

Effect of Red Betel Leaf Extract (*Piper crocatum*) as Feed Additive on Ileal Characteristic and Intestinal Microflora in Broiler Chicken

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Abstract— The purpose of this research was to evaluate the effect of red betel leaf extract (RBLE) as feed additive on the ileal characteristics and microflora population in broiler chicken. The method was in vivo experiment by using Completely Randomized Design. One hundred and sixty eight day old chicken strain Cobb allocated into 4 treatments and 6 replications. The treatments consisted of T0= basal feed (control). T1=basal feed + 0.5% RBLE. T2=basal feed + 1% RBLE. and T3=basal feed + 1.5% RBLE . The measured variable were: ileal length and weight, pH and viscosity of ileal content, population of microflora consisted of *Lactobacillus*, *Escherichia coli*, dan *Salmonella sp*. The data were analyzed with ANOVA and continued with Duncan’s multiple range test. The results showed that there were no significant different ($P>0.05$) effect of treatment toward intestinal characteristic aspects, but it significantly affected all microflora population. Conclusion of this research was addition of RBLE up to 1.5% does not cause a negative effect on intestinal characteristics, but improve non-pathogenic microflora population in broiler chicken.

Keywords— Red betel. feed additive. ileal characteristic. intestinal microflora. broiler.

I. INTRODUCTION

Success keys in poultry farming business was influenced by three most important factors: provision of superior breeds, fulfillment of nutrient requirements and good operational managements. All of those aspects have to be implemented for high production. Therefore, cost of feed usually dominate the production cost.

Farmers are expected to produce healthy poultry products in response to government regulation on ban of the use of antibiotic growth promoter (AGP). The main reason is it might cause microbial resistance and leave antibiotic residue in animal product that could be dangerous for human. In consequent, AGP replacer in animal feed is urgently required. Recently, there has been an increase in interest to use phytobiotic which is a natural growth promoter, to replace AGP. Phytobiotic might be defined as whole part of plant in the forms of powder or extract that have one or more pharmacological effects [1].

Red betel leaf is one of betel leaf types found throughout the countries, including Indonesia. Red betel leaf has bioactive compounds, mainly flavonoid. Other components are alkaloid, polyphenol, tannin and essential oils. The volatile oils showed a pharmacological functions, such as

anti-bacterial, antifungal, anti-inflammatory, antioxidant [2]. Addition of red betel leaf extract is expected could inhibit proliferation of pathogenic bacteria such as *Salmonella sp.* and *Escherichia coli*, then might improve intestinal microbial balance leading to improvement of nutrient adsorption and animal productivity. In fact, flavonoid is also known as antioxidant source which could protect cells from free radicals. In the previous experiment, addition of green betel leaf juice 5-12.5 ml/bird/day could increase hen day production and decrease FCR of laying hens [3]. Considering that red betel leaf contained higher flavonoid [7], this leaf might be expected to improve performance of broiler. The current research aims to evaluate the use of red betel leaf as feed additive in broiler on ileal characteristic and microbial count.

II. MATERIALS AND METHODS

A. Materials

Materials used in this research were 168 DOC strain Cobb with average initial body weight of 43.66±2.18g. The chicks have been already vaccinated in the hatchery. Twenty four unit cages were used, each has size of 1m² equipped with feeder and drinker, lamp. The room is heated by using gasolec heater during brooding period. The formula and nutrient content of basal diets were described in the following Table:

TABLE 1. Formula and nutrient content of basal feed

Raw materials %	Starter	Finisher
Yellow Corn	56	60
Soybean meal	24.99	24.99
Meat bone meal	9.60	9.60
Limestone	4.60	4.60
Dicalcium phosphate	0.12	0.12
Fish meal	4	0
Sodium bicarbonate	0.072	0.072
Lysine	0.12	0.12
Salt	0.10	0.10
Premix	0.40	0.40
Nutrient content		
Moisture (%)	10.36	10.63
Crude protein (%)	23.50	20.10
Crude fiber (%)	2.81	3.18
Crude Fat (%)	4.55	4.13
Metabolizable energy (cal/g)	3870	3839

Basal feeds were formulated to meet nutrient requirements provided by Cobb nutrition management guide. The basal feeds were divided into 2 types: starter feed (1-21 days) and finisher feed (22-35 days).

