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MICROBIAL COMPOSITION OF HIND GUT, DIGESTABILITY AND GROWTH RATE OF LOCAL RABBIT WITH FEED FERMENTATED BANANA PEELS (*ACUMINATA BALBISIANA*) SUPPLEMENTATION

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Abstract

The research was conducted to determine the bacterial composition of the hindgut, feed digestibility and growth rate of local rabbits aged 5 to 17 weeks. 96 rabbits were used in the experiment using a randomized block design (CRBD) with 4 treatments and 8 blocks, each experimental unit containing 3 rabbits. Blocks were carried out based on differences in the initial weight of the experiment. The treatments in the experiment were: feed without using fermented banana peels (R0), 5% fermented banana peels (R1), 10% fermented banana peels (R2) and 15% fermented banana peels (R3). The results showed that lactic acid bacteria and total bacteria in rabbit hindgut R3, R2 and R1 were higher ($P < 0.05$) than R0. All treatments had no significant effect ($P > 0.05$) on total Coliform and *E. Colli*. The dry matter digestibility and growth rate of local rabbits receive feed treatment R3, R2 and R1 were higher ($P<0.05$) than R0. It was concluded that feed containing fermented banana peels produced better bacterial composition on hindgut, feed digestibility and rabbit growth rate than without fermented banana peels.

Key words: fermented banana peel, hindgut bacteria, growth rate, local rabbits.

Introduction

The success of raising rabbits is being able to provide a feed that meets the standard nutrient needs, low prices because feed costs can reach 80% of the total production cost. Feed ingredients that have sufficient nutrients at a relatively cheap price and are abundantly available can be obtained from agricultural waste. Banana peel (Musa cv) is a potential agro-industrial waste as a feed ingredient for rabbits (Imam and Akter, 2011). Banana peels contain antibiotics that can suppress the development of mycobacteria (Sivestre, 2016). According to Yin *et al*., (2008), the bioactive content of dopamine and ascorbic acid in banana peels functions to reduce stress caused by environmental factors. Mokbel and Hashinaga, (2005) stated that the antioxidant activity of β-carotene can reduce free radicals at animals by 31.70% and some mineral content in banana peels such as potassium 78.10%, calcium 19.20%, iron 24.30% and manganese 24.30%. Okareh *et al.*, (2015) statedcalcium and manganese minerals can help the process of bone growth and calcification and the preparation of the body's skeleton. Banana peels can be used as animal feed ingredients, especially in the process of bone growth as body support, which requires high amounts of calcium in this process.

Bio fermentation using probiotics as a fermenter is an interesting application because of the growing concern about bacterial resistance to antibiotics, as well as antibiotic residues in livestock products (Tang *et al*., 2018). Preliminary research found that unfermented banana peel supplementation at a level of 9% can improve feed digestibility and final weight of local rabbits (Nuriyasa *et al*., 2018).

Rabbit feed containing fermented grape wine waste with *Aspergilus niger* can increase the use-value of grape wine waste and can improve the performance of local rabbits (Mahardika-Atmaja *et al*., (2016). Coffee pulp bio-fermentation with *Aspergilus niger* can reduce the crude fiber content of coffeepulp and its use in feed can reduce feed conversion (Nuriyasa *et al*., 2015).

Aspergillus niger as a probiotic has been shown to improve nutrients of agro-industrial waste, increase feed efficiency and increase animal performance. This study aims to determine the microbial composition of the hindgut, to evaluate the effect of use fermented banana peels as a rabbit feedon the microbial composition of the hindgut, feed digestibility and growth rate of local rabbits.

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Materials and Methods

Animals and Experimental Designs

Ninety-six local male rabbits aged five weeks were used in the randomized block design (CRBD) experiment with 4 treatments and 8 blocks, each experimental unit containing 3 rabbits. Blocking was done based on differences in body weight at the start of the study. Microbial composition variables include: total bacteria (CFU / g), E. Colly (CFU / g) and Collyform (CFU / g) lactic acid bacteria (CFU / g). Feed digestibility includes dry matter digestibility, digestibility of energy and protein digestibility. Growth variables included: final body weight, weight gain, feed consumption, feed conversion ratio.

Feed Treatment

All treated feeds were atthe same level of energy and the same level of protein. The feed was formulated according to the nutritional requirements of rabbits (Mc.Nitt, 1996) for 12 weeks of the experiment. The feed ingredients and chemical composition of the feed are shown in table 1 and table 2.

