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Effect of Adding *Cassia alata* L. Leaf Extract as Feed Additive on Ileal Histological Response in Broiler

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Abstract**—** *The aim of this research was to evaluate the extract of Cassia alata L. leaf which was used as a feed additive in broilers on the intestinal histomorphological response. The materials were 200 broiler chicks strain Cobb CP 707 produced by PT Charoen Pokphand Indonesia Tbk, which were not distinguished by sex and kept for 35 days. The average of chick body weight was 40.41 ± 5.93 grams. The method was experiment arranged in a completely randomized design. The treatments given consisted of the addition of tetracycline antibiotics added to basal feed as a positive control (P0(+)), basal feed without any additional antibiotics or extracts (P0(-)), the respective addition of 0.4% of Cassia alata L. Leaf extract (P1), 0.8% extract (P2), and 1.2% extract (P3) to basal feed. Parameters measured included height of villi, apical width, basal width, crypth depth, villous surface area, and number of villi. Data were analyzed using analysis of variance (ANOVA) if there were differences between treatments followed by Duncan's Multiple Range Test. The results showed that the treatment gave a significant effect (P<0.05) to the number of ileal villi, but not other variables. The conclusion is addition of 0.8% extract in broiler feed gave the best results to influence to intestinal development by improving villous surface area and number of villi.*

Keywords**—** *Broiler, extract, Cassia alata L., intestinal development*.

I. Introduction

Broiler farming continues to grow in Indonesia, the broiler population in 2018 was 3,138 million, and increased in 2019 it increased to 3,170 million, indicating an increase of about 10%. In addition, consumption of broiler meat in 2019 was still low at about 5.7 kg per capita per year. The development of the broiler industry then becomes one of the main pillars of animal protein providers in Indonesia, because the price of broiler meat is a lot cheaper than other types of meat [1]. This population increase is certainly required to maximize production to meet consumer needs. Antibiotic supplementation in feed is used to maintain health and stimulate the immune system of broilers so that production can be optimal [2]. Antibiotics given through feed and used to stimulate growth are called AGP (Antibiotic Growth Promoter). Long-term use of AGP can cause bacterial resistance and might cause antibiotic residues in broiler meat products [3], so it is necessary to use other AGP alternatives.

AGP alternatives can be in the form of probiotics, prebiotics, phytobiotics, enzymes, organic acids, and so on. According to Ganguly [4], phytobiotics are defined as additional compounds that are the result of secondary plant metabolites (could be in the forms of either nutritive or non-nutritive, or anti-nutritional factors) which have effect on enhancing livestock productivity through improving feed properties, improving digestive tract health, by controlling pathogenic bacteria, increasing production performance, and improving the quality of livestock products.

One of the plants, the *Cassia alata* L. could be a phytobiotics of which the leaves contain tannins, saponins, alkaloids, steroids, flavonoids, and anthraquinone compounds. These phytochemical compounds can act as antifungal, antibacterial, and immunomodulatory [5]. Therefore, extraction rather than the use of *Cassia alata* fluor is attempted to improve the content of active substances. One method developed in our laboratory is Microwave-Assisted Extraction (MAE) would be applied to extract *Cassia alata* fluor. The rich extractable flavonoid is expected to be able to act as antimicrobial agent enable to improve the balance of intestinal microflora and has a positive effect toward broiler production. Based on the above study, researchers are interested in conducting research by conducting experiments on the biological evaluation of feed on the histological changes of the ileum in broiler.

II. Material Methods

Broiler chicks were kept at the Tholabie Islamic Boarding School, Puncak Indah Buring, Kedung Kandang District, Malang Regency. Intestinal preparations was carried out at the Pathology and Anatomy Laboratory, Faculty of Medicine, and further observation of intestinal prepared was done at the Molecular Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya.

The material used in this study was day old chick (DOC) broiler strain Cobb CP 707 produced by PT Charoen Pokphand Indonesia Tbk. which did not differentiate between male and female sex (unsex), Cassia alata L. leaf extract, intestinal preparations, and a microscope. A total of 200 broilers were reared for 35 days and were placed in 20 plots.

This study using *Cassia alata* L. leaf extract used a completely randomized design (CRD) with treatment P0 (+) = Commercial feed with the addition of 0.1% tetracycline antibiotics, P0 (-) = Commercial feed without additional treatment, P1 = Feed commercial feed with the addition of 0.4% *Cassia alata* L. leaf extract, P2 = Commercial feed with the addition of 0.8% *Cassia alata* L. leaf extract, P3 = Commercial feed with the addition of 1.2% *Cassia alata* L. leaf extract.

There were 5 treatments and each treatment was repeated 4 times, so a total of 20 experimental units. Each unit was occupied by 10 chicks. Feed and drinking were given ad libitum. The method of adding *Cassia alata* L. leaf extract in feed was to mix it homogeneously using a sprayer of the feed. The feed used in this study was a basal feed formulation based

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on the nutritional requirements of broilers in the starter and finisher periods.

