



Effect of Using Xylanase and Inulin in Feed on Caecum Histology of Broiler Chickens

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Abstract. This research to determine the effect of xylanase and inulin in feed on pH, viscosity and cecum histology. This research used 100 unsexed broilers. The research began on 15-day-old chickens. This research used 5 treatments and 4 groups, that's : T0 Control Feed (without using of xylanase and inulin), T1 (0.01% xylanase + 0.2% inulin), T2 (0.01% xylanase + 0.4% inulin), T3 (0.2% xylanase + 0.2% inulin) and T4 (0.02% xylanase + 0.4% inulin). The parameters measured included pH, viscosity, crypt depth, villi height and villi width in the broiler cecum. The data was statistically analyzed using analysis of variance (ANOVA) from a randomized group design and then the significant difference continued with Duncan's Multiple Range. The results showed that the treatment of the addition of xylanase and inulin had no impact on cecum pH, viscosity, length and width of villi as well as crypt depth ($P>0.05$) in the cecum broiler section. The conclusion of the addition of xylanase and inulin did not affect on histology in the cecum broiler.

Keywords: broiler, cecum, enzymes, histology, prebiotic

1 Introduction

The broiler industry faces major challenges in simultaneously improving production efficiency and poultry health. The digestive health of broilers is critical to achieving optimal growth and high productivity. One approach being studied to improve digestive health is to modify feed composition. In this research, the enzymes xylanase and inulin emerged as two feed additives that have the potential to provide significant benefits. Enzymes can be produced from all living cells, both animal, plant and microbial cells [1]. Xylanase is an enzyme that can break down xylan, thereby increasing fiber digestibility and nutrient absorption. Meanwhile, prebiotics are undigested food ingredients that benefit the intestinal microflora by selectively stimulating the growth or activity of one or more bacteria found in the colon (large intestine) which can improve intestinal health [2]. Inulin is a carbohydrate that functions as a prebiotic. Inulin is an oligosaccharide, called fructan and is a polymer containing fructose groups with glycosidic bonds [3]. Inulin is easily soluble in air and cannot be digested by digestive enzymes, so that in the large intestine the inulin structure does not change. The prebiotic inulin

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functions as a substrate for good bacteria in the intestine, improving the balance of microbiota and supporting intestinal health.

Enzymes are proteins that act as biocatalysts in chemical reactions in the metabolic system. With enzymes, the metabolic process in the body can take place quickly [4]. One of the enzymes commonly used in agricultural waste-based feed as an energy source is xylanase [5]. The xylanase enzyme plays a very important role in chicken digestion, especially as an additive in animal feed. Xylanase breaks down xylan, an important part of hemicellulose in plant fiber. Chicken feed that is rich in fiber, such as wheat bran and straw, usually contains a lot of xylan. Without xylanase, chickens have difficulty digesting this fiber efficiently, which can reduce nutrient availability and inhibit growth. Adding xylanase to chicken feed can improve digestion by breaking down xylan into more easily digested oligosaccharides, thereby improving nutrient absorption and supporting chicken growth. Xylanase is an extracellular enzyme that has the ability to hydrolyze xylan (hemicellulose) into xylooligosaccharides and xylose [6]. Xylanase hydrolyzes the β -1,4-glycosidic bonds of xylan, breaking it down into smaller units such as xylose and oligosaccharides. Overall, adding xylanase to chicken feed accelerates the breakdown of indigestible fiber components, increases digestion efficiency and nutrient absorption, and supports chicken gut health. Xylanase enzymes work to degrade NSP into xylose and arabinose in the ileum, jejunum and duodenum which play a role in reducing viscosity so that nutrient digestibility and has a positive impact on chicken performance [7]. The addition of xylanase enzymes also improves feed conversion, AME and increases digestibility in the ileum [8].

