Short Research Article

Effect of Turmeric (*Curcuma domestica*) Powder and Multi-Enzyme Supplementation on Intestinal Villus Morphology in Broiler Chickens

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ABSTRACT

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| **Aims:**This study aimed to evaluate the effect of turmeric powder (Curcuma domestica Val.) and multi-enzyme supplementation on intestinal villus morphology, particularly villus height, surface area, and number, in broiler chickens.  **Study Design:** The experiment was conducted using a Completely Randomized Design (CRD) with five dietary treatments and six replications per treatment.  **Place and Duration of Study:** The research was carried out at the Poultry Production Laboratory, Faculty of Animal Science, Universitas Brawijaya, Indonesia, over a period of 6 weeks.  **Methodology:** This study employed a Completely Randomized Design (CRD) with five treatments and five replications. A total of 300 day-old Lohmann broiler chickens were randomly allocated into five treatment groups: P0 (control diet), P1 (basal diet + 0.5% turmeric powder), P2 (basal diet + 1.0% turmeric powder), P3 (basal diet + 0.5% turmeric powder + 0.1% multienzyme), and P4 (basal diet + 1.0% turmeric powder + 0.1% multienzyme). Each experimental unit consisted of 12 birds per pen, resulting in 60 birds per treatment group. At day 35, jejunum samples were collected from one bird per replicate (n=5 per treatment) for histological examination. Tissue sections were prepared using standard paraffin embedding and stained with Hematoxylin-Eosin (H&E). Villus height, surface area, and number were measured under 40× magnification using image analysis software. Data were analyzed using one-way ANOVA followed by Duncan's Multiple Range Test at P<0.05 significance level.  **Results:** There were no statistically significant differences (P > 0.05) in villus height, surface area, or number among treatment groups. However, descriptively, treatment P4 (1.2% turmeric + enzyme) showed the highest average values for villus height (673.76 µm), surface area (1056.16 µm²), and number (9.12 per transverse cut), compared to the control group.  **Conclusion:** Although statistical significance was not observed, the combination of turmeric powder and multi-enzyme supplementation showed a positive numerical trend in improving the morphology of intestinal villi in broiler chickens. This suggests potential benefits in gut health and nutrient absorption efficiency |

*Keywords: turmeric flour, multienzyme, villus height, villus surface area, villus number, broiler chicken, gut morphology*

1. INTRODUCTION

Broiler chickens are a high-producing poultry breed known for their rapid growth and efficient meat production, making them a promising source to fulfill the community's animal protein needs. Their short growth phase, typically between 28–35 days, makes broilers an economically viable livestock option compared to others. According to Statista (2023a), chicken stock in Indonesia amounted to approximately 3.48 billion heads in 2022, demonstrating the massive scale of broiler production in the country. This substantial population reflects the growing demand for chicken meat, influenced by rising population numbers, income levels, and public awareness of the importance of animal protein consumption. The growing consumption is evidenced by per capita poultry meat consumption that stood at 11.63 kg in 2022, while in 2023 the consumption increased to 12.58 kg (Knowledge Sourcing Intelligence, 2024). Moreover, the affordable price of chicken meat contributes to its popularity. This significant growth is also supported by improved management, including high-quality feed provision in both quantity and nutritional value, with 310 establishments involved in broiler chicken production in Indonesia in 2023 (Statista, 2023b).