The *Cassia alata* L. leaf powder was obtained from a local supplier. The procedure of extraction was by preparing 1 part of leaf powder immersed in 0.6 part of 70% ethanol (w/v), then the simplicia was extracted following the MAE (Microwave Assisted Extraction) method [6].

The procedure to obtained digesta samples was as follow: 1) choosing 1 chicken at 35 days-old represented the population of each experimental unit; 2) the selected chickens were slaughtered; 3) the small intestine was separated; 4) cutting 3-4 cm from the end ileum to the ileocecal junction; 5) the ileal content was then taken out by inserting and slowly pushing a syringe containing water; 6) the ileal content sample was finally put into a drug pot contained 10% formalin (pro-analyst) until the sample completely submerged and; 7) labelled the samples accordingly.

The variables measured included villi length, villous crypt depth, apical villi width, villous basal width, and intestinal surface area by calculating the ileal area using the intestinal mucosa histology method (light microscopy) [7].

The data obtained in the study were tabulated and analyzed for variance using a completely randomized design. The data that has been analyzed and there are differences are further tested using Duncan's Multiple Range Test.

III. Results and Discussions

Measuring the histomorphological or microscopic condition of the villi is done to see the development of the villi related to the absorption of nutrients. The wider the surface, the absorption of nutrients in the feed will also be more optimal, so body weight gain should also increase [8].

The effect of adding *Cassia alata* L. leaf extract to broiler feed on intestinal histomorphological conditions (villous height, apical villi width, villous basal width, crypt depth, villous surface area, and the number of villi) during the study can be seen in Table I.

TABLE I. The effect of treatment on the ileal histological changes in broiler.

Treatment	P0(+)	P0(-)	P1	P2	P3
Villi Height (µm)	549 ± 145	542 ± 167	649 ± 18	672 ± 81	700 ± 109
Apical Villi Width (µm)	45 ± 8,8	68 ± 20	53 ± 23	62 ± 20	59 ± 11
Villous Basal Width (µm)	84 ± 37	97 ± 45	61 ± 13	87 ± 42	74 ± 15
Crypt Depth (µm)	151 ± 39	102 ± 13	134 ± 4	139 ± 26	136 ± 49
Villi Surface Area (µm)	1550 ± 536	1349 ± 672	1456 ± 185	1616 ± 412	1580 ± 176
Number of Villi (/transversal cut)	57 ± 13[a]	53 ± 7[a]	64 ± 6[a]	71 ± 8[b]	70 5[b]

The results of the analysis of variance treatment with additions *Cassia alata* L. leaf extract in broiler diet showed a significant difference ($P<0.05$) in the number of villi, but had no significant effect on ileal length, villi height, in crypts, apical width, basal width, and villous surface area.

Villi Height

The higher the villi are an indication that the absorption process is also more efficient [9]. The height villi of the broilers ileum in this study showed that the highest treatment was the addition of *Cassia alata* L. leaf extract with a concentration of 1.2% (P3), which was an average of 700 µm, the next sequence was P2 with an average height of 672 µm, P1 with a height of 649 ±18 µm, then positive control (P0(+)) 549±145 µm, and the lowest was negative control treatment (P0(-)) as high as 542±167 m. The results could be due to the negative control or P0(-) did not receive additional antimicrobial compounds, resulting in free competition between pathogenic and non-pathogenic bacteria. The unfavorable microflora conditions will affect the process of feed absorption, so that the nutrients needed for the formation of villous cells, especially protein, are less available. This is supported by Kataren [10] who explains the role of protein as a cell-forming, replacement for damaged cells, and firming body tissues.

The results of statistical analysis showed that the treatment given did not have a significant effect on the height of the ileal villi. This could be an indication that the addition of *Cassia alata* L. leaf extract was not effective in increasing ileal height. The cause of this could be possible because the concentration of the *Cassia alata* L. leaf extract was not high enough, so it was not able to increase the villi height optimally and the absorption process was not optimal. This statement is supported by Rahmah et al. [11] who explained that the active substances in herbs up to a level of 1.5% have not been able to work optimally to suppress the growth of pathogenic bacteria in the digestive tract because the production of organic acids is low. Another possibility that could cause no significant effect was because all of the chickens whose intestines were collected were 35 days old, so there was no difference in the height of the ileal villi. According to Sun et al [12] broilers that are more than 28 days old will not effect the height of the intestinal villi.

Apical and Basal Width of Villi

The results of the analysis of variance on the apical width and basal width parameters of the villi did not show a significant difference. The treatment with the highest average apical width was P0(-) or negative control with a value of 68 µm, then P2, P3, P1, and P0(+) respectively had a width of 62 µm, 59 µm, 53 µm, 45 µm. P0(-) also has the highest average basal width variable (97 µm), then P2 with a width of 87 µm, P0(+) 84 µm, P3 74 µm, and P1 61 µm. The negative control (P0(-)) had the highest apical and basal width of the ileal villi. The apical and basal width of these villi affects the surface area of the villi which serves as a place for nutrient absorption.

