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Influence of Turmeric (*Curcuma domestica*) and Acidifier in Feed on the Ileal Development and Characteristics of Broiler

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Abstract:

This study aimed to investigate the effect of turmeric (Curcuma domestica) and acidifier in broiler feed on the ileal development and characteristics of the broiler. The study used 240 unsexed Cobb strain broilers (one day old). It was carried in a farmer's poultry house located in Buring, Kedungkandang District, Malang City. The study was conducted for five weeks, from November 12, 2022, to December 18, 2022. The study used six treatments including P0: basal feed, P1: basal feed + 2% turmeric flour + 0% acidifier, P2: basal feed + 1.5% turmeric flour + 0.5% acidifier, P3: basal feed + 1% turmeric flour + 1% acidifier, P4: basal feed + 0.5% turmeric flour + 1.5% acidifier, and P5: basal feed + 0% turmeric flour + 2% acidifier. Each treatment was repeated 4 times. Data were analyzed using analysis of variance (ANOVA), and if there were differences between treatments, followed by Duncan's Multiple Range Test. The results showed that the treatment had a significant effect ($P < 0.05$) on the length and weight of the broiler ileum. In addition, it also had a very significant effect ($P < 0.01$) on the characteristics of the ileum, including the number of villi, villus height, crypt depth, basal villus width, apical villus width, and villus surface area. Based on the results of the study, it can be concluded that a ratio of 1% turmeric and 1% acidifier feed additive resulted in the best responses.

Keywords **—Turmeric, acidifier, villi, small intestine, ileum and broiler.**

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I. INTRODUCTION

The increasing consumption of broiler meat must be matched by the existing broiler production. One of the supporting factors for success in broiler management is feed. Quality feed can meet the needs of livestock in terms of quality and quantity.

Broilers are almost impossible to show maximum productivity without feed additives. Feed additives are materials added to poultry feed that are not part of the basic nutrients in the feed formulation and are used as an effective effort to increase productivity [1].

Antibiotics are one of the feed additives commonly used by farmers to increase broiler productivity. The use of antibiotics as growth promoters reaches up to 80% in the livestock sector [2]. The improper and continuous use of antibiotics can result in harmful residues in the final product when consumed by the community. The Indonesian government, through Minister of Agriculture Regulation No. 14/PERMENTAN/-PK.350/5/2017, has banned the use of antibiotics in Indonesia since 2018. There is a need for a solution to replace antibiotics towards a healthier Indonesia.

Turmeric and acidifiers are feed additives commonly used as a substitute for antibiotics.

Adding turmeric to the feed has anti-fungal, anti-bacterial, immunomodulatory, and antioxidant activities [3]. Turmeric can be used as a natural antibiotic due to its ability to inhibit and suppress the growth of pathogenic bacteria in the digestive tract of poultry. Turmeric can increase the relaxation of the small intestine, reducing intestinal contractions, so that the digesta stays longer, and the absorption process of nutrients is more complete.

Acidifiers are organic and inorganic acids intentionally added to feed as feed additives. The principle of acidifiers is to increase the acidity of the digestive tract, inhibiting the growth of pathogenic microorganisms [4]. Acidifiers have the potential to become feed additives and antibiotic alternatives because they can kill pathogenic bacteria like *Escherichia coli* and *Salmonella sp.,* in the digestive tract.

The nutrients that enter the body of a broiler undergo the process of absorption in the digestive tract, specifically in the small intestine. The broiler's small intestine consists of three parts, namely the duodenum, jejunum, and ileum [5]. The small intestine is the main organ where digestion [6] and nutrient absorption [7] occur. The intestine's ability to absorb nutrients is determined by the development of the digestive organs [8], which correlates with the chicken's growth rate [9]. A longer small intestine and a larger villus surface area indicate good villus development and may have an impact on higher body weight [10].

