The Effectiveness of Biacid (Organic Acid and Essential Oil) as Substitute for Antibiotics on Ileal Characteristics of Broilers

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Abstract. The research was aimed to evaluate the characteristics of ileal broiler with the inclusion of Biacid as feed additive on broiler feed. The material was used 900 DOC broiler with average body weight 45.34±4.403 g and coefficient of diversity 9.71 % and reared for 35 days. The method that used in the research was experiment using completely randomized design with 4 treatments and 5 replications. The feed treatments consisted of P0= basal feed without Biacid, P1= basal feed + Biacid 0.075 %, P2= basal feed + Biacid 0.100 %, and P3= basal feed + Biacid 0.125 %. The variables measured were villus number, villus height, villus surface area, and crypt depth. The data was analyzed use Analysis of Variance (ANOVA) and Duncan’s Multiple Range Test (DMRT). The result showed that the inclusion of Biacid had a significant effect (P<0.05) on villus high and crypt depth, but not significant effect (P>0.05) on villus number and villus surface area. The conclusion research that is addition 0.075 % Biacid give the best result from villus total, villus surface area, and crypt depth at level.

1. Introduction
Feed costs reach 60-70% of the total production costs, and by increasing feed efficiency used the feed cost can decrease optimally. Feed efficiency is achieved when the conditions of the animal's digestive tract are optimal in digesting and absorbing food substances. One of the methods used to optimize the condition of the digestive tract of livestock is the inclusion of feed additives. Feed additive is an ingredient that can affect livestock health and nutrition conditions even though the material is not containing a nutrients. Feed additives are usually used in commercial farms in the form of antibiotics that aim to support growth, increase performances, productivity, and efficiency of the feed. The use of synthetic antibiotics that are not allowed with the particular dose can have a negative impact on livestock, left the residue of antibiotics left in the meat. The Minister of Agriculture of the Republic of Indonesia has issued a ban on the use of antibiotics that has been stated in the Minister of Agriculture Regulation No. 14 of 2017. some researcher had been advised to be wise to use synthetic antibiotics in livestock have deficiencies, that the use of antibiotic doses must be considered because excessive use is feared to leave residues in the body chicken besides increasing production costs.
Essential oils and acidifier can improve the efficiency of broiler feed to produce livestock products without residue. The use of acidifier can optimize the intestinal pH to be low so that absorption of food substances can be maximized. Organic acids such as propionic acid, citric acid, fumaric acid and formic acid have been recognized as acidifiers which have a positive influence on growth [1]. Acidifier can reduce the pH of the digestive tract, and suppress pathogenic bacteria, thereby improving the number and height of villi [2]. The inclusion of acidifier can improve and optimize the work of the small intestine so that non-pathogenic bacteria in the small intestine reproduce well and cause absorption in the small intestine to occur optimally. Emma, Sjofjan, Widodo, and Achmanu [2] total lime acid can increase the height of villi and the number of broiler villi because total lime acid can reduce ileal pH which supports the growth of non-patogenic bacteria.

Essential oil functions as an antibacterial agent by limiting the growth of gram-negative pathogens such as Eschericia coli and Salmonella sp. [3]. Setyanto [4] volatile oil can stimulate digestive tract enzymes, so that the amount of enzymes that come out causes the stomach to empty quickly and chickens will consume more feeds. Kurniawan, Widodo, and Djunaidi [5] that study of utilization of noni fruit powder and essential oil to improve morphology of the small intestine so that it improves digestion and absorption of nutrients in the digestive tract. Many commercial feed additive products we find on farms. "Biacid" is a feed additive product that contains an acidifier and essential oil.

Based on the description above, it is necessary to conduct research to find out what level shows the best results from the use of acidifier and essential oil contained in the Biacid product as an additive feed in feed on the number of villi, villi height, villi width, and crypt depth.

2. Materials and methods

2.1. Time and location

This research was conducted 18 November- 29 December 2018 at Sumbersekar field Laboratory of animal science, and at Nutrition Laboratory of animal science, UB. Observation of the number of villi, villi height, crypta depth and area of the villi ileum in the anatomic pathology laboratory of the Faculty of Medicine, UB on 15 January 2019.

2.2. Research material

This study used 900 unsexed DOC (day old chick) broilers of Lohmann MB 202 broilers, and reared for 38 days. The DOC was obtained from the hatchery factory of PT. Japfa Comfeed, Kediri. The average body weight of the entire sample used was 45.34 ± 4.403 g/head with a coefficient of diversity of 9.71%.