Livestock production is influenced by several key factors, including genetics, husbandry, and feed. Feed plays a crucial role, both economically and in terms of productivity, accounting for 60–70% of total production costs (Intelia, 2025). This high proportion of feed costs makes nutritional management a critical component of profitable livestock operations. The heavy reliance on imported raw materials such as corn, soybeans, and fish meal negatively impacts the poultry industry in Indonesia, making feed prices highly dependent on international market dynamics. This dependency on imports creates considerable fluctuations in feed prices, significantly affecting production costs and market prices, which poses challenges for the sustainability of Indonesia's poultry sector. The high volume of soybean imports also increases foreign exchange expenditure, further straining the economic viability of local producers. To address these challenges and optimize production efficiency, feed cost efficiency must be improved through enhanced nutrient digestibility and absorption in the digestive tract. This approach directly aligns with the economic pressures faced by producers, as improved feed conversion efficiency can reduce the overall cost burden while maintaining or improving production performance. One of the primary strategies to improve feed efficiency is the use of feed additives (McDonald et al., 2011). Feed additives are supplementary ingredients added to animal feed with the aim of enhancing nutritional value, improving feed utilization efficiency, digestive function, nutrient absorption, and maintaining gut health. These additives also support growth acceleration, immune enhancement, improved production performance, and overall animal health (Windisch et al., 2008; Castanon, 2007). They can be in the form of vitamins, minerals, amino acids, enzymes, probiotics, prebiotics, antioxidants, synbiotics, phytobiotics, and other functional substances. In broiler farming, disease outbreaks are common, prompting farmers to use antibiotics as treatment and to reduce mortality. However, the use of antibiotics as feed additives can lead to residues that pose risks to humans through the development of antibiotic-resistant bacteria (Marshall & Levy, 2011). This concern led the Indonesian government to implement regulatory measures to restrict antibiotic use in animal feed. The prohibition was established through Ministry of Agriculture Regulation No. 14 of 2017, which prohibits antibiotic use according to the Classification of Veterinary Drugs, with the regulation becoming effective from January 1, 2018. To comply with this policy and maintain production efficiency, the adoption of alternatives such as natural feed additives (probiotics, prebiotics, phytobiotics, organic acids, and essential oils) and enhanced biosecurity measures has become necessary (Huyghebaert et al., 2011; Castillo et al., 2006). These alternatives not only help maintain animal health and performance but also contribute to sustainable livestock production practices that align with consumer demands for antibiotic-free animal products.

Feed additives can be administered in the form of phytobiotics and enzymes, representing innovative approaches to sustainable livestock production. Phytobiotics are natural plant-derived additives used in livestock feed to improve animal health and productivity (Windisch et al., 2008). Phytogenic feed additives (phytobiotics) are defined as plant-derived or biological origin feed ingredients used in the diets to improve the productivity of livestock or poultry, encompassing a wide range of botanical compounds including essential oils, herbs, oleoresins, and plant extracts. These compounds possess antimicrobial properties that help control the growth of pathogenic bacteria in the digestive tract while maintaining beneficial gut microbiota. The in vitro minimum inhibitory concentration assay showed strong antibacterial activity of the EO product, thymol, and carvacrol against pathogenic Escherichia coli, C. perfringens, and Salmonella strains, and weak activity towards beneficial Lactobacillus strains. This selective antimicrobial action is particularly valuable as it promotes gut health without disrupting the beneficial bacterial populations essential for optimal digestion. Phytobiotics also stimulate digestive enzyme production and help maintain gut microbiota balance, thereby enhancing nutrient digestion and absorption (Yang et al., 2009). The bioactive compounds present in phytobiotics, such as essential oils and phenolic compounds, can improve intestinal morphology by increasing villus height and crypt depth, which directly correlates with enhanced nutrient absorption capacity (Hashemipour et al., 2013). As reported by Kiczorowska et al. (2017), phytobiotics in poultry nutrition have been shown to promote growth, improve feed efficiency, enhance intestinal morphology, and act as antimicrobials, thus boosting immune defenses. These multifaceted benefits make phytobiotics particularly attractive for modern livestock production systems seeking to optimize performance while maintaining animal welfare standards. Thymol and carvacrol as natural essential oils and phenol compounds are components derived from some medicinal plants, such as thyme and oregano species. Modern research indicates that "supplementing broiler chickens' diet with a phytogenic product containing a mixture of equal parts thymol and carvacrol at four different amounts increased body weight gain and feed efficiency, and reduced feed intake" (Kiczorowska et al., 2024). Improved body weight gain and feed efficiency was detected in broilers with other essential oils such as thymol and cinnamaldehyde, essential oils present in oregano, coriander, clove and star anise, demonstrating the consistent efficacy of these compounds across different studies and formulations. The mechanism of action of thymol and carvacrol involves multiple pathways, including membrane disruption in pathogenic bacteria, antioxidant activity, and immunomodulatory effects. Research has shown that increased TJP gene expression and improved intestinal barrier function were observed in thymol and carvacrol-treated broilers challenged with Clostridium, indicating their role in maintaining gut integrity under pathogenic challenges.Due to concerns over antibiotic resistance caused by excessive antibiotic use in animal feed, phytobiotics offer a safer and more environmentally friendly alternative (Huyghebaert et al., 2011). The European Food Safety Authority (EFSA) and other regulatory bodies have recognized the potential of phytobiotics as natural alternatives to antibiotic growth promoters, leading to increased research and commercial application in livestock production systems worldwide.