The process of breaking down food in the small intestine cannot be separated from the role of microorganisms present in it. Microorganisms in the poultry digestive tract play an important role in growth and development, producing energy from breaking down fatty acids and affecting the morphology of intestinal villi [11]. After the process of breaking, the resulting nutrients are absorbed through the villi in the small intestine, then distributed through capillary blood vessels and lymph nodes throughout the body [12]. The size of the small intestine villi is closely related to the potential for nutrient absorption; the longer the small intestine villi, the more effective the nutrient absorption [13].

Based on the function and usefulness of each feed additive, this study aims to determine the effect of ratio between turmeric and acidifier on the ileal development and characteristics of broiler.

II. Material and Methods

The study was conducted from November 12 to December 18, 2022, in a farmer's poultry house located in Buring Village, Kedungkandang District, Malang City. The material used was 240 Day-Old Chick (DOC) broilers of the Cobb strain without distinguishing gender (unsexed). The average body weight of the broiler DOC used was 40.55±1.77 grams, with a coefficient of variation (CV) value of 4.35%. The cage used was a battery cage measuring 100×100×70 cm. Each experimental cage unit was filled with 10 broilers. Feed was provided *ad libitum*. The basal feed given was commercial feed produced by PT. JapfaComfeedTbk. The nutritional content of the basal feed in this study is presented in Table 1.

TABLE I. Nutritional content of basal feed

Nutrient Content	Feed code (Period)	
	BR-1 (Starter)	BR-2 (Finisher)
Water (%)	max. 12	max. 12
Ash (%)	max. 7	max. 7
Crude protein (%)	21-23	19-20
Metabolizable energy (Kcal/kg)	min. 3.000	min. 3.100
Crude fat (%)	min. 5	min. 5
Crude fiber (%)	max. 5	max. 5
Calcium (Ca) (%)	0,8-1,1	0,8-1,1
Phosphor (P) (%)	min. 0,50	min. 0,45
Aflatoxin (µg/kg)	max. 50	max. 50

Source: PT. JapfaComfeedTbk.

The turmericused was in the form of powder and bought from UPT. Materia Medica,Batu City, whilethe acidifier was obtained from Poultry Shop.The research methodology utilized in this study was an experiment by using a Completely

Randomized Design (CRD) with six treatments as follows:

T0 : Basal feed without additive.
T1 : Basal feed + 2% turmeric + 0%acidifier
T2 : Basal feed + 1,5% turmeric + 0.5%acidifier
T3 : Basal feed + 1% turmeric + 1.0%acidifier;
T4 : Basal feed + 0,5% turmeric + 1.5%acidifier;
T5 : Basal feed + 0% turmeric + 2.0%acidifier.

Each treatment was repeated four times.The chickens were raised for 35 days.

The variables measured in this study were the development of the broiler ileum, including thelength and weight of the ileum, as well as the characteristics of the broiler ileum, including the number of villi, villus height, crypt depth, basal villus width, apical villus width, and villus surface area. Sampling was performed by slaughteringone broiler from each experimental unit, removing visceral organs, and taking a sample of the ileum from the small intestine. The length and weight of the ileum were measured without digesta. Villus samples were taken by cutting 4-5 cm from the ileum, which were then stored in formalin solution (4% concentration) in a medicinal pot. The villus samples were later brought to the laboratory for preparation.

The preparation of the samples involved cutting 4 µm thick sections of the small intestine lumen using a microtome and placing them on a slide for staining using the Hematoxylin-Eosin method. The villus samples were then taken to the laboratory for the measurement of the number of villi, villus height, crypt depth, basal villus width, apical villus width, and villus surface area. The number of villi was determined by counting them in the histopathology sample preparation using an Olympus BX51TF DIC microscope with an Olympus DP 12 projector at a magnification of 4x. Villus height was measured using calibrated Image Raster software at the appropriate microscope magnification. Crypt depth was measured from the base (Lamina propria) to the villus base, and villus surface area was calculated using the formula c + d/d x a (where a = villus height, c = basal villus width, and d = apical villus width).

The collected data were then tabulated and analyzed using Analysis of Variance (ANOVA). Treatments that showed significant effects ($P<0.05$) were subsequently subjected to further analysis using Duncan's New Multiple Range Test.