The cages used in this study were 20 plots. Each plot was filled with 45 broilers equipped with one hanging feeder and two drinker bells. Equipment used during the study included cage sealing equipment (bamboo, raffia, wire, knives, scissors and steel pliers), brooding equipment (LPG-fueled infrared heaters placed in cages, feed mixing equipment (small buckets, shovels) , plastic scales and scales), additional equipment (stationery, digital scales, documentation tools, disinfectants, feed containers, drinking containers, thermometers and hydrometers, as well as final research equipment (ovens, test tubes, glazing covers, glazing objects, port film, scalpels, micro tweezers, xylol tubes, and microscopy) The materials used for research are physiological HCL, 10% formalin, HE dyes, xylol, alcohol (70,80,90% and absolute), 1% acid alcohol eosin and paraffin.

The feed provided is starter period feed by self mixing feed based on nutritional requirements of broilers for the starter phase and using commercial food from PT. Japfa Comfeed Indonesia. The composition of the mixed feed and the nutrient content of both feeds can be seen in Table 1. Mixed feed is given at 1-12 days of age while complete feed (BR 1) is given at 13-38 days. Feeding and drinking water in an ad lib.
Table 1. Basal feed nutrient

| Nutrient            | Content       |
|---------------------|---------------|
| Water %             | Max. 12       |
| Crude Protein (%)²  | 22.20         |
| Crude Fat (%)¹      | Min 5         |
| Crude Fiber (%)¹    | Maks 5        |
| ash (%)¹            | Maks 7        |
| Calcium (%)¹        | 0.8-1.1       |
| Phosphor (%)¹       | Min. 0.5      |
| Energy (kal/g)³      | 4259          |

Notes: ¹ Based on label
² Based result analysis at Nutrition Laboratory, animal science University of Brawijaya
³ Based result analysis at Nutrition Laboratory, animal science and fisher department Blitar

2.3. Research method

2.3.1. Research design experiment

The method of this research was in vivo experiment with a completely randomized design (CRD) with 4 treatments and each treatment had 5 replications using 20 broilers. The treatments are given by following:

- P0: Basal Feed + without the addition of Biacid
- P1: Basal Feed + Biacid 0.075%
- P2: Basal Feed + Biacid 0.100%
- P3: Basal Feed + Biacid 0.125%.

2.3.2. Variable measured

The variables observed in this study were the number of villi, villi height, villi area, and crypta depth. Data was collected by taking 3 chickens from each treatment. Intestinal sampling is done by cutting 3 cm from the ileo caecal junction to the ileum 4 cm long, then cleaned with physiological NaCl using a syringe slowly to remove the ileal digesta and put in a bottle containing 10% formalin solution to test the number of villi and height small intestine villi [6]. The stages of the procedure for making small intestinal histology preparations and observations of small intestine villas according to [7, 8, 9] as follows: The process of cutting tissue in the form of macroscopic, the process of blocking and cutting of tissue, the process of deparaffinization, the process of coloring (HE), the level of alcohol dyeing, clearing, and mounting with slime and deckgloss. Furthermore, it was analyzed under a DIC Olympus BX51TF light microscope using a reader application, Olyvia, to determine the number of villi, villi height, villi area, and crypta depth. The villi measurements can be illustrated according to Figure 1.

![Figure 1](image)

*Figure 1. a = villi height b = crypta depth, c = basal villi width and d = apical villi width* [10]
2.4. Data Analysis

The data were analyzed with Microsoft Excel 2010. Based on Completely Randomized Design analysis (CRD). Mathematical model of Complete Random Design:

\[ Y_{ij} = \mu + \tau_i + \varepsilon_{ij} \]

- \(Y_{ij}\) = Introductory value in the treatment of the jth test
- \(\mu\) = General value
- \(\tau_i\) = Effect of i\text{-}treatment
- \(\varepsilon_{ij}\) = Effect of trial error from first treatment and j\text{-}birthday
- \(i = 1,2,3,4\) and 5
- \(j = 1,2,3,4\) and 5

3. Results and Discussion

The inclusion effect of Biacid treatment containing acidifier and essential oil on the number of villi, villi height, villi area, and crypt depth can be seen in Table 2.