Furthermore, phytobiotics demonstrate additional benefits beyond antimicrobial activity, including antioxidant properties that can improve meat quality and shelf-life, stress reduction capabilities, and potential prebiotic effects that support beneficial gut microbiota proliferation (Hashemi & Davoodi, 2011). These comprehensive benefits position phytobiotics as valuable tools in the transition toward antibiotic-free livestock production systems.

One herbal plant widely used as a phytobiotic is turmeric (Curcuma domestica Val.), which contains the active compound curcumin with known antibacterial properties. According to Jubair et al. (2021), antimicrobial compounds are specialized chemicals produced by living organisms in low concentrations that can inhibit critical processes within microorganisms. Curcumin possesses broad-spectrum antibacterial activity effective against both Gram-positive and Gram-negative bacteria, and it also has antiviral and pro-apoptotic (anti-tumor) effects. Furthermore, curcumin has been shown to stimulate digestive processes by influencing pancreatic enzyme secretion. Research demonstrates that curcuminoids can affect pancreatic lipase activity, which is crucial for fat digestion (Li et al., 2022). This mechanism improves digestion, increases feed digestibility, and speeds up digestive transit time, which in turn enhances feed intake in broilers.

In poultry, enzymes play a vital role in digestion, metabolism, and nutrient absorption. Farmers often supplement broiler diets with enzymes either through feed or drinking water. Enzymes are considered safe and beneficial, as they not only improve feed quality but also enhance meat nutritional value for human consumption. Supplemental enzymes help maintain gut microbial balance by ensuring that more nutrients are digested and absorbed in the small intestine, limiting the nutrients available for pathogenic bacteria in the large intestine. This reduces digestive disturbances and promotes overall gut health.

The application of multi-enzyme complexes in livestock feed has been extensively studied and proven effective. Each enzyme has a specific function in breaking down hard-to-digest components, making nutrients more bioavailable. The addition of enzyme complexes such as protease, cellulase, and hemicellulase has been shown to significantly improve livestock performance. These enzymes work synergistically to break down complex feed substances, increasing feed efficiency and supporting animal growth. By breaking down complex feed compounds, digestion becomes more effective, and nutrient absorption is optimized.

Therefore, supplementation of broiler diets with a combination of turmeric and multi-enzymes can serve as a natural alternative to antibiotics. The antimicrobial effect of turmeric enhances intestinal relaxation, slows peristalsis, and extends ingesta retention time in the small intestine, allowing for more efficient nutrient absorption. Meanwhile, multi-enzymes break down fiber into simpler, more absorbable components, improving gut health and productivity as indicated by intestinal microflora composition.

Based on this rationale, research is needed to evaluate the effects of combining turmeric powder (Curcuma domestica Val.) and multi-enzymes on antibacterial activity and the intestinal characteristics of broiler chickens.

2. material and methods

**2.1 Study Design and Experimental Animals**

This research was conducted using a Completely Randomized Design (CRD) with five treatments and five replications. A total of 300 day-old Lohmann broiler chickens were used, with 12 birds placed in each pen (1 m × 1 m × 0.5 m). The chickens were housed under standard management conditions with ad libitum access to feed and water. The study was conducted over a 35-day rearing period in a controlled environment with 24-hour lighting for the first week, then 16 hours of light and 8 hours of darkness thereafter.