III. Results and Discussions

The effect of the addition of turmeric flour and acidifier in feed on ileum length, ileum weight, and broiler ileum characteristics, including the number of villi, villi height, crypt depth, basal villi width, apical villi width, and villi surface area are presented in Table 2.

TABLE II. The effect of treatments on the ileal development and characteristics of broiler.

Variables	Treatments					
	P0	P1	P2	P3	P4	P5
IleumLength (cm)*	83.75±2.36^{a}	85.25±2.99^{ab}	89.25±4.03^{bc}	92.25±2.50^{c}	91.75±5.56^{c}	84.75±3.59^{ab}
Ileum Weight(g)*	17.83±1.62^{a}	18.18±0.64^{a}	19.75±1.20^{b}	20.03±0.92^{b}	19.90±1.49^{b}	18.38±0.57^{ab}
Numberof Villi**	61.75±1.71^{A}	65.89±1.32^{A}	72.88±2.04^{B}	74.34±2.51^{BC}	78.20±3.33^{C}	70.98±1.98^{B}
Villi Height (µm)*	602.40±9.34^{a}	642.60±10.66^{b}	653.13±11.49^{b}	675.25±21.93^{b}	662.48±53.48^{b}	644.13±9.57^{b}
Crypt Depth (µm)**	156.78±8.25^{A}	162.33±3.00^{A}	170.40±4.59^{AB}	182.65±9.86^{B}	177.25±3.45^{B}	167.38±9.37^{AB}
Basal Villi Width (µm)**	124.50±10.54^{A}	128.25±7.80^{AB}	139.50±9.71^{AB}	159.50±6.86^{C}	144.25±3.50^{BC}	135.50±9.88^{AB}
Apical Villi Width (µm)**	41.50±1,29^{A}	43.10±1.30^{AB}	46.97±2.66^{B}	47.18±1.59^{B}	46.84±2.63^{B}	46.26±1.93^{B}
Villi Surface Area (μm^2)**	2412.22 ±184.94^{A}	2554.47 ±108.55^{A}	2601.94 ±235.00^{AB}	2960.89 ±184.57^{B}	2701.70 ±136.74^{AB}	2535.32 ±202.78^{A}

Note:

(*) Significant effect (P<0.05) indicated by different superscripts in the same column (a-c)
(**) Highly significant effect (P<0.01) indicated by different superscripts in the same column (A-C)

a). The effect of the treatment feed on ileal length and weight

The digestive tract is an intermediary organ between the internal and external environment with the main function of digestion and nutrient absorption. Therefore, the morphological characteristics of the digestive tract, especially the small intestine in broilers, determine the function of the intestine in broiler growth and development [18]. A healthy digestive tract is characterized by the development of the weight and length of the digestive tract and theoptimal development of intestinal villi [19]. The ability of the intestine to absorb nutrients is determined by the development of the digestive tract organs that correlate with the level of broiler growth [8].

The treatment has a significantly different effect on ileum length and weight (Table 2). The best treatments for increasing the length and weight of the ileum small intestine are found in treatments P3 and P4. Statistically, there is no difference between these two treatments, but they are significantly difference from other treatments. This result is likely due to each treatment feed having reached its maximum limit. If given in excess concentration, it will not provide a significant effect and will be wasted through excreta. This is in line with research conducted by [20], which shows that the compound curcumin can stimulate the gallbladder to produce bile fluid that is rich in lipase, amylase, and protease enzymes that are useful for optimal nutrient absorption in the digestive tract.

