Effect of Feeding A Combination of Probiotic Feed Additives, Phytobiotics, And Microencapsulated Acidifiers On Broiler Villi Characteristics

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Abstract. This study aims to determine the effectiveness of the addition of natural feed additives on the production performance and quality of broilers. The sample used in this study was DOC (Day Old Chicken) Cobb strain with the trademark CP 707 as many as 75 heads. The method used was experimentally designed using a completely randomized design with three treatments and five replicates using five broilers per replicate. The treatments were T0 = basal feed, T1 = basal feed with 0.5% natural feed additive, and T2 = basal feed with 1.0% natural feed additive. The variables used in this study include villous length, number of villi, and depth of villous crypts. Data were analyzed with ANOVA and continued with Duncan's multiple range test. The results showed that the test of villi characteristics using combination of probiotics-phytobiotics-acidifier microencapsulation gave a significantly different effect ($P<0.05$) on the number and depth of villi crypts, but gave a very significantly different effect ($P<0.01$) on villi length. The use of a combination of probiotics-phytobiotics-acidifiers gives effective results on the characteristics of intestinal villi in the ileum section giving good results at the level of use of 1.0% and can be used to replace antibiotics.

1 Introduction

Indonesian people's need for animal protein is increasing day by day. The majority of protein nutrition fulfillment comes from chicken meat. The broiler population in Indonesia from 2016 to 2021 has increased significantly with a percentage of 52.5% [1]. This increase was triggered by the interest factor and the efficiency of meat acquisition in its maintenance. Broilers are able to produce meat relatively quickly with a maintenance period of 4 to 5 weeks, are rich in nutrients, and the feed efficiency rate in the form of the Feed Conversion Ratio (FCR) is quite high, reaching 1.6 to 1.8 [2]. The meat content in 1 broiler is 37 grams of protein, 295 Kcal of energy, and 14.7 grams of fat.

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The challenge comes when the Indonesian government through the Ministry of Agriculture officially bans the use of Antibiotic Growth Promoters (AGP) as animal feed additives. The prohibition of the use of antibiotics as feed additives is regulated in article 16 of MOA No. 14/2017 concerning the classification of veterinary drugs. The ban on the use of AGP in feed makes farmers doubt the efficiency of feed conversion by poultry. AGP is an antibiotic that is widely used to increase growth and reduce disease in poultry [3]. Feed additives play an important role in improving production performance in chickens by increasing the absorption capacity of the intestine (ileum) of the necessary nutrients so that ration consumption will increase accompanied by additional body weight. The growth of pathogenic bacteria can be suppressed by antibiotics accompanied by an increase in the population of beneficial bacteria in the intestine which stimulates the formation of acidic compounds and creates an uncomfortable environment for the growth of pathogenic bacteria. Antibiotics are discontinued because they trigger microorganisms to become resistant to antibiotics [4].

The national poultry sector requires innovation in the form of breakthrough feed additives that are of good quality but still use natural ingredients that are not at risk of causing resistance. Natural feed additives can be a solution to the problem of banning the use of AGP because it is considered to have the ability that is not much different from antibiotics in maintaining the health of the digestive tract of poultry, especially broilers [5]. Natural feed additives are predicted to increase in the future so that research on the effectiveness of natural feed additives with a combination of probiotics, phytobiotics, and acidifiers based on microencapsulation technology through Microwave Assisted Extraction (MAE) as a feed additive to replace AGP for improving the characteristics of broiler villi. This study aims to determine the effectiveness of the addition of natural feed additives on the characteristics of broiler villi.