Composition of Bacteria on Hind Gut

The hind gut fluid is taken from the cecum of the rabbit, placed in a plastic clip, then taken to the laboratory for testing. Sterilize the appliance using an oven at 170°C for 1 hour, while sterilizing PCA (*Plate Count Agar*) and EMBA (*Eosin Methyl Blue Agar*). and test tube using an autoclave for 15 minutes at a temperature of 121°C. The sample dilution is carried out by placing 1 g gram of digests liquid into a test tube containing 9 ml of distilled water, shaking it so that it is homogeneous, the dilution is continued until the dilution reaches 10^8. To determine the total Escherichia coli and Coliform total plate count (TPC) and lactic acid bacteria using the Pour Plate Method. Samples were pipette 1 ml from each dilution 10^1, 10^2, 10^3, 10^4 to 10^8 and put into sterile Petri dishes. Added 12 ml to 15 ml of medusa sterile EMBA for testing Escherichia coli, coliform and lactic acid bacteria. The total plate count (TPC) used PCA media, which had been cooled in a water bath to reach a temperature of 45°C ± 1°C, into each Petri which already contained 1 ml of sample. For the sample and media to be completely mixed, the plates were rotated following the number 8 rotation. After the media became solid, then it was incubated in the best position in the incubator for 24 hours at 37°C ± 1°C. The total colony per gram sample can be calculated following the formulation Fardiaz, (1989):

$$(\text{TPC}) = \frac{\text{number of colonies per plate}}{\text{dilution factor}} \times 1$$

Table 1: Composition of research feed ingredients.

Feed ingredients	Percentage of feed Ingredients			
	R0	R1	R2	R3
Yellow corn	37.2	39.4	39.0	39.6
Coconut mill	9.0	10.0	10.4	6.0
Fish mill	11.5	8.6	9.0	8.0
Tapioca mill	11.4	9.7	11.4	9.4
Soybean mill	11.0	13.3	13.0	14.0
Rice ban	11.02	8.0	4.8	11.8
Elephant grass	7.3	7.2	6.1	2.6
Salt	0.25	0.3	0.3	0.3
Mineral mix	0.5	0.5	0.5	0.3
Bone mill	0.65	0.5	0.5	0.5
Fermented banana peel	0	2.5	5.0	7.5
Total (%)	100	100	100	100

The number of lactic acid bacteria (BAL) was calculated using the same method as calculating the TPC but the calculation of lactic acid bacteria using MRSA media. After 24 hours, LAB colonies growing on the Petri dish can be seen using a Quebec colony counter. The LAB colony calculation is calculated by the following formula:

$$(\text{BAL}) = \frac{\text{number of colonies per plate}}{\text{dilution factor}} \times 1 \frac{\text{number of colonies per plate}}{\text{dilution factor}} \times 1$$

The feed digestibility was calculated following the total collection method. Feces were collected for 7 days, dried under the light to dry weight then oven at 60°C for 24 hours. Proximate analysis was performed on feed and feces. Digestibility is calculated by the formulation:

$$(\text{Digestibility}) = \frac{\text{nutrient consumption - faecal nutrient}}{\text{nutrient consumption}} \times 100\%$$

Data analysis

All data obtained from this study will be analyzed by variance. If there are significantly different results ($P < 0.05$), it will be followed by Duncan's multiple range test at the 5% level (Steel and Torrie, 1991).

Results and Discussion

Total bacteria in the hind gut of rabbit that fed rations R3 was the highest, namely 7.8×10^3 CFU / g, while total bacteria in the hind gut of rabbit fed R2, R1 and R0 were 8.23%, 13.95%, lower ($P>0.05$) than those of rabbit fed R3, respectively. The group fed R0 was 44.85% lower ($P < 0.05$) than R3 (Table 3).

The group fed R3 caused a total coliform of 6.8×10^3 CFU / g, while total coliform of rabbits fed R2, R1 and R0 were 4.41%, 8.82% and 11.76% lower ($P > 0.05$) than R3 respectively. The group fed R3 feed produced a total of *E. Colli* 1.8×10^3 CFU / g, while the treatment of R2, R1 and R0 was 5.56%, 16.67% and 33.33% lower

Table 2: Proximate analysis and nutrient content of the feed.