The administration of *Curcuma* extract at a dosage of 2-4% /kg of feed for 3-6 weeks can increase body weight and optimize the metabolic process, making feed utilization more efficient. Other studies have shown that the use of synthetic acid at a level of 0.8-1.2% as an acidifier in the step-down period of starter and finisher feed has an effective effect in increasing the weight and length of the small intestine, especially the duodenum, and the final body weight of broilers [21]. The use of encapsulated acidifiers or herb-acidifier combinations as feed additives to increase lactic acid bacteria, histological characteristics of the intestine, and serum, while also reducing *Escherichia coli* and *Salmonella sp.* The encapsulated herb-acidifier combination has been shown to have the same effect in stabilizing gut microflora [22].

b). The effect of treatment on the number of ileal villi

Villi are small protrusions found on the mucosa of the small intestine [23], with hundreds to millions of villi present [24]. The number of villi is closely related to the intestine's ability to absorb nutrients from feed [25]. The more villi in the intestine, the larger the nutrient absorption area, resulting in an increase in broiler chickens' body weight [26]. The treatment had a significantly different effect (Table 2) on the number of ileal villi in broiler chickens. The results of this study differ from those of [14], which explained that adding natural acidifiers at a concentration of 0.8% did not have a significant effect on the number of villi. Many factors support the increase in the number of villi in the intestine, one of which is the microflora found within it [27]. The expansion of the nutrient absorption area depends on the dominant microflora in the intestine. Lactic acid bacteria (LAB) are non-pathogenic bacteria that can prevent the attachment and erosion of intestinal villi by pathogenic bacteria such as *Escherichia coli*and*Salmonella sp.* [28]. The administration of acidifiers in feed can lead to the dominance of LAB, as acidifiers can create an acidic environment reducing the number of pathogenic bacteria colonies that are intolerant to acid. In this way, the destruction of villi by pathogenic bacteria can be stopped.

The increase in the number of villi correlates with the resulting length of the ileum. The increase in weight and length are accompanied by an increase in the size of the small intestinal cavity, which affects the absorption process [29]. The

growth performance of chickens highly depends on digestion and nutrient absorption, meaning that longer intestines will enhance absorption and produce maximum growth. The best feed treatment for increasing the number of villi in this study was found in treatment P4 (Table 2), which involves the addition of 0.5% turmeric and 1.5% acidifier. This result may have occurred because the dosage of each feed additive has reached its maximum limit, so if given at a higher concentration, it would be excreted by the broiler [30]. The administration of turmeric (*Curcuma longa*) at 0.5% (5 g/kg feed) in the feed might provide the maximum effect on broiler performance.

Turmeric has antibacterial properties that can suppress the growth of pathogenic bacteria [31]. The antibacterial properties of turmeric can provide an antagonistic role (resistance) against the growth of pathogenic bacteria and can also promote the growth of beneficial bacteria [32]. The addition of 1% turmeric in feed can reduce 48% of the total bacteria in the small intestine of broiler chickens [33]. This statement suggests that using more than 1% turmeric alone may result in a higher number of bacteria in the small intestine dying, including not only pathogenic bacteria but also non-pathogenic ones. Therefore, the use of a single type of herbal ingredient should be combined with other feed additives to achieve a synergic effect in improving broiler performance.

Another study shows that the use of synthetic acids at levels of 0.8-1.2% as acidifiers in step-down feed for the starter and finisher periods has an effective impact on increasing the weight and length of the small intestine, especially the duodenum, and the final body weight of broilers [21]. The use of encapsulated acidifiers or a combination of herb-acidifiers as feed additives to increase lactic acid bacteria, intestinal histological characteristics, and serum, while reducing *Escherichia coli* and *Salmonella sp*., has been proven to have a similar effect in stabilizing gut microflora [22]. Acidifiers are beneficial in protecting feed from microbes and fungi that can interfere with the digestive process, as well as increasing fiber and protein absorption during digestion [34].

c). The effect of treatment on the height of ileal villi

The increase in villi height and width is related to the villi surface area for nutrient absorption into the bloodstream [23]. An increase in villi height in the duodenum, jejunum, and ileum of broiler chickens is consistent with the improvement of digestive function and absorption due to the expanded nutrient absorption area. The small intestine of broiler chickens with a heavier body weight is characterized by a longer and wider small intestinal absorption area.