Broilers are very sensitive to feed ingredients that contain non-starchy polysaccharides (NSPs). This is because feeds containing non-starch polysaccharides (NSP) have a direct impact on digestive rate, gut health, and microbiome composition. NSP polysaccharides determine nutrient utilization and productive performance, so the use of NSP needs to be considered in feed formulation. The addition of more xylanase will further increase the degradation of xylan, which results in more XOS production and consequently improves performance. The success of xylanase use may be as much due to the production of prebiotic oligosaccharides, and its impact on the composition of the microbiota, as it does to viscosity reduction [9]. The effect of NSP use on broiler performance can be reduced by xylanase supplementation. The dose of xylanase administration depends on the proportion of NSP formulated in the feed. Higher NSP levels in feed supplemented with 3,000 U/kg xylanase led to an increase in body weight and bird feed intake. Improved performance is associated with the influence of higher NSP levels to meet the required enzyme-substrate concentrations which ultimately facilitates a decrease in intestinal viscosity and increases nutrient utilization [10].

Prebiotics are substrates that are selectively utilized by microorganisms in the host that can cause health-enhancing effects [11]. Prebiotics contain oligosaccharides that stimulate microbial growth in the digestive system and can provide health effects on digestion. Thus, prebiotics act as substrates that support the growth of good bacteria and produce changes in the composition of the intestinal microflora [12]. MOS prebiotics in feed affect broiler chicken nutrition, increase the absorption of nutrients in the intestine and ensure good performance [13]. The main prebiotics used consist of oligosaccharides such as fructooligosaccharide (FOS), galactooligosaccharide (GOS) and

xyloseoligosaccharide (XOS). Some examples of prebiotics are inulin and fructooligosaccharides (FOS) [14]. Inulin can be classified as soluble dietary fiber and functions as a prebiotic. Inulin provides many health benefits, such as improving gut health [15]. Inulin is a type of carbohydrate that cannot be digested by the body but can selectively stimulate the growth and activity of good bacteria in the digestive tract, thus functioning as a prebiotic. Almost all inulin in the intestine can be fermented by lactic acid-producing microbes so that the final product becomes SCFA (short chain fatty acids) or short chain fatty acids where in this condition causes the intestinal pH to decrease which ultimately inhibits the growth and activity of pathogens, while non-pathogenic bacteria, especially *Lactobacillus*, can grow well [16]. Inulin cannot be digested by digestive tract enzymes but can be fermented by bacteria in the intestine so that it has a positive effect on digestive tract health and affects broiler fat and carcass [14] [17]. The addition of prebiotics from inulin acts as a substrate utilized by beneficial bacteria in the digestive tract [18]. The addition inulin combined with tributyrin was able to improve the balance of microflora in the caecum. The combination of tributyrin and inulin can reduce cases of diarrhea and death in weaning rabbits [19]. Inulin can alter the gut microbiota of broilers and is beneficial in the development of chickens. Supplementation of 1, 2, and 4% inulin is able to increase the lipid metabolism and immune activity of broilers. Age-dependent changes in the cecum microbiota, and the inclusion of inulin at all levels change its composition markedly. Increased populations of *Lactobacillus johnsonii* and *Bifidobacterium* correlated with bird weight gain, suggesting that these bacteria in particular play an important role in the metabolism of inulin caecum [20].

In poultry, the digestive tract consists of the beak, esophagus, proventriculus, ventricle, small intestine, cecum, rectum, cloaca, and anus, and additional organs consist of the pancreas and liver. The digestive tract of broiler chickens consists of the esophagus, crop, glandular stomach (proventriculus), muscular stomach (ventriculus), intestines (small intestine, large intestine and blind intestine) and ends in the cloaca [21]. Anatomically and physiologically, the digestive system in poultry is a simple digestive system, therefore poultry is very dependent on enzymes released by its digestive organs to digest feed so that it is easily absorbed by the body [22]. High chicken productivity can be achieved with good internal organ conditions. Organs that function in increasing chicken productivity include the liver, pancreas, stomach, and intestines [23]. The chicken digestive tract cannot digest crude fiber. Only about 20% of crude fiber is digested through the cecum, and nutrients in undigested crude fiber are excreted through feces. The cecum is a part of the intestine that functions as a place for microbial digestion with the aim of digesting nutrients that are not absorbed in the small intestine such as fiber [24]. Poultry that experience cecum development have the ability to utilize fiber better [25].