The extraction of red betel leaf was carried out according to the previous method [4]. Red betel leaf powder was macerated for 24 hours with ethanol 70%. Ratio of red betel with ethanol was 1:6. After that red betel leaf was extracted in the microwave oven for 10-15 minutes at controlled temperature 40°C and then cooled down until reach room temperature. After that the liquid extract of red betel leaf was obtained by filtering with sterile muslin cloth.

B. Experimental Design

The birds were allocated into 4 treatments and 6 replications per treatment, each replication used 7 birds. They were reared for 35 days. The experimental design was completely randomized design with the treatments given were:

T0 = basal feed

T1 = basal feed + 0.5% red betel leaf extract

T2 = basal feed + 1% red betel leaf extract

T3 = basal feed + 1.5% red betel leaf extract

C. Variables Observed

The variables observed in this study were:

a). Ileal characteristics included intestinal length and weight, intestinal content pH and viscosity. The ileal part of intestine was taken, cut and weighed by scale, while the length was measured by ruler. The pH value and viscosity of ileal digesta was carried out according to the previous method [5], which was taking 1 g of digesta in ileal and then add with aquadest up to 10 ml in volume. The mixture was then centrifuged at 3000 rpm for 5-10 minutes. Supernatant from centrifugation results was separated and put into beaker glass for 2 measurements, pH by pH meter and viscosity by viscometer.

b). Total population of *Escherichia coli*, *Salmonella* sp. and *Lactic Acid Bacteria* (LAB). Calculation of total bacteria used the previously explained Total Plate Count (TPC) method [6].

D. Statistical Analysis

All data obtained in this research analyzed with one way analysis of variance (ANOVA) and continued with Duncan’s Multiple Range Test (DMRT) if there were significant differences.

III. RESULTS AND DISCUSSION

A. Effect of Red Betel Leaf Extract (*Piper crocatum*) on Ileal Characteristic. The result of red betel extract addition in broiler feed to the ileal characteristic is showed in Table 2:

TABLE 2. Effect of red betel extract addition to the ileal characteristic

Treatments	Ileal length (cm)	Ileal weight (g)	pH value	Digesta Viscosity (cP)
T0	25.56 ± 3.795	6.42 ± 2.072	6.82 ± 0.117	876.50 ± 263.29
T1	27.46 ± 7.014	9.48 ± 3.419	6.78 ± 0.172	1064.83 ± 108.12
T2	26.96 ± 3.603	11.7 ± 8.594	6.78 ± 0.160	731.50 ± 231.86
T3	25.9 ± 3.209	8.38 ± 1.543	6.70 ± 0.155	215.32 ± 215.32

A.1 Ileal length and length

Based on analysis of variance, the treatments did not show a significant effect on ileal length of broiler chicken (P<0.05). Numerically, the data between the treatments also did not show specific pattern. The respective ileal length data from the lowest to the highest values were T0, T3, T2, and T1. Based on the analysis of variance, the treatment did not show a significant effect on ileal weight and the lowest ileal weight was 6.42 g (T0) and the highest was 11.7 g (T2). Previous report indicated that the use of sanguarine herb increased relative length of the small intestine [10,11], but not the weight. In addition, it also increased *Lactobacillus*, but not *oli* form [10]. This might relate to the effect of herb or herb extract to stimulate growth of intestine.

A.2 pH and viscosity values

Table 2 showed the data of average pH taken from ileal content. The highest ileal pH was shown by T0 (without the addition of feed additive) with an average of 6.82 ± 0.11, while the lowest pH was for T3 with an average of 6.70 ± 0.15. Based on analysis of variance, the treatments did not show a significant different effect on ileal pH (P<0.05). The respective values of pH data from the highest to the lowest were T0, T1, T2, and T3. The average results on viscosity showed that the feed treatment did not have a significant effect (P> 0.05) on the

digesta viscosity. However, it can be concluded that in treatment P1 the best results with viscosity reached 1064.83 ± 108.12. Previous report indicated that viscosity of ileal content of broiler significantly reduced, but no change in pH value, due to addition of thymol and carvacol in broiler feed [12].