Probiotics are live microorganisms in the form of bacteria that when used properly and sufficiently will have a positive effect on the host (poultry) [6]. Phytobiotics are natural bioactive components or substances derived from plants such as curcumin which contains terpenoids, alkaloids, glycosides and phenolics that are used as antimicrobial, antiparasitic, anticoccidial and immunostimulants in the poultry field [7]. Acidifiers are acidic substances such as benzoic acid, fumaric acid, and formic acid that can increase the pH of the intestinal environment so as to inhibit pathogenic microflora to grow on the intestinal mucosa and morphology of poultry, increase immunity, reduce pathogenic activity and balance the bacterial population in the intestine with acidic substances [8]. The protection process of each natural feed additive component is carried out by microencapsulation technique. Microencapsulation is a coating process carried out to protect natural bioactive compounds from quality deterioration when passing through processing and storage in various conditions for a long period of time [9].

The combination of probiotics, phytobiotics, and acidifiers is very appropriate to be applied because acidifiers are able to create an acidic atmosphere in the small intestine, so that non-pathogenic bacteria are able to maximize their activity. The non-pathogenic bacteria will produce digestive enzymes, with the addition of phytobiotics will further maximize enzyme performance in the presence of essential oils from garlic. Further research is needed to determine the effect of using a mixture of probiotics, phytobiotics, and acidifiers in the form of microencapsulation as a feed additive on the intestinal characteristics of broilers in terms of the number of villi, length of small intestinal villi, and depth of crypts.

2 Material and Method

2.1 Time and Place

Chicken rearing was carried out at the Malang Regency, Indonesia with a rearing period of 32 days. The process of making natural feed additives was carried out at the Animal Nutrition and Food Laboratory, Faculty of Animal Husbandry, Universitas Brawijaya. Histopathological testing of intestinal villi characteristics was conducted at the Research and Diagnostic Laboratory of the Healthy Animal Clinic, Tidar, Malang.

2.2 Tools and Materials

Tools used in this study include rotary evaporator, aluminum foil, whattman no 1 filter paper, oven, blender, microwave assisted extraction, mixer grinding, analytical scales, beaker glass, funnel, ceramic plate, knife, scissors, thermometer, erlenmeyer flask, basin, glass stirring rod, ziplock plastic, rubber band, black plastic, jerry can bottle, laboratory mask, dropper, volume pipette, potfilm, spoon, rearing cage, chicken feed, chicken drinker, hanging scale, hand gloves, 1 mm plywood, saw, name label, and rope.

Materials needed in the study included 75 one-day-old broilers (DOC) of CP 707 strain, garlic flour, yellow turmeric powder, apple vinegar, molasses, EM 4 farm, skim milk powder, gum arabic, distilled water, 96% ethanol, BHT, whey powder, tissue, formalin, hand sanitizer, and commercial rations produced by Pokphand.

2.3 Research Stage

The probiotics used in the study were EM-4 farms that had been cultured with molasses to produce optimal starter bacteria. Liquid probiotics were homogenized with skim milk in a ratio of 2:1 and plates with a thickness of 0.5-1 cm were taken. Probiotics were then subjected to an oven at 30-40^{o}C for 36-48 hours to form half-wet dough. In the next stage, the half-wet dough was shelled by hand and subjected to oven at 30-40^{o}C for 36 hours and then grinding for 3-5 minutes. The processed probiotics were then mixed with gum arabic and water in a ratio of 1:1:2 and then oven at 30-40^{o}C for 36-48 hours and then grinded again for 3-5 minutes.

The phytobiotics used were curcumin extract and garlic that had been processed into flour. Garlic flour mixed with curcumin extract in a ratio of 3: 1 and then added 0.075% BHT plays a role in preventing oxidation of compounds in the ingredients [10]. On the other hand, apple cider vinegar was mixed with distilled water in a ratio of 1:4. Phytobiotics were mixed with apple vinegar solution in a ratio of 1:1. Encapsulation was done with gum and whey in the ratio of 4:1 of the total ingredients. The phytobiotic and acidifier mixture was homogenized together with the encapsulant with a mixer at speed 5 for 15 minutes. The mixed solution was poured and leveled in a container and then put into Microwave Assisted Extraction (MAE) at 55^{o}C for 2-6 minutes. The distribution of probiotic, phytobiotic and acidifier combinations of feed additive products are shown in Table 1.