The digestive tract is one of the important organs to support the growth and development of chickens [26]. The main point at the beginning of chicken maintenance focuses on maximizing the performance of the digestive tract, especially in organs that have the main function of nutrient absorption. The histological structure of the cecum shows more muscle layers and prominent mucosa, supporting its function in fermentation and absorption of food waste. The cecum is a part of the intestine that functions as a place for microbial digestion with the aim of digesting nutrients that are not absorbed

in the small intestine such as fiber [27]. In healthy livestock, the composition of the digestive tract microflora is relatively constant, but if there is an imbalance in the microflora, it can cause colonization of pathogenic microorganisms that can cause disease. The presence of beneficial bacteria in the cecum needs to be maintained because it affects the population of beneficial bacteria that can have implications for the health of the chicken digestive tract and increase nutrient absorption. Giving xylanase will catalyze the xylan structure into shorter chains and the breakdown of the cell wall matrix. Changes in the chemical structure of xylan and the physicochemical properties of digesta can cause changes in intestinal health parameters, especially the composition and diversity of intestinal microbiota [28].

This research wants to determine the effect of synergy of prebiotic forms of inulin and enzyme (xylanase) in producing XOS and FOS which produce non-pathogenic bacteria and produce Short Chain Fatty Acids (SCFA). Two oligosaccharides will be fermented in the broiler's large intestine (caecum). Therefore, the part observed in this study is focused on the histopathology of the broiler caecum.

2 Materials and Methods

2.1 Research Tools and Materials

This research used 100 day old chick which were raised for 35 days. These chickens were not differentiated by sex (unsexed), after the brooding period was completed (15 days), the chickens were given additional feed treatment using xylanase enzymes and inulin prebiotics. The entire proximate composition of the basal diet which includes moisture, crude protein, crude fat, crude fiber, and ash content was examined after it was created to analyzed the nutritional needs of broilers. Xylanase was supplemented at levels providing approximately 500 IU/kg feed, while the inulin used had a purity of $\geq 98\%$.

2.2 Research Methods

This research used a experiment method with a Randomized Block Design (RBD) design. This design has one treatment factor, namely the addition of a combination of feed additives in the form of inulin, xylanase enzymes and inulin prebiotics to broiler feed. Initial body weight served as the blocking factor, in order to minimize variation and increase accuracy, chickens were grouped into blocks according to comparable starting weights. This treatment factor has 5 different levels of administration. Xylanase 0.01% + Inulin 0.2%, Xylanase 0.01% + Inulin 0.4%, Xylanase 0.02% + Inulin 0.2%, Xylanase 0.02% + Inulin 0.4% and without the addition of feed additives (control). This study involved 20 experimental units, with each unit consisting of 4-5 chickens. Each treatment level has 4 groups, so that a total of 100 chickens were used. The treatments include:

T0: Control Feed (without using of xylanase and inulin)

T1: Feed + Xilanase 0.01% + Inulin 0.2%

T2: Feed + Xylanase 0.01% + Inulin 0.4%

T3: Feed + Xylanase 0.02% + Inulin 0.2%

T4: Feed + Xylanase 0.02% + Inulin 0.4%

2.3 Research Tools and Materials

The schematic timeline of experimental procedure is presented in Figure 1.

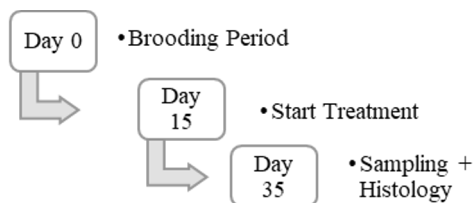


Fig. 1. Schematic timeline of the experimental procedure from day 0 to day 35 indicating feed phases and sampling points

pH measurement procedure. The caecum will be taken and placed in a fixation solution such as 10% formalin to maintain its histological structure and for the fixation buffer (pH 7.0), then the pH of the caecum will be measured using a pH meter that has been calibrated to measure the pH. The first step in preparing fresh material for making histological preparations is fixation. Fixation is an important step in making whole preparations and sections [29].

Crypt Depth Procedure. For crypt depth, the caecum will be thinly sliced and stained with hematoxylin-eosin, then observed using a microscope to observe the crypt structure in the caecum. After that, the crypt depth will be measured using microscope software or a micrometer on the microscope. The general histological structure is identified by hematoxylin-eosin (HE) staining and the distribution of neutral carbohydrates is identified by Periodic Acid Schiff (PAS) staining [30].