The treatment had a significantly different effect on ileal villi height (Table 2) compared to the control treatment (P0). Based on these results, it can be concluded that the addition of single feed additives or a balance between turmeric and acidifiers has an impact on ileum villi height. [35] Turmeric, which contains curcumin, can trigger intestinal epithelial cells to stimulate more Antimicrobial Peptides (AMPs), which are peptide proteins that function as a defense mechanism against harmful pathogenic bacteria [31]. The antibacterial properties of turmeric can suppress the number of pathogenic bacteria and increase beneficial bacteria [36]. Turmeric can play a role in controlling and limiting the growth of colonization from various pathogenic and non-pathogenic bacterial species in chicken intestines, resulting in a balanced gut microbial ecosystem. In addition to turmeric, acidifiers containing organic acids play a role in increasing ileum villi in broilers [37]. Acidifiers help maintain pH and create an acidic environment suitable for the growth and development of beneficial bacteria (lactic acid bacteria or LAB), allowing them to dominate the digestive tract.

Many factors affect the height of intestinal villi, including stress levels, chemical compounds in the intestine, feed factors, disease infections, and the colonization of beneficial microbes in the gut [38]

and [39]. The colonization of beneficial bacteria (lactic acid bacteria or LAB) can halt the damage to intestinal villi caused by pathogenic bacterial colonies [40] by repairing the villi surface and releasing bioactive compounds. This process leads to the strengthening of the gut barrier, which directly modifies the function of epithelial cells, including cytokine and chemokine release. The antibacterial properties of herbal ingredients can play a role in resisting pathogenic bacterial colonies and preventing intestinal villi damage [32]. The increase in villi height is due to the increased short-chain fatty acids induced by probiotics or LAB. These short-chain fatty acids can stimulate the proliferation of intestinal epithelial cells, resulting in a larger nutrient absorption area [41].

d). Effect of treatment on ileal crypt depth

Crypts are temporary storage sites for feed nutrients resulting from the breakdown of feed nutrients before being distributed to other organs through blood vessels [42]. The depth of the crypts is one measure of the maximum digestive process, with higher crypt depth values indicating more feed nutrients being absorbed [23]. The absorption of nutrients is greatly influenced by the cells present in the small intestinal villi, as these cells have the ability to absorb nutrients in the feed. These cells are formed in the crypts as rapidly developing mature cells, also known as immature proliferative cells, which slowly differentiate and move up toward the villi [43]. Low villi and decreased crypt depth will reduce the activity of enzymes secreted by the villi tips [24]. The ratio of villi height to crypt depth is an indication of an increasingly larger area for nutrient absorption [44]. The shortening of the small intestinal villi is associated with a decrease in nutrient absorption, intestinal gland secretion, and decreased performance.

Table 2 shows that the addition of turmeric and acidifiers significantly affects the depth of ileal crypts in broiler chickens. The best treatment in this study was found in treatment P3, which increased crypt depth by 16.67% compared to the control feed's crypt depth or P0. Based on this, it can be concluded that the administration of a ratio between turmeric and acidifiers at a level of 1% for each in feed can synergize and perform their roles in improving the absorption process of feed nutrients, as evidenced by the crypt depth.

Several supporting factors for the development of crypt depth can be used as an indication of the results obtained, one of which is the domination of beneficial bacteria, which is one of the reasons for the difference in results obtained. This is because beneficial bacteria can maximize nutrient breakdown and maintain the integrity of intestinal villi from erosion caused by pathogenic bacteria. Previous reports [40] and [15] explain that the colonization of beneficial bacteria (lactic acid bacteria or LAB) can halt the damage to intestinal villi caused by pathogenic bacterial colonies by repairing the villi surface and releasing bioactive compounds, leading to the strengthening of the gut barrier, which directly modifies the function of epithelial cells, including cytokines and chemokine release.