Table 1. Size distribution of probiotic, phytobiotic and acidifier combination feed additive products

Median (µm)	Mean (µm)	Modul (µm)	Absorbansi (µm)	Std Dev
13,39	9,86	17,14	0,85	0,48
13,53	9,87	17,14	0,85	0,49

The research test design conducted was an in vivo experiment using a completely randomized design (CRD). The sample in this test was CP 707 starter broilers with a maintenance period of 32 days. Data analysis used RAL with 3 treatments with 5 replicates and each replicate used 5 CP 707 starter broilers. Feed and drinking water were given *ad libitum* in a controlled manner with additional encapsulated feed additives with the measurements in Table 1. The treatments carried out in feeding the chickens were as follows:

- T0 : Feed without using natural additives combination of probiotics- phytobiotics- acidifier
- T1 : Feed with the addition of 0.5% probiotic-phytobiotic- acidifier combination natural additives
- T2 : Feed with the addition of 1.0% probiotic- phytobiotic- acidifier combination natural additives

2.4 Research Procedure

Observation of intestinal histopathology preparations in treated experimental animals, namely the characteristics of intestinal villi in broilers, the parameters observed were the number of villi, the length of the villi, and the depth of the crypt at 5LP (Field of View) with different magnifications of 100x which were then averaged, which were observed directly on the image and automatically using Image Raster software. This observation used a light microscope (Nikon Eclipse type Ei) with the help of an Optilab Microscope Camera connected to a computer. Data collection techniques obtained from qualitative and quantitative data are concluded based on descriptive data analysis. The data obtained were analyzed using Analysis of Variance (ANOVA) with the Complete Randomized Design (RAL) method. If the conditions are met or there is a difference, it is further analyzed using Duncan's Multiple Range Test (UJBD) to see which treatment group has a significant difference.

3 Results and Discussion

Data on the results of research on the effect of the level of use of encapsulated natural feed additives on the number of villi, length of villi, and depth of cysts in the small intestine of the ileum of 32-day-old broilers are shown in Table 2.

Table 2. Mean data on the number of villi, length of villi, depth of crypts, and surface area of villi

Treatment	Variables measured		
	Number of villi	Villi length (µm)	Kripta depth (µm)
T0	5,24±0,6^{a}	552,06 ± 47,23^{a}	225,10 ± 18,14^{a}
T1	5,88±0,9^{b}	613,55 ± 40,42^{b}	243,40 ± 23,56^{ab}
T2	6,06±0,2^{bc}	652,69 ± 33,41^{bc}	264,66 ± 15,28^{bc}

$^{a-c}$ Superscript letters in the same column indicate a very significantly different effect (P<0.01)
$^{a-b}$ Superscript letters in the same column indicate significant differences (P<0.05)