The effect of giving *Cassia alata* L. leaf extract on ileal crypt depth can be seen in table I. The table shows that there is an increase in ileal crypt depth along with the increase in the concentration of the extract, which in succession from the highest are P0(+), P2, P3, and P1 with average values of 151 µm, 139 µm, 136 µm, and 134 µm, and the lowest was in the negative control, which was 102 µm.

Research on plant extracts given to broilers conducted by

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Sugito et al [13] found that administration of jaloh bark extract (*Salix tetrasperma* Roxb) had a better protective effect in maintaining the morphometrics of chicken intestine villi compared to chickens not given the extract. Research on the effect of *Cassia alata* L. leaves has not been reported so far, but the active compound in Cassia Alata L. leaf extract can reduce pathogenic bacteria in the digestive tract of chickens. Dahanakurr et al. [14] also reported that in some plants many active compounds have the effect of inhibiting the growth of pathogenic bacteria in the digestive tract. Inhibited pathogenic bacteria will provide a greater opportunity for non-pathogenic bacteria to develop properly. Colonization of pathogenic bacteria attached to the intestinal epithelium can damage the protective mucosal layer and cause damage to epithelial cells, including villi [15].

Crypt Depth

The results of statistical analysis showed that the treatment given had no significant effect on the ileum crypt depth. These results of the addition of *Cassia alata* L. leaf extract in broiler feed are not effective in increasing the crypt depth of the broiler ileum. Research conducted by Sun et al [11] and Smirnov et al [12] also gave the same results, villi height and crypt depth had no effect after the chickens were more than 28 days old. However, numerically the addition of *Cassia alata* L. leaf extract could increase the depth of crypts more than the negative control. That can occur due to the presence of phytochemical compounds in the *Cassia alata* L. leaf extract which can be antimicrobial, especially on pathogenic bacteria, so it can increase the population of non-pathogenic bacteria. This statement is supported by Wresdiyati et al. [16] which state that the thickness of the intestinal mucosa will increase when probiotics (nonpathogenic bacteria) are in good condition and will have an impact on increasing villi height and crypt depth, because probiotics can produce short chain fatty acids. This increase in short chain fatty acids can stimulate the proliferation of epithelial cells.

Surface Area of the Villi

The administration of *Cassia alata* L. leaf extract did not significantly affect the surface area of the villi, but numerically there was an increase in the surface area of the villi along with the concentration of the extract given. The highest surface area was treated with 0.8% *Cassia alata* L. leaf extract (P2) with an average area of 1616 µm, then P3 with an area of 1580 µm villi, then P0(+) 1550 µm, and P1 which had an area of 1456 m, while the lowest is P0(-) which has an area of 1349 µm. These results can be caused by the activity of antimicrobial compounds present in the *Cassia alata* L. leaf extract which can suppress the development of pathogenic bacteria, to create a good environment for non-pathogenic bacteria (probiotics) to thrive in the digestive tract of chickens. Gunal et al. [17] also explained that the increase in villi surface area was caused by the increase in short chain fatty acids induced by lactic acid bacteria. Short chain fatty acids produced by the fermentation process of probiotic bacterial strains can stimulate the multiplication of intestinal epithelial cells, because short-chain fatty acids are a phospholipid component of the intestinal epithelial membrane. This statement is also by the opinion of Lenhardt et al. [18], increasing LAB population will result in the production of more short chain fatty acids, and antimicrobial substances that are antagonistic to the growth of pathogenic bacteria. Lactic acid bacteria, especially Lactobacillus sp. can produce short chain fatty acids, one of which is butyric acid which has a role to increase small intestinal epithelial cells, namely goblet cells which function to protect the small intestinal mucosa from damage.

Number of Villi

The results showed that the addition of *Cassia alata* L. extract had a significant effect ($P<0.05$) on the number of ileal villi. The highest number of villi was found in chickens treated with 0.8% *Cassia alata* L. leaf extract (P2), which was 71±8/transverse cut, then sequentially, 1.2% *Cassia alata* L. leaf extract (70±5/transverse cut), 0.4% extract (64±6/transverse cut), chickens treated with tetracycline antibiotics (57±13/transverse cut), while the lowest number of villi was in the negative control 53±7/transverse cut. Treatment with the addition of tetracycline antibiotics had a smaller number of villi than the addition of *Cassia alata* L. leaf extract. This indicates that the use of *Cassia alata* L. leaf extract has a better impact than tetracycline on the parameters of the number of intestinal villi in the ileum. This increase in the number of villi could be due to the *Cassia alata* L. leaf extract being able to suppress the growth of pathogenic bacteria so that it can support the growth of non-pathogenic bacteria [19].

Non-pathogenic bacterial colonization can also repair the villi surface and can release bioactive compounds, resulting in an improvement in the intestinal barrier which directly modifies the function of epithelial cells including cytokines, so that the absorption process becomes more optimal [20]. Gunal et al [17] also explained that the increase in villi was caused by short chain fatty acids produced by probiotics or lactic acid bacteria. These short chain fatty acids can stimulate the multiplication of intestinal epithelial cells.

IV. Conclusion

The study concluded that 0.8% of *Cassia alata* L. leaf extract in broiler feed gave the best results in terms of the surface area of the villi, and the number of villi.

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