Energy (Kcal/Kg)**	3037.87	3012.20	3023.02	3032.83

* Based on the Analysis at the Laboratory of Nutrition and Feed Science Calculation Result, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang.

**ME (metabolizable energy) was predicted based on Bolton formulation as follow: 40.81 {0.87 [CP + 2.25 crude fat + nitrogen-free extract] + 2.5} (Sugiharto et al., 2017).

FLWF: Fermented Lime Waste Flour.

Table 2. Formulation and nutrient composition of broiler chicken feed 22-35 days old

Ingredients	FLWF (%)			
	0	1	2	3
Yellow corn	59.40	58.40	57.90	57.20
Soybean Meal	28.50	28.20	28.00	28.00
Fish flour	6.40	6.60	6.60	6.50
bran	1.70	1.90	1.90	1.90
Fermented Lime Waste Flour (FLWF)	0.00	1.00	2.00	3.00
Soybean Oil	3.00	2.90	2.60	2.40
Premix	0.20	0.20	0.20	0.20
$CaCO_3$	0.20	0.20	0.20	0.20
D –L Methionine	0.40	0.40	0.40	0.40
L – Lysine	0.20	0.20	0.20	0.20
Nutrient content				
Crude Protein*	20.04	20.05	20.05	20.08
Crude Fiber (%)*	4.29	4.50	4.70	4.89
Ether Extract*	3.02	3.05	3.05	3.03
Ash (%)*	5.04	5.17	5.24	5.31
Metabolic Energy (Kcal/Kg)**	3102.30	3108.28	3108.26	3111.76

*Based on the Analysis at the Laboratory of Nutrition and Feed Science Calculation Result, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang.

**ME (metabolizable energy) was predicted based on Bolton formulation as follow: 40.81 {0.87 [CP + 2.25 crude fat + nitrogen-free extract] + 2.5} (Sugiharto et al., 2017).

FLWF: Fermented Lime Waste Flour.

The next step is to take 1 ml of each dilution using a volume pipette and then pour it into a petri dish filled with medium. The next stage is the incubation process of the medium filled with samples in an incubator with a temperature of 38ºC for 48 hours for lactic acid bacteria and 24 hours for coliform bacteria. Calculation of growing bacteria using a colony counter (Halimatunnisroh et al., 2017; Mareta et al., 2020).

Calculation of total lactic acid bacteria using the total plate count (TPC) method, then compiling the calculation results based on the standard plate count (SPC) method. Calculation of the total lactic acid bacteria and coliform using the formula (Fardiaz, 1993):

$$\text{Total Lactic Acid Bacteria} = \text{Number of colony} \times \frac{1}{\text{dilution factor}}$$

$$\textit{Coliform} = \text{Number of colony} \times \frac{1}{\text{dilution factor}}$$

Statistical Analysis

The data were analyzed for variance using the SPSS 19.0 program. The data presented is also calculated standard error of the mean (SEM). A significant effect between treatments is continued with Duncan's difference test (Steel and Torrie, 1989).

RESULT AND DISCUSSION

The following shows the research results on the value of duodenal pH, jejunal pH, ileal pH, total ileal lactic acid bacteria, and ileal Coliform due to the use of feed additives in the form of fermented lime waste flour (*Citrus aurantifolia*) in broiler chicken feed.

Small Intestine pH Value

Data on the pH value of the small intestine of broiler chickens fed with fermented lime waste flour (FLWF) are presented in Table 3 below.