| treatments | Villi Number (unit/ lumen) | Villi height (µm) | Crypt depth (µm) | Villi area (µm²) |
|------------|-----------------------------|-------------------|-----------------|-----------------|
| P0         | 47.0±3.9                    | 522.8±9.1a        | 174.8±4.1a      | 1586.1±191.1    |
| P1         | 55.0±2.3                    | 558.4±34.9ab      | 194.3±12.7c     | 1673.9±233.4    |
| P2         | 46.8±5.5                    | 500.5±76.8a       | 177.8±6.7ab     | 1286.9±440.2    |
| P3         | 52.2±4.2                    | 638.7±53.1b       | 190.9±6.0bc     | 1440.1±373.0    |

Note: Different superscripts in the same column showed significant differences (P <0.05)

3.1. Effect of treatment on the number of villi ileum

The results of the average analysis of the number of broil ileum villi can be seen in Table 2. The average number of broil ileum villi in Table 2 based on the lowest to highest number during the study was P2 (Biacid 0.100%) of (46.83 ± 5.48) units / transversal cut, P0 (without Biacid) of (47.00 ± 3.97) units / transversal cut, P3 (Biacid 0.125%) of (52.17 ± 4.19) units / transversal cut, and the highest number of villi ileum at P1 is (Biacid 0.075%) of (55.00 ± 2.29) units / transversal cut. Inclusion of Biacid in feed can be known its effect on the number of villi ileum by analyzing the variety of observations of the number of villi. The results of the analysis of the variety of observations of the number of villi ileum in Appendix 3 shows that the addition of Biacid to broiler feed gives no significant difference (P> 0.05) on the number of villi ileum, so the results of the analysis of variance are not followed by Duncan's Multiple Range Test to determine difference between treatments.

Fitasari [11] states that the small intestine is the main place of absorption of nutrients. First, liquid digesta can easily pass through the small intestinal epithelium that is on the surface which will then be carried into the bloodstream. Second, the small intestine is high enough for the surface area to occur. Third, there are millions of villas in the small intestinal epithelial mucosa. Villiile is denser in the duodenum and jejunum and the amount decreases in the ileum. Research shows the number of villi in the P1 treatment obtained the highest results with the addition of 0.075% Biacid into the feed, this shows that Biacid which contains Essential oil and Acidifier can affect the number of intestinal villi.Emma, Sjofjan, Widodo, and Achmanu [2] stated that lime acid can increase the height of villi and the number of broiler villi, although not significantly. The increased height of villi and the number of villi is caused by the total lime acid can reduce ileal pH so that it strongly supports the growth of nonpathogenic bacteria. This was confirmed by Wresdiyati, Laila, Setiorini, Arief, and Astawan [12] stating that maintaining the balance of intestinal microflora can increase the defense of the animal's immune system.
Inclusion of Biacid affects the number of villi that will affect nutrient absorption. Fitasari [11] mentions that low viscosity will cause a higher number of small intestine villi and stimulate higher villi height because low viscosity of feed will lead to more optimal absorption of nutrients. The increase in the number of villi in the form of encapsulation is due to the higher content of active substances in the form of encapsulation compared to the form without encapsulation [6].

P0 treatment number of villi is lower than P1 treatment with an additional level of 0.075% Biacid, this is due to the presence of pathogenic bacteria that can damage cells in the intestine so that it can affect the number of intestinal villi. Wresdiyati, Laila, Setiorini, Arief, and Astawan [12] stated that in the digestive tract of the small intestine, the pathogenic bacteria that often causes disturbances is *Enterobacteriaceae* *Escherichia coli* (EPEC). This is supported by Zalizar, Satrija, Tiuria, and Astuti [13] stating that widespread damage to epithelial cells in the digestive tract of infected worms can lead to the replacement of functional cells with immature and non-functional cells, thus forming imperfect intra cellular complexes. Sjofjan, Natsir, and Ardiati [14] stated that the decrease in viability causes a higher pH and lower viscosity of digesta, resulting in a faster digesta rate and allows a decrease in the process of digestion and absorption of food substances. This decrease in absorption activity causes the number and height of chicken intestine villi to not develop properly.

### 3.2. Effect of treatment on villi ileum height

The results of the average analysis of the height of broil ileum villi can be seen in Table 2. The average height of broil ileum villi in Table 2 based on the shortest to the highest data in a row during the study is P2 (500.49 ± 76.77) µm, P0 (522.84 ± 9.05) µm, P1 (558.44 ± 34.87) µm, and the highest villi ileum height at P3 was (638.71 ± 53.10) µm. Inclusion of Biacid in feed can be seen its effect on the height of the villi ileum by analyzing the variety of observations of villi height.