**2.2 Feed Preparation and Treatment Groups**

**2.2.1 Turmeric Flour Preparation**

Fresh turmeric rhizomes (Curcuma domestica Val.) were obtained from local markets, cleaned, and sliced into thin pieces (2-3 mm thickness). The sliced turmeric was dried in an oven at 60°C for 48 hours until the moisture content reached approximately 10%. The dried turmeric was then ground using a hammer mill and sieved through a 60-mesh screen to obtain turmeric flour. The turmeric flour was analyzed for curcumin content using High-Performance Liquid Chromatography (HPLC) and contained 3.2% curcumin.

**2.2.2 Multienzyme Supplement**

Commercial multienzyme supplement (Enzyme-Pro®) containing protease (10,000 U/g), amylase (8,000 U/g), cellulase (2,000 U/g), and lipase (1,500 U/g) was used in this study.

**2.2.3 Treatment Groups**

The experimental treatments were as follows:

* **T0 (Control)**: Basal diet without turmeric flour or multienzyme supplement
* **T1**: Basal diet + 0.5% turmeric flour
* **T2**: Basal diet + 1.0% turmeric flour
* **T3**: Basal diet + 0.5% turmeric flour + 0.1% multienzyme supplement
* **T4**: Basal diet + 1.0% turmeric flour + 0.1% multienzyme supplement

The basal diet was formulated to meet the nutritional requirements of broiler chickens according to NRC (1994) standards, containing 21% crude protein and 3,000 kcal/kg metabolizable energy for the starter period (0-21 days) and 19% crude protein and 3,100 kcal/kg metabolizable energy for the finisher period (22-35 days).

**2.3 Villus Histomorphology Observation**

Histomorphological examination of the small intestine was conducted at the end of the rearing period (day 35). One bird per replicate was randomly selected and humanely euthanized following institutional animal care guidelines. A segment of the jejunum (approximately 3 cm in length) was collected from the middle portion, cleaned with physiological saline (0.9% NaCl), and immediately fixed in 10% neutral buffered formalin for 24–48 hours.

Tissue processing was performed using the standard paraffin embedding method (Bancroft & Gamble, 2008). Fixed tissues were dehydrated through ascending concentrations of ethanol (70%, 80%, 90%, 95%, and 100%), cleared with xylene, embedded in paraffin wax, and sectioned at 5 μm thickness using a rotary microtome (Leica RM2125RT). The sections were mounted on glass slides and stained using hematoxylin-eosin (H&E) staining following standard protocols. Histological slides were observed using a light microscope (Olympus CX23) at 40× magnification.

**2.4 Parameters Observed**

For each slide, five randomly selected fields were examined, and the following parameters were measured:

* **Villus Height (μm)**: Measured from the base of the villus (at the crypt opening) to the tip of the villus using image analysis software (ImageJ) with calibrated measurements
* **Villus Width (μm)**: Measured at the middle portion of the villus
* **Villus Surface Area (μm²)**: Calculated using the formula:

Villus surface area = 2π × (villus width/2) × villus height

* **Number of Villi**: Counted directly from five randomly selected fields per slide under 40× magnification

**2.5 Statistical Analysis**

The data obtained were analyzed using one-way Analysis of Variance (ANOVA) with SPSS software version 25.0. Normality and homogeneity of variance were tested using Shapiro-Wilk and Levene's tests, respectively. When significant differences (P < 0.05) were found, post-hoc analysis was conducted using Duncan's Multiple Range Test to determine the differences among treatment groups. Results were presented as means ± standard error of the mean (SEM).

3. results and discussion

**3.1 Villus Height**

The results of villus height measurements following turmeric flour and multienzyme supplementation are presented in Table 1. The villus height values, from highest to lowest, were observed in T4 (673.76 ± 142.46 μm), T3 (652.86 ± 108.00 μm), T2 (619.57 ± 125.11 μm), T1 (597.29 ± 73.25 μm), and T0 (501.72 ± 91.16 μm), respectively. Statistical analysis revealed that the addition of multienzyme and turmeric flour supplementation had no significant effect (P > 0.05) on villus height in broiler chickens.