B. Effect of Red Betel Leaf Extract (*Piper crocatum*) on Intestinal Microflora.

The role of microflora in the intestine is important to maintain health of the chicken. The additional effect of red betel leaf extract (*Piper crocatum*) on the population of intestinal microflora in broiler is showed in Table 3.

TABLE 3. Population of intestinal microflora (*Lactobacillus*, *Escherichia coli* and *Salmonella* sp.)

Treatments	<i>Lactobacillus</i> (log CFU/ ml)	<i>Escherichia coli</i> (log CFU/ ml)	<i>Salmonella</i> sp. (log CFU/ ml)
T0	1.70 ± 0.38 ^a	9.12 ± 0.25 ^d	5.24 ± 0.24 ^d
T1	2.56 ± 0.28 ^b	8.22 ± 0.28 ^c	4.26 ± 0.11 ^c
T2	3.30 ± 0.15 ^c	7.36 ± 0.34 ^b	2.46 ± 0.16 ^b
T3	5.36 ± 0.20 ^d	5.56 ± 0.24 ^a	1.40 ± 0.18 ^a

Notes: The different superscripts in the same row showed highly significant differences (p<0.01)

Table 3 showed an average total of non-pathogenic bacteria (*Lactobacillus*) and pathogenic bacteria (*Escherichia coli* and

Salmonella sp.). Addition of red betel leaf extract as natural growth promoter was expected to increase the non-pathogenic bacteria. In this experiment, the highest total of non-pathogenic bacteria was shown by T3 which has an average was 5.36 ± 0.20 log CFU/ ml, while, the lowest total of non-pathogenic bacteria was shown by T0 with an average of 1.70 ± 0.38 log CFU / ml. The result of analysis of variance showed that the application of red betel leaf extract in feed gave a highly significant effect ($p < 0.01$) to the population of non-pathogenic bacteria (*Lactobacillus*).

Average data from the microflora population showed that the higher application of red betel leaf extract leads to increase total *Lactobacillus* bacteria. According to previous research, the chicken given phytobiotics could increase a total of *Lactobacillus* along with decreasing pathogenic bacteria in the digestive tract. The growth of *Lactobacillus* bacteria depends on the pH environment because non-pathogenic bacteria tend to produce acid and grow in an acidic environment. The stability of total *Lactobacillus* bacteria in the digestive tract requires an appropriate environmental conditions, particularly an acidic pH environment [8].

Table 3 also showed that the highest population of *Escherichia coli* was shown by T0 with an average of 9.12 ± 0.25 log CFU/ ml, meanwhile the lowest population was shown by T3 with an average of 5.56 ± 0.24 log CFU/ ml. Similarly, the highest number of *Salmonella* sp. bacteria was shown by T0 with an average of 5.24 ± 0.24 log CFU/ ml, but the lowest population was shown by T3 with an average of 1.40 ± 0.18 log CFU/ ml.

The result of analysis of variance showed a highly significant effect ($p < 0.01$) of addition of phytobiotics in the feed to the population of pathogenic bacteria, especially *Escherichia coli* and *Salmonella* sp. The number of pathogenic bacteria from both types of gram-negative bacteria was 14.36 log CFU / ml then reduced to 6.96 log CFU/ ml. the addition of red betel extract in the feed at different levels can reduce the population of pathogenic bacteria in ileal part of intestine. This is similar to the previous report [8] that increasing levels of turmeric and ginger reduced the number of *E. coli*, and *Salmonella* sp. The research employed onion as feed additive in broiler also reported similar results [9]. This indicated a tendency of decrease in the number of *Escherichia coli* and *Salmonella* sp., suggesting for leveling up use of turmeric and ginger in feed of broilers. Addition of turmeric and 0.8% ginger gave the best effect due to the lowest number of *E. coli* and *Salmonella* sp. in the ileum, so the number of pathogenic bacteria reduced in the intestine.

IV. CONCLUSION

Based on research results, it could be concluded that addition of red betel leaf extract up to 1.5 % in broiler diet did not affect ileal characteristics, but population of microflora alters preferably by increasing not pathogenic bacteria and decreasing pathogenic one.

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