The antibacterial properties of herbal ingredients can play a role in resisting pathogenic bacterial colonies and preventing intestinal villi damage [32] and [41]. The increase in villi height is due to the increased short-chain fatty acids induced by probiotics or LAB. These short-chain fatty acids can stimulate the proliferation of intestinal epithelial cells, resulting in a larger nutrient absorption area. In addition to the role of turmeric, acidifiers also play a role in the domination of beneficial bacteria. As explained by [14], the addition of an acidifier from lime at a level of 0.8% can increase the number of villi, increase ileal villi height and crypt depth in broiler chickens. This is because lime acid can lower the ileum pH, which

greatly supports the growth of beneficial bacteria such as LAB [22]. The use of encapsulated acidifiers or herbal-acidifier combinations as feed additives to increase lactic acid bacteria, intestinal histological characteristics, and serum, while reducing *Escherichia coli* and *Salmonella sp*. The encapsulated herbal-acidifier combination has been proven to have the same effect in stabilizing gut microflora [21]. The use of synthetic acid at a level of 0.8-1.2% as an acidifier in the starter and finisher step-down period feed provides an effective effect in increasing the weight and length of the small intestine, especially the duodenum, and the final body weight of broilers.

e). The effect of treatment on the surface area of ileal villi

The surface area of the villi is the area of nutrient absorption performed by the digestive organ of the small intestine. The higher the surface area of the small intestine villi, the larger the area for nutrient absorption from a feed. The results of studies [46]; [47]; and [48] show that the increase in the size of villi and depth of crypts in the small intestine of broiler chickens is followed by an increase in the surface area of the small intestine villi. The surface area of absorption has a strong correlation with the process of nutrient absorption from feed [50]. A large intestinal surface area will increase the ability to absorb nutrients from feed during the digestion process.

Table 2 shows that the addition of turmeric and acidifiers has a very significant difference effect on the surface area of ileal villi in broiler chickens. The increase in villi height and width is closely related to the surface area of villi produced for the absorption of nutrients that enter the bloodstream [51]. The increase in villi height and depth of the avian digestive tract crypts is positively correlated with digestive function and absorption capacity. The wider the villi, the more nutrient absorption occurs, which ultimately leads to increased growth and carcass weight [52]. The increase in villi width is in line with the growth of chickens, as villi function to expand the surface area of the small intestine and have a significant impact on the food absorption process.

The increase in villi height is related to the increase in the number of epithelial cells surrounding it [44]. Villi height can be linked to the active process of epithelial cell division in the small intestine. This can be linked to the increase in the surface area of the small intestine villi for nutrient absorption. Conversely, shortening of small intestine villi is associated with decreased nutrient absorption, intestinal gland secretion, and performance decline [38]. The surface area of the villi can be caused by the ability of small intestine epithelial cells to maintain the integrity of the intestinal villi from attacks by pathogenic bacteria and toxic compounds that can damage the villi [53]. A large small intestine villi size will maximize the process of nutrient absorption.

The best treatment in this study is found in treatment P3, which is the addition of 1% turmeric and 1% acidifier. Increasing the amount of single turmeric (2%) and single acidifier (2%) in treatments P1 and P5 did not have an effect compared to the control treatment. This result is in line with the study conducted by [54], where giving turmeric in broiler feed at doses of 0.1%, 0.2%, and 0.4% resulted in a decrease in feed consumption as to the dose given increase. This is due to the effect of essential oil on the function or work of the digestive tract, especially the small intestine, and it is toxic when given in excessive doses [21]. The administration of synthetic or natural citric acid at the appropriate dose effectively lowers the overall pH of the digestive tract. The use of synthetic citric acid at a level of 0.8% as an acidifier in the starter and finisher step-down period feed is effective in reducing the pH of the small intestine and the

number of *Escherichia coli* bacteria while increasing the population of BAL. The addition of biacid as a feed additive containing acidifiers and essential oils in broiler feed at a level of 0.075% can affect the number and surface area of the small intestine villi as well as the depth of the crypts.

IV. Conclusion

The addition of turmeric and acidifiers in broiler feed with a concentration of 1% turmeric and 1% acidifier can increase the number of villi, villi height, crypt depth, basal width, and apical width, resulting in a larger surface area beneficial for improving nutrient absorption from feed.

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