Table 3. The pH value of the small intestine of broiler chickens fed FLWF

Parameter	FLWF (%)				SEM	P
	0	1	2	3		
Duodenum	6.06	6.18	5.90	6.10	0.04	0.12
Jejunum	6.28	6.64	6.38	6.54	0.10	0.62
Ileum	6.46	6.58	6.43	6.44	0.07	0.90

FLWF: Fermented Lime Waste Flour; SEM: Standard Error of Mean

The results of statistical analysis showed that the use of fermented lime waste did not affect (P > 0.05) the pH value of each segment of the small intestine (Table 3). In this study, the pH value of the small intestine was still in the normal range. Another role of citric acid is to maintain the pH value of the digestive tract remains in normal conditions. The pH value of the duodenum is in the range of 5.88-6.23, the pH value of the jejunum is in the range of 5-6, and the pH value of the ileum is in the range of pH 6-7 (Jamilah et al., 2014; Hamid et al., 2018).

The crude fiber content also influences the decrease in pH in the digestive tract in the feed. Mabelebele et al. (2014) stated that there are crops and gizzards in the front of the digestive tract. The higher fiber content of feed will undergo a fermentation process and cause more acidic conditions in the crop, thus affecting the pH of the next digestive organ. The fermentation process can result in several organic acids such as lactic, acetic, propionic, butyric, and total SCFA (Short Chain Fatty Acid) (Gomez et al., 2019). In this study, the crude fiber content between treatments (Tables 1 and 2) was relatively the same, so that the pH of the small intestine was not significantly different (P>0.05).

Total Ileum Lactic Acid and Coliform Ileum Bacteria

Data on total lactic acid bacteria and ileal coliforms of broiler chickens fed with fermented lime waste flour (FLWF) are presented in Table 4 below.

Table 4. Total lactic acid bacteria and ileal coliforms of broiler chickens given FLWF

Parameter	FLWF (%)				SEM	P
	0	1	2	3		
Total Ileum Lactic Acid Bacteria (log CFU/g)	10.69	10.38	10.44	10.88	0.11	0.39
Coliform of Ileum (log CFU/g)	8.52[a]	5.90[b]	6.28[b]	6.23[b]	0.35	0.02

Notes: a, b Different superscripts in the same line show significant differences (p<0.05).
FLWF: Fermented Lime Waste FlourSEM: Standard Error of Mean

The results of statistical analysis showed that the addition of fermented lime waste did not affect the total lactic acid bacteria (P>0.05) (Table 4) but decreased the Coliform population (P<0.05) (Table 4). Non-pathogenic bacteria such as lactic acid bacteria are more resistant to organic acids than pathogenic bacteria like Coliform. The citric acid in lime waste reduces Coliform bacteria by disrupting the balance of bacterial cells until lysis occurs. Based on the opinion of Markazi et al. (2019) stated that the cell membrane of pathogenic bacteria can be disrupted in the presence of organic acids by lowering the pH of the cytoplasm. An imbalance of protons and anions occurs. Pathogenic bacteria will respond by releasing protons so that bacterial cells lose energy and cause cell death. The decrease in ileal coliforms can also be caused by fermented feeding. Widodo et al. (2015) stated that efforts to improve the condition of the intestinal microflora could be made by giving a fermented feed.

Fascina et al. (2017) stated that the administration of organic acids could reduce pathogenic bacteria in the digestive tract of broiler chickens. The decrease in gastrointestinal pathogenic bacteria can increase the effectiveness of using nutrients for growth and body resistance. The decrease in Coliform can also be caused by pectic oligosaccharides resulting from the breakdown of pectin fibers from lime waste flour. Pectic oligosaccharides have the potential as prebiotics that provide nutrients for beneficial bacteria and can suppress the development of pathogenic bacteria in the digestive tract (Babbar et al., 2017). The decrease in pathogenic bacteria was due to pectic oligosaccharides acting as antimicrobials (Li et al., 2016). The antimicrobial mechanism in inhibiting the development of pathogenic bacteria is by interfering with bacterial cell wall synthesis, protein, and nucleic acid synthesis and inhibiting bacterial metabolism (Reygaert 2018).

CONCLUSION

The use of fermented lime waste flour at a 1% level can reduce ileal Coliform but has not been able to reduce the pH of the small intestine and increase the total lactic acid bacteria.

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