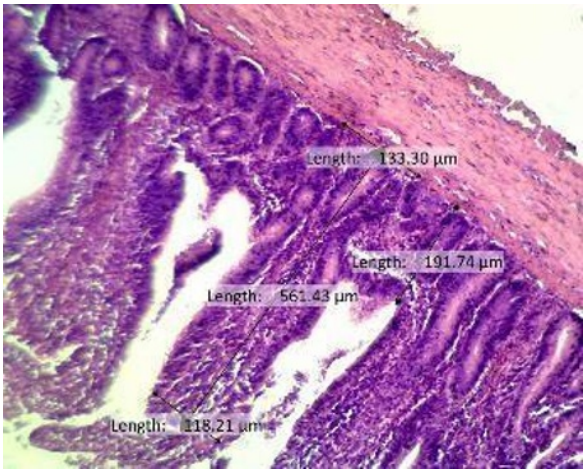

Fig. 1. The effect of combined feed additive at 0% level

Fig. 2. The effect of combined feed additive at 0,5% level

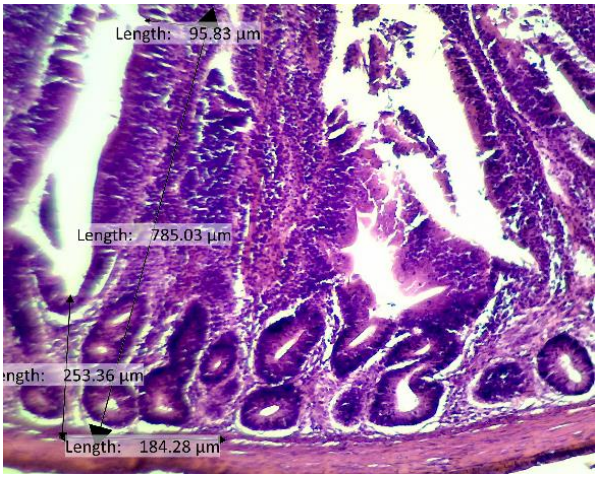

Fig. 3. The effect of combined feed additive at 1% level

3.1 Effect of Treatment on the Number of Broiler Villi

Based on the results of statistical analysis of feed treatment given showed a significantly different effect (P<0.05) on the number of villi. The average number of villi of the small intestine of broilers in Table 2 shows that the lowest number of intestinal villi in the addition of a mixture of probiotics, phytobiotics, and acidifiers is the 0% level (5.24 ± 0.6), and the highest length of small intestinal villi is the 1% level (6.06 ± 0.2). The results of the analysis of variance showed that the provision of feed additives mixed with probiotics, phytobiotics, and acidifiers at the level gave a significantly different effect (P>0.05) on the number of villi. This is thought to be because the addition of feed additives will produce enzymes that can increase digestion so as to optimize the acid atmosphere and improve the condition of villi, especially the ileum. The number and length of villi can affect the surface area of the absorption field in the small intestine so that it directly affects the characteristics of the intestine and absorption of the digestive tract will be more optimal (Zainuddin and Balqis, 2018) [11]. The results of research by Emma, et al (2013) state that the addition of lime acid can increase the height of villi and the number of villi through a decrease in ileal pH so that it is very supportive of the growth of non-pathogenic bacteria [12].

3.2 Effect of Treatment on the Length of Broiler Villi

Based on the results of statistical analysis of the feed, the treatment given showed a very significantly different effect (P<0.01) on the length of the villi. The average length of small intestinal villi of broilers in Table 2 shows that the lowest length of intestinal villi in the addition of a mixture of probiotics, phytobiotics, and acidifiers is the 0% level (552.06 ± 47.23) micrometers, and the highest length of small intestinal villi is the 1% level (652.69 ± 33.41) micrometers. The results of the analysis of variance showed that the provision of mixed feed additives of probiotics, phytobiotics, and acidifiers at the level gave a very significantly different effect (P<0.01) on villi length. This is probably because in acidic conditions the number of pathogenic bacteria is reduced due to the content of phytobiotics as an additional feed that is able to increase beneficial bacteria in the digestive tract by lowering the pH in the digestive tract. Acidic conditions in the small intestine will cause the number of pathogenic bacteria to decrease, while the number of non-pathogenic bacteria increases so that this can stimulate the development of intestinal villi length. The dayak onion phytobiotics play a role in increasing feed efficiency by reducing the population of bacterial pathogens in the digestive tract which will more effectively increase the development of the broiler small intestine [13]. The encapsulated form tends to be better because it is able to protect the material so that it is not degraded before reaching the small intestine so that it is more optimal in the absorption of food substances, this is indicated by the average villi height of the treatment group for the addition of turmeric and ginger mixture both flour and encapsulation is 584.73 µm, while the control feed is 532.00 µm [14].