The results of analysis of observations of the height of the villi ileum in Appendix 4 shows that the addition of "Biacid" to the broiler feed significantly different (P<0.05) on the height of the villi ileum. The results of the analysis of variance were then continued with Duncan's Multiple Range Test to determine differences between treatments. The highest villi was in the P3 (638.71 ± 53.10) and the shortest in the P2 (500.49 ± 76.77µm), but in the P0 (522.84 ± 9.05) with P2 (500.49 ± 76.77 µm) there was no difference in notation between the three treatments. This shows that Biacid which contains Essential oil and Acidifier can affect the height of villi ileum. Intestinal histomorphology can reflect health status in farm animals. Increased villi height and crypta depth in the duodenum are indicative of good health status in livestock [15]. This is supported by Apriliyani, Djaelani, and Tana [7] stating that the high size of high villi can maximize the process of absorption of nutrients from feed, so that in the optimum absorption process the nutrients used in metabolic activities will be more optimum. Followed by Fitasari [11] that after food substances are absorbed into the villi, then it will go to the circulation system in the spleen and blood, so that it will support the growth of livestock. Emma, Sjofjan, Widodo, and Achmanu [2] stated that lime acid can increase the height of villi and the number of broiler villi, although not significantly. The increased height of villi and the number of villi is caused by the total lime acid can reduce ileal pH so that it strongly supports the growth of non patogenic bacteria.

The results showed that the treatment of P3 with the addition of 0.125% Biacid higher than P0, it is means that Biacid can stimulate villi height optimally and can affect the optimal absorption of nutrients. This is in line with the statement that the provision of fermented soybean pulp can stimulate an increase in the size of the villi ileum [16]. Wresdiyati, Laila, Setiorini, Arief, and Astawan [12] that *L. fermentum* improves the health and defense of the host intestinal mucosa by several mechanisms, including by attaching very well to epithelial cells and the surface of the intestinal mucosal cells, so that it can inhibit attachment. Inclusion of Biacid affects the number of villi that will affect nutrient absorption. This is in line with Fitasari [11] stating that low viscosity will cause a higher number of small intestine and stimulate higher villi height because low viscosity of feed will lead to more optimal absorption of nutrients.
3.3. Effect of treatment on the ileum crypt depth

The results of the average analysis of the depth of broiler crypt ileum broiler can be seen in Table 2. The average depth of broiler crypt ileum in Table 2 based on the lowest to highest crypt depth in a row during the study is P0 (174.83 ± 4.06), P2 (177.82 ± 6.66), P3 (190.94 ± 6.00) µm, and the highest at P1 (194.32 ± 12.72)µm. Inclusion addition of Biacid in feed can be known its effect on the depth of crypt ileum by analyzing the variety of observations of crypt depth.

The results of the analysis of the various observations of the depth of crypt ileum in Appendix 5 shows that the addition of Biacid to broilers' feeds had a significantly different effect (P<0.05) on the depth of crypt ileum. The results of the analysis of variance were then continued with Duncan's Multiple Range Test to determine differences between treatments. The highest crypt depth was found in the P1 (194.32 ± 12.72) and the shortest in the P0 treatment (174.83 ± 4.06 µm). This shows that Biacid which contains Essential oil and Acidifier can affect the depth of crypt ileum. Hidayat, Harimurti, and Yusiat [16] that intestinal histomorphology can reflect health status in farm animals with increasing villi height and crypta depth in the duodenum is an indication of good health status in livestock. This was confirmed by Emma, Sjofjan, Widodo, and Achmanu [2] that the use of total lime acid as an acidifier in feed can improve the characteristics of the small intestine, at a level of 0.8% the total lime acid responds well to the characteristics of the small intestine of livestock.

P3 treatment got the highest results with an average crypt depth of 190.94 µm, that in administering Biacid in the level of inclusion of 0.125% Biacid can suppress the growth of non-pathogenic bacteria thereby increasing the depth of crypt. Essential oil extracted from plants can be used to suppress non-pathogenic bacteria in which there are active compounds as antibacterial. This is in line with the statement of Abdel, Kehraus, Hippenstiel, and Sudekum [17] that herbal plants known as medical plants can function as antibacterial because they contain active compounds that are not integrated with the outer membrane of pathogenic bacteria so that material in bacterial cells will be released out of cells. Added by the explanation of Wresdiyati, Laila, Setiorini, Arief, and Astawan [12] states that L. fermentum improves the health and defense of the host intestinal mucosa with several mechanisms, including by attaching very well to epithelial cells and the surface of the intestinal mucosa, so that it can inhibits attachment. Olnood, Beski, Choct, and Iji [18] that probiotics affect the intestinal microstructure more consistently, affect the height of villi to the ratio of crypt depth in the ileum compared to control feed.