Feed treatment	Nutrient					
	Metabolizable Energy (Kkal/kg)	Crude Protein (%)	Ca (%)	Pav (%)	Fat (%)	Crude fiber (%)
R0	2606.54	16.018	0.66	0.47	6.35	6.72
R1	2605.92	16.08	0.58	0.41	6.47	7.02
R2	2607.18	16.08	0.61	0.42	6.38	6.81
R3	2615.47	16.04	0.54	0.38	7.62	6.31
Mc Nitt *et al*. Standards, (1996)	2600.00	16.0	0.3	0.2	5.0	10.0

Note: (R0): ration without fermented banana peels; (R1): 5% fermented banana peels; (R2): 10% fermented banana peels; (R3): 15% fermented banana peels.

(P>0.05) than R3. Lactic acid bacteria in hindgut of rabbits fed R3 was the highest (5.8 × 10^3 CFU / g), while the treatment of R2 and R1 was 6.89% and 29.31% higher (P>0.05), respectively. Rabbits treated with rations using 15% fermented banana feel (R3) caused lactic acid bacteria 37.93% highest (P<0.0) than rabbit fed R0. *Aspergillus niger* as a feed fermenter has the function of suppressing the development of Coliform and *E. Colli* bacteria so that it is not significantly different between treatments, although the growth rate of rabbits fed with fermented banana peels is higher (P<0.05) than control (Table 3), according to the opinion of Mountzouris*et et al*., (2010). *E. Colli* bacteria must be maintained within normal limits because of their role in degrading food in the digestive tract. The hindgut of rabbits, namely the cecum and colon are the places for the growth of bacteria that function as proteolysis. These bacteria attack the undigested protein into skatole, indole, phenol, fatty acids, hydrogen sulfide and amino acids. Hydrolyzes cellulose into glucose units, then converted into volatile fatty acids, especially acetate, propionate and butyrate (Kompiang, 2009). Existence *Aspergillus niger* in animal feed ingredients functions as a probiotic which can increase the number of lactic acid bacteria which will affect the digestive process of rabbits and as an antimicrobial (Yirmaga, 2013). Ohimain *et al.,* (2012) found that feed fermentation with *Aspergillus niger* was able to increase the number of lactic acid bacteria in the digestive tract of animals. Lactic acid bacteria are high, causing a low hydrogen potential (pH) in the digestive tract so that it can control the development of bacteria or a coliform and *E. Colli* (McNaught and MacFie, 2000), as shown in table 3.

Table 3: The effect of using fermented banana peels on the composition of hindgut microorganisms, digestibility and growth speed of local rabbits.

Variable	Treatment				
	R0	R1	R2	R3	SEM3
Composition of Hind Gut Bacteria					
TPC (CFU/g)	2.41×10^{5b}	3.76×10^{5a}	4.01×10^{5a}	4.37×10^{5a}	1.14
Total Coliform (CFU/g)	6.0×10^{3a}	6.2×10^{3a}	6.5×10^{3a}	6.8×10^{3a}	1.95
Total *E. Colli*. (CFU/g)	1.2×10^{3a}	1.5×10^{3a}	1.7×10^{3a}	1.8×10^{3a}	0.11
Lactic Acid Bacteria (CFU/g)	3.6×10^{3b}	4.5×10^{3a}	5.4×10^{3a}	5.8×10^{3a}	1.03
Feed Digestibility					
Dry Matter Digestibility (%)	66.12^{b}	67.05^{a}	67.49^{a}	67.84^{a}	0.42
Digestible energy (%)	73.45^{a}	73.86^{a}	73.93^{a}	74.28^{a}	2.95
Protein Digestibility (%)	78.75^{a}	79.13^{a}	79.45^{a}	80.09^{a}	1.07
Growth rate					
Initial weight (g)	385.26^{a}	383.98^{a}	390.02^{a}	386.78^{a}	11.07
Final body weight (g)	1916.78^{b}	2096.46^{a}	2122.76^{a}	2176.38^{a}	18.32
Weight grain (g/day)	18.23^{b}	20.37^{a}	20.63^{a}	21.30^{a}	1.97
Feed consumption (g/day)	80.58^{a}	80.94^{a}	81.06^{a}	82.45^{a}	4.08
Feed conversion	4.42^{a}	3.97^{b}	3.92^{b}	3.87^{b}	0.96

R0: Feed without using fermented banana peels; R1: Feed using 5% fermented banana peels; R2: Feed using 10% fermented banana peels; R3: Feed using 15% fermented banana peels; The same superscript on the same line shows no significant difference (P> 0.05)and different superscripts on the same line indicate significant differences (P <0.05); SEM:*Standard Error of the Treatment Means*