Viscosity Procedure. In viscosity, the caecum will be measured for viscosity with a viscometer using an appropriate method such as a rotational or capillary viscometer. A viscometer is a tool used to determine the viscosity value of a fluid. Viscosity is also called the level of thickness of a liquid [31]. Viscosity was measured by initially homogenizing cecal material and then diluting it with distilled water (aquadest) at a 1:10 ratio (w/v). The diluted sample was subsequently centrifuged at 3000 rpm for 5 to 10 minutes to isolate the solid residues. The supernatant obtained was gathered and utilized for viscosity assessment [32].

Intestinal Villi Procedure (cecum). The caecum was collected on day 35 post-prandial from each treatment group intestines that have been differentiated for each treatment for 1-2 cm, then the cecum will be rinsed using distilled water. Then the sample is fixed in 10% formalin solution for 24-28 hours to prevent tissue damage. After that, the

sample is dehydrated using gradual ethanol (70-100%) and inserted into liquid paraffin for hardening, the sample will be sliced (3-5 μM) using a microtome. The sample will be attached to a glass object and stained with hematoxylin-eosin to clarify the structure of the villi. The stained sample will be observed under a microscope with a magnification of 10x-40x. The height and width of the villi are measured using a microscope scale or measurement software.

2.4 Research Variables

The variables used in this study were pH, crypt depth, viscosity, villus height, basal villus width, apical villus width and villi surface area in broiler cecum. All data were examined using Analysis of Variance (ANOVA) in IBM SPSS Statistics version 26. Notable variations among treatment means were analyzed further utilizing Duncan's Multiple Range Test at the 5% significance threshold.

3 Results and Discussion

3.1 pH caecum

The caecum in broiler chickens plays an important role in the fermentation of undigested nutrients, especially fiber, through beneficial microbial activity. Chickens have two cecum, located at the junction of the small intestine and large intestine, where microbial fermentation occurs to produce short-chain fatty acids (SCFA) consisting of acetate, propionate, and butyrate, which have important roles for intestinal health and energy metabolism. The results of fermentation in the cecum will also affect the development of the intestinal immune system through the mucosa and epithelial cells and the overall efficiency of nutrient utilization [33]. The pH of the cecum serves as a marker of beneficial microbial fermentation activity. A low caecum pH usually indicates increased SCFA production, so that microbial development can be maximized. In this context, inulin is a prebiotic, which can enhance the growth of good bacteria such as Bifidobacteria and Lactobacillus, while xylanase, a non-starch polysaccharide degrading enzyme, can break down complex fibers into fermentable substrates, thereby increasing SCFA production [34].

The ANOVA test value on caecum pH is shown in Table 1. Based on the study, the results of data analysis showed that the adding of xylanase enzymes and inulin prebiotics in feed had not significant effect, which showed that the treatment had a very significant difference ($P > 0.05$) on broiler cecum pH. The treatment that had no effect was caused by a dose that was too low so that it was less than optimal in influencing the pH of the cecum, a higher dose was needed to provide a more optimal effect. Previous research explained that the addition of xylanase in basal feed with a dose of 0.03% can reduce the pH value in the digestive tract [35], so that when compared to the dose of xylanase used, it is still relatively low. The treatment time of only 15 days caused fermentation in the digestive tract to not occur optimally, so that the SCFA produced was not enough to reduce the pH value of the cecum.

Table 1. Caecum pH Data.

Treatment	Variable	
	pH	Viscosity (mPa's)
T0	7.17 ± 0.49	6.52 ± 1.25
T1	6.95 ± 0.18	5.37 ± 0.42
T2	7.14 ± 0.66	4.37 ± 0.96
T3	7.24 ± 0.14	6.42 ± 2.52
T4	6.95 ± 0.45	4.65 ± 1.83

3.2 Caecum Viscosity

The ANOVA test value on caecum viscosity is shown in Table 1. Based on the study, the results of data analysis showed that the administration of xylanase enzymes and inulin prebiotics in feed did not provide any difference, F count < F table 0.05 showed that the treatment did not provide any difference ($P > 0.05$) on broiler cecum viscosity. The absence of effect of xylanase and inulin supplementation on cecal viscosity values in this study was due to the feed content used. The main target of xylanase is non-starch polysaccharides (NSP) such as arabinoxylan, which are abundant in wheat feed compared to corn, so that the NSP content in corn feed is not too high. The low NSP content causes insufficient substrate availability so that xylanase activity is limited which directly results in minimal viscosity reduction. The unchanged viscosity value is proven by previous research that a dose of 0.03% xylanase in corn feed can affect the viscosity value of digestion [35]. The lack of effect of inulin fermentation also resulted in no decrease in the digestion viscosity value. Inulin requires active fermentation by beneficial gut bacteria such as lactobacilli and bifidobacteria to produce short-chain fatty acids (SCFAs) that lower pH and potentially affect viscosity. The microbial population had not yet developed to its maximum by day 35, especially since the doses used tended to be low (0.2 - 0.4%).