The control group P0 (174.83 ± 4.06 µm) decreased in this study, presumably due to the presence of pathogenic bacteria that can damage the intestinal characteristics. Zalizar, Satrija, Tiuria, and Astuti [13] stated that inflammation occurs in the digestive tract causing chickens to be unable to digest and utilize feed properly, so that growth is disrupted. This is reinforced by Kurniawan, Widodo, and Djunaidi [5] stating that bio active compounds and essential oils from noni fruit powder can contribute to enhancing the morphology of the small intestine so that changes in the structure and morphology of the villi thus increasing the performance of poultry production which plays an important role in digestion and absorption of nutrients in the digestive tract. Sari, Wahyuni, Hammy, Jalaluddin, Sugito, and Mas Yitha [16] stated that the histological picture and histo morphometric data of small intestine showed that the provision of fermented soybean dregs could stimulate an increase in the size of livestock small intestine villi.

3.4. Effect of treatment on villi ileum area

The results of the average analysis of the wide range of broiler ileum villi can be seen in Table 2. The average area of broiler ileum villi in Table 2 based on the lowest to highest successive area during the study is P2 (1286.96 ± 440.17), P3 (1440.08 ± 373.00), P0 (1586.05 ± 191.08), and the highest area of villi ileum at P1 (1673.89 ± 233.35µm²). Inclusion of Biacid in the powder form in the feed can be known its effect on the area of villi ileum by analyzing the variety of observations of villi area.

The results of the analysis of observations on the extent of villi ileum shows that the addition of Biacid to broiler feed gives no significant difference (P>0.05) on the area of villi ileum. So the results of the analysis of variance were not followed by Duncan's Multiple Range Test. Apriliyani, Djaelani,
and Tana [7] state that the cross-sectional area of the intestine tenue can affect the ability of digestion and absorption of nutrients and the cross-sectional area is influenced by the size of the height and width. This is reinforced by the statement that nutrient absorption will be more efficient if the absorption area is wider and wider [15].

P1 with 0.075% Biacid have an average of 1673.89 µm2 gives higher compared to P0, it is suspected that the pathogenic bacteria in the villi are smaller because Biacid contains essential oil which is used to suppress non-pathogenic bacteria. Pathogens in the digestive tract of the small intestine that often cause disturbances are Enteropathogenic Escherichia coli (EPEC) [12]. This can damage the characteristics of the small intestine so that it will interfere with the performance of the small intestine to absorb nutrients in livestock. Zalizar, Satrija, Turiya, and Astuti [13] stated that damage to epithelial cells in the digestive tract of infected worms could result in the replacement of functional cells with immature and non-functional cells to form intracellular complexes that are not perfect. This is reinforced by Markovic, Sefer, Krstic, and Petrujkic [19] that the protection of villi from damage caused by pathogens or rough food makes the need for cell turnover in the intestine to be reduced, so that damage to the villi causes increased speed of replacement of enterocytes that require energy and protein so that it can inhibit growth, development of other tissues, and organ systems.

P2 treatment gave the lowest results with an average value of 1286.96 µm2, it was suspected that in the treatment there was a digestive disorder which caused the villi could not develop properly. Inflammation in the digestive tract causes chickens to be unable to digest and utilize their food properly, so that growth is disrupted [13]. This is reinforced by the statement that a heavy dose of A. galli worm infection caused the surface area of the small intestine villi starter chicken to be 20% smaller than in the group without infection and slowed growth by 12.31% [13]. This causes a decrease in viability so that it causes a higher pH and lower viscosity of digesta which results in a faster digestion rate and allows a decrease in the process of digestion and absorption of food substances [14]. The appropriate pH will activate a number of enzymes to reform glucose, protein and lipid polymers into simple structures so as to increase absorption of food substances [14].

4. Conclusion
Inclusion of Biacid as feed additive containing acidifier and essential oil in broiler feed at level of 0.075% gives the best results in terms of the number, depth of crypt, and area of ileum villi.

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