3.3 Effect of Treatment on the Depth of Broiler Small Intestine Kripta

The results of statistical analysis of feed treatment given showed a significantly different effect (P<0.05) on the depth of villous crypts of broilers. The average depth of villous crypt of broilers in Table 2. shows that the lowest villus crypt depth in the addition of a mixture of probiotics, phytobiotics, and acidifiers is the 0% level (225.10 ± 18.14), and the highest villus crypt depth is the 1% level (264.66 ± 15.28). The results of the analysis of variance showed that the provision of mixed feed additives of probiotics, phytobiotics, and acidifiers at the level gave a significantly different effect (P<0.05) on the depth of villous crypts. This is thought to be because absorption is considered optimal during the digestion process.

Acidifiers and probiotics given are very helpful in improving the digestive process through lowering pH and improving the digestive system so that non-pathogenic bacteria are able to function optimally, while organic acids are able to stimulate pancreatic secretion so as to improve digestion, absorption, and retention of protein and amino acids. The supplementation of *Eschericia hirta* and acidifiers at the level can increase the depth of the broiler crypts, which is 186 μm [15].

4 Conclusion

Natural feed additives, such as combinations of probiotics, phytobiotics, and microencapsulated acidifiers, were studied for their effects on the characteristics of the villi in the ileum section of the small intestine. Adding natural feed additives at the encapsulated level in the feed increased the number, length, and depth of villi. The combination of probiotics, phytobiotics, and encapsulated acidifier at a 1.0% level in the feed showed the most significant effect compared to the 0.5% level and the control treatment.

The combination of probiotics and phytobiotics as feed additives to replace antibiotics can affect the condition of eggs. The addition of a 0.5 % - 0.75 % combination of phytobiotics and probiotics can improve the external quality of duck eggs.

References

1. Badan Pusat Statistik. Populasi Ayam Ras Pedaging menurut Provinsi (Ekor), (2022).
2. S. Pakage, B. Hartono, Z. Fanani, B. A. Nugroho, D. A. Iyai, J. A. Palulungan, Ollong, D. Nurhayati, D, *JSIP* **15**, 383-389, (2020).
3. L. K. Froebel, S. Jalukar, T. A. Lavergne, C. D. Coufal, T. Duong, J. Appl. Poult. Res **29**, 765–774 (2020).
4. F. Akbar, P. Anwar, I. Infitria, JAC **4**, 27-35 (2022).
5. Hidayat, C, Rahman, JITRO **6**, 188-213 (2019).
6. M. F. Ferdous, M. S. Arefin, M. M. Rahman, M. M. R. Ripon, M. H. Rashid, M. R. Sultana, M. T. Hossain, M. U. Ahammad, K. J. Rafiq, *J. Adv. Vet. Anim. Res* **6**, 409 – 415 (2019).
7. W. A. E. Ghany, J. World. Poult. Res **10**, 571 – 579 (2020).
8. H. M. Ramadhan, E. Widodo, I. H. Djunaidi, EAS. J. Vet. Med. Sci **2**, 50 – 54 (2020).
9. N. Mehta, P. Kumar, A. K. Verma, P. Umaraw, Y. Kumar, O. P. Malav, A. Q. Sazili, R. Domínguez, J. M. Lorenzo, Appl. Sci **12**, 1424 (2022).
10. S. Rohmawati, D. R. Pangestuti, L. Widjajanti, JKM **5**, 307-314 (2017).
11. Z. Zainuddin, Z, U. Balqis, JIM **2**, 283-287 (2018).
12. W. M. S. M. Emma, O. Sjofjan, E. Widodo, Achmanu, J. Vet **14**, 105-110 (2013).
13. S. Rijal, N. Humaidah, D. Suryanto, J. Dina. Rek **6**, 362-367 (2023).
14. M. H. Natsir, E. Widodo, Muharlien, Bul. Pet **40**, 1-10 (2016).
15. S. R. Hashemi, I. Zulkifli, H. Davoodi, M. Hair Bejo, T. C. Loh, Iran. J. Appl. Anim. Sci **4**, 95-103 (2014).