This finding aligns with previous research indicating that moderate levels of herbal supplementation may not always produce significant morphological changes in intestinal structure. According to Alagawany et al. (2018), the effectiveness of phytogenic feed additives on intestinal morphology depends on various factors including dosage, duration of supplementation, and the specific bioactive compounds present. The lack of significant differences might be attributed to insufficient concentrations of enzymes or turmeric flour to induce substantial structural changes in the intestinal mucosa. Morphological adaptations such as increased villus height typically require prolonged and sustained stimulation; therefore, suboptimal dosages or short administration periods may result in minimal changes, with additive effects yielding only marginal improvements.

The competitive dynamics between beneficial and pathogenic bacteria in the gut may also influence the effectiveness of feed additives. Pandit et al. (2018) suggested that suboptimal growth of lactic acid bacteria (LAB) in the small intestine, which competes with Escherichia coli for nutrients, may limit LAB performance in enhancing nutrient absorption through villus elongation and surface expansion. This bacterial competition could potentially reduce the morphological benefits of dietary supplements.

Although statistically non-significant, treatment T4 (1.0% turmeric flour + 0.1% multienzyme) achieved the highest villus height descriptively. Yadav and Jha (2019) explained that LAB colonization in the small intestine remains suboptimal due to nutrient competition with E. coli, preventing complete establishment in the villus area and limiting their role in enhancing absorption capacity. Despite suboptimal dosing in this study, turmeric supplementation demonstrates potential benefits on intestinal morphology. Recent research confirms that turmeric's antimicrobial properties can suppress pathogenic bacteria while supporting beneficial microbiota, contributing to improved digestive health in poultry (Kiczorowska et al., 2024).

**3.2 Villus Surface Area**

The effects of turmeric flour and multienzyme combinations on villus surface area are presented in Table 1. Surface area values increased progressively from T0 (768.91 ± 198.93 μm²), T1 (927.05 ± 146.67 μm²), T2 (998.47 ± 187.06 μm²), T3 (1030.64 ± 224.42 μm²), to T4 (1056.16 ± 234.97 μm²). Statistical analysis indicated no significant effect (P > 0.05) of supplementation on villus surface area.

Although not statistically significant, the numerical trend showed that feed supplementation with turmeric and multienzymes resulted in greater villus surface area compared to the control group. This suggests that enzyme addition positively influences intestinal morphology, particularly the villi structure, which is crucial for nutrient absorption efficiency. Expanded villus surface area directly correlates with enhanced nutrient uptake capacity from intestinal digesta. The efficacy of multienzyme supplementation depends on appropriate dosage and feed composition compatibility. Enhanced nutrient availability through enzymatic breakdown stimulates epithelial cell proliferation, increasing both villus length and width, thereby expanding the absorptive surface area.

Contemporary research demonstrates that microbial growth is enhanced by enzymatic activity that breaks down complex carbohydrates into simple sugars and proteins into amino acids, both essential for microbial nutrition (Borda-Molina et al., 2018).

The bioactive compounds in turmeric, particularly curcumin, possess antimicrobial properties that can suppress pathogenic bacteria such as Salmonella spp. Turmeric also contains curcuminoids with antioxidant properties and essential oils with antimicrobial and anti-inflammatory effects (Dhama et al., 2015). By reducing pathogenic microbial populations, normal gut flora can flourish, supporting mucosal integrity and promoting villus development. Curcumin additionally stimulates intestinal epithelial cell proliferation, promoting villus growth and elongation, which enhances nutrient absorption efficiency. Furthermore, turmeric supports the growth of beneficial gut microbiota, indirectly improving villus structure and function (Kiczorowska et al., 2024).

**3.3 Villus Number**

The results of villus number evaluation following turmeric flour and multienzyme supplementation are shown in Table 1. Villus counts, from lowest to highest, were recorded as T0 (6.44 ± 0.91), T2 (7.88 ± 3.68), T3 (8.15 ± 2.54), T1 (8.24 ± 1.26), and T4 (8.44 ± 1.87). Statistical analysis revealed no significant differences (P > 0.05) in villus number among treatments.