Rabbits treated with rations without fermented banana feel (R0) caused the lowest digestibility of dry matter namely 66.12%. The dry matter digestibility in rabbit fed rations R1, R2 and R3 was 1.41%, 2.07% and 2.60% higher (P<0.05) respectively than that of rabbit fed ration R0. The metabolic energy in rabbits treated ration R3 was 74.28%, while R2, R1 and R0 were 0.47%, 0.57% and 1.52% lower (P>0.05) respectively. Rabbit treated ration R3 caused the highest protein digestibility (80.09%), while group fed R2, R1 and R0 caused protein digestibility 0.80%, 1.19% and 1.67% lower (P>0.05) respectively. The use of fermented banana peels in the feed caused the dry matter digestibility, digestibility of energy

and protein digestibility to be higher than the control feed (R0). The bioactive content of banana peels such as alkaloids, tannins, flavonoids, triterpenoids, saponins (proximate analysis) can be antioxidant and antibacterial pathogens so that they can increase metabolism and absorption of nutrients. *Aspergilus niger* can be a probiotic that can increase enzymatic processes in the digestive tract which have an impact on the intestinal micro flora and better digestibility Ohimain and Ofongo, (2012). Pathogenic microbes in the digestive system can harm animals because they can damage the cell walls of the digestive tract which interfere with the absorption of nutrients and reduce the digestibility of feed (Kompiang, 2009). The results of this study found that the dry matter digestibility of local male rabbits ranged from 66.12% - 67.84%. The results of this study are still in the normal range because Prasad *et al*., (1996) found that the dry matter digestibility of rabbits ranged from 63.74 to 76.06%. The digestible energy of local male rabbits were 73.88%. The same research results were obtained by Prasad *et al.,* (1996) where the Soviet chinchilla rabbit had digestible energy ranging from 66.17% to 77.79%. The average protein digestibility of local male rabbits was 79.36%. Other researchers, namely Ramchurn *et al.,* (2000) obtained an average protein digestibility of 74.2 ± 2.23%.

Rabbits treated fed R3 produced the highest final body weight, namely 2176.38g, while R2 and R1 produced final body weight were 2.46% and 3.67% lower (P>0.05) respectively than rabbit fed R3. The final body weight of rabbit fed R0 was 11.93% lower (P <0.05) than the rabbit fed R3. Treatment ration R3 resulted a bodyweight gain was 21.30 g /day, while rabbit fed R2 and R1 were 3.15% and 4.37% lower (P> 0.05) respectively and rabbit fed R0 was 14.41% lower (P<0.05) than R3. The use of fermented banana peels causes higher lactic acid bacteria than without fermented banana peels. Lactic acid bacteria, which is one of the proteolysis bacteria that produces proteolysis enzymes around the cell wall, cytoplasm membrane and inside cells (Wikandari *et al*., 2012). According to Moningkey *et al.*, (2016), lactic acid bacteria produce cellulolytic enzymes that break down cellulose into glucose, causing the organic substances in this case cellulose and hemicellulose to decrease. Kompiang, (2009) states that lactic acid bacteria can also synthesize B vitamins which can be absorbed by the intestine. This results in higher feed digestibility and animal growth. The treatment of rabbit fed R0 caused the lowest feed consumption, namely 80.58 g /day, while the rabbits fed rations R1, R2 and R3 were 0.45%, 0.60% and 2, 32% higher (P>0.05) respectively. Feed consumption was not significantly different because the feed was made with the same energy and protein content, according to the opinion Puger and Nuriyasa, (2019).

Rabbit fed R3 resulted inthe lowest conversion ratio value was 3.87, while rabbit fed R2 and R1, caused conversion ratio was 1.29%, 2.58% higher (P> 0.05) and the group fed R0 was 14.21% higher (P <0.05) compared group fed R3. According to Moningkey *et al*., (2016) *Aspergillus, niger* can produce cellulolytic enzymes that break down cellulose into glucose, causing organic substances in feed, namely cellulose and hemicellulose to decrease. This condition results in better feed digestibility (Table 3) and higher feed efficiency. Chen *et al.,* (2009) stated that probiotics can produce protease, amylase, cellulose and lipase enzymes which can increase the digestibility of protein, carbohydrate and lipid nutrients in animals so that animal performance becomes higher.

Conclusion

It was concluded that the use of fermented banana peels in rabbit feed at the level of 15% could improve the hindgut microbial composition, increase feed digestibility and increase the growth of local male rabbits.

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