Despite the absence of statistical significance, numerically, feed supplemented with turmeric and multienzymes resulted in higher villus counts compared to the control group, with T4 showing the highest count and T0 the lowest. Contemporary research emphasizes that multienzyme supplementation helps maintain gut health, improve feed utilization efficiency, and reduce feed costs by breaking down complex nutrients into simpler, more readily absorbable forms (Bedford and Cowieson, 2012). Studies have noted that multienzymes facilitate nutrient metabolism while supporting morphological adaptations in the digestive tract (Cowieson and Roos, 2016).

Histologically, intestinal villi play crucial roles in nutrient absorption efficiency. Longer, more numerous, and denser villi increase the total surface area available for efficient nutrient uptake. Increased villus height reflects expanded absorption capacity, while increased villus number indicates healthy and active intestinal tissue renewal, directly improving growth performance through optimized nutrient utilization. Research has demonstrated that phytobiotics, plant-derived feed additives known for their antimicrobial, antioxidant, immunomodulatory, and growth-promoting properties, have emerged as promising natural alternatives to conventional growth promoters.

Turmeric flour functions as a natural feed additive that supports gut health maintenance. Its bioactive compounds stimulate digestive enzyme activity and promote beneficial bacterial colonization, indirectly enhancing histological structure including villus number. Research has explained that reduced villus numbers may result from pathogenic bacterial activity (Alagawany et al., 2018). The curcumin and essential oils present in turmeric help minimize epithelial cell damage caused by oxidative stress or pathogenic infections, thereby preserving intestinal mucosa integrity. Studies have demonstrated that curcumin promotes appetite by accelerating gastric emptying and increasing bile secretion, thus supporting overall digestive function (Li et al., 2022).

4. Conclusion

The supplementation of turmeric powder (Curcuma domestica Val.) and multi-enzyme in broiler chicken feed had no statistically significant effect (P>0.05) on villus height, surface area, or villus number. However, descriptive data showed that diets enriched with these additives—particularly at the highest inclusion level (1.5% turmeric powder and 0.15% multi-enzyme)—consistently yielded improved intestinal morphology indicators. This suggests that turmeric and multi-enzyme supplementation may positively influence gut health and nutrient absorption, albeit marginally. Turmeric’s antibacterial properties and the enzymatic breakdown of complex nutrients may enhance the intestinal environment by supporting beneficial microflora growth, improving epithelial integrity, and increasing the absorptive surface of the small intestine. Although not statistically conclusive, these findings support the potential of phytobiotic and enzymatic supplementation as natural feed additives in broiler diets to support gut development and productivity.

Ethical approval

This study does not involve human participants and thus ethical approval and informed consent are not applicable.

**Disclaimer (Artificial Intelligence)**

Option 2:

Author(s) hereby declare that generative AI technologies have been used during the writing or editing of this manuscript.

Details of AI usage:

1. AI Technology Used: Claude Sonnet 4 (Anthropic) and ChatGPT (OpenAI)
   * Purpose: Assistance in discussion development and manuscript revision
   * Usage: Improvement of sentence structure, grammar, paragraph organization, and enhancement of scientific writing clarity

Statement: All scientific content, data, research results, and conclusions remain the original intellectual work of the authors. AI was used solely for language improvement and structural editing, not for data analysis or research interpretation.

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Table 1. Effect of Turmeric Flour and Multienzyme Supplementation on Intestinal Villus Morphology in Broiler Chickens

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| --- | --- | --- | --- | --- |
| **Observation Variables** | | | | |
| **Treatment** | **Vilus Number** | **Vilus Height** | **Vilus Surface Area** |
|  | **(per transveral cut)** | **(µm)** | **(µm)** |
| P0 | 6.44±0.91 | 501.72±91.16 | 768.91±198.93 |
| P1 | 8.24±1.26 | 597.29±73.25 | 927.05±146.67 |
| P2 | 7.88±3.68 | 619.57±125.11 | 998.47187.06 |
| P3 | 8.52±2.54 | 652.86±108.00 | 1030.64±244.42 |
| P4 | 9.12±2.32 | 673.76142.46 | 1056.16±234.97 |