3.3 Cecum Crypt Depth

The crypt depth test values are in Table 2. Based on the study, the results of data analysis showed that the provision of xylanase enzymes and inulin prebiotics in feed did not provide any difference ($P > 0.05$) with an average crypt depth from the lowest to the highest being T1 (42.63 ± 1.78), T2 (49.04 ± 7.19), T0 (50.64 ± 3.37), T4 (50.94 ± 8.95), T3 (52.12 ± 5.53). Cryptic depth has a correlation with the area of the villi. Crypts are used as one thing that indicates the wide part of the nutrient absorption area in the intestine. The highest crypt depth value was 52.12 at T3 with the addition of 0.02% xylanase + 0.2% inulin, while the lowest value was 42.63 at T1 with the addition of 0.01% xylanase + 0.2% inulin. The administration of inulin and xylanase that did not affect the depth of the cecum crypts was caused by the insufficient dosage of feed additives. The dosage levels of additives used in this study (inulin of 0.2–0.4% and xylanase of 0.01–0.02%) were too low to produce measurable histological changes in the

cecum, doses that were too low would not have a significant effect on the morphology of the cecum. Previous studies have shown that higher doses of inulin or xylanase enzyme are often required to produce a significant impact on caecal morphology, with 1% inulin supplementation improving chicken performance [36], while 0.5% inulin supplementation improved digesta [37].

Table 2. Average effect of xylanase enzyme and prebiotic inulin on cecum.

Treatment	Variable				
	Crypt Depth (μm)	Vili Height (μm)	Basal Width of Vili (μm)	Apical Width of Vili (μm)	Surface Area of Vili (μm)
T0	50.64 \pm 3.37	172.06 \pm 36.94	52.18 \pm 4.52	37.80 \pm 3.22	331.1 \pm 30.72
T1	42.63 \pm 1.78	153.62 \pm 17.30	50.71 \pm 4.83	39.94 \pm 13.72	319.2 \pm 46.56
T2	49.04 \pm 7.19	176.71 \pm 39.29	47.90 \pm 7.91	40.35 \pm 6.43	373.7 \pm 97.66
T3	52.12 \pm 5.53	168.69 \pm 13.99	34.23 \pm 4.21	33.04 \pm 3.61	348.2 \pm 56.42
T4	50.94 \pm 8.95	161.18 \pm 38.44	42.48 \pm 7.03	38.56 \pm 4.58	3.6 \pm 109.4

3.4. Vili Height

Vili height is an indicator of the absorption area of nutrients in the small intestine of broiler. Table 2 shows the results of the analysis of the average villus height in the intestines of broiler with the administration of xylanase enzymes and prebiotics. Based on the research that has been done, the results of data analysis on each treatment showed that there was no significant effect ($P > 0.05$) on the height of the villus in the intestines of broiler.

The height of the villus in the treatment produced an average value from the lowest, namely (T1) 153.62 \pm 17.30 μm , (T4) 161.18 \pm 38.44 μm , (T3) 168.69 \pm 13.99 μm , (T0) 172.06 \pm 36.94 μm , (T2) 176.71 \pm 39.29 μm . The results of this analysis are thought to be due to the level of administration of the combination of xylanase enzymes and prebiotic inulin being relatively low so that it does not have an effect on villi height (Figure 2). The unaffected villi height by the addition of xylanase and inulin is due to the cecum being a relatively stable organ. The caecum has a dense microbial population and an established mucosal architecture. In contrast to the small intestine, which responds rapidly to dietary interventions, the cecum is less sensitive to short-term or moderate dietary changes, especially at the cellular or structural level such as crypt depth. Too low a dose combined with the cecum being difficult to influence results in no change in the height of the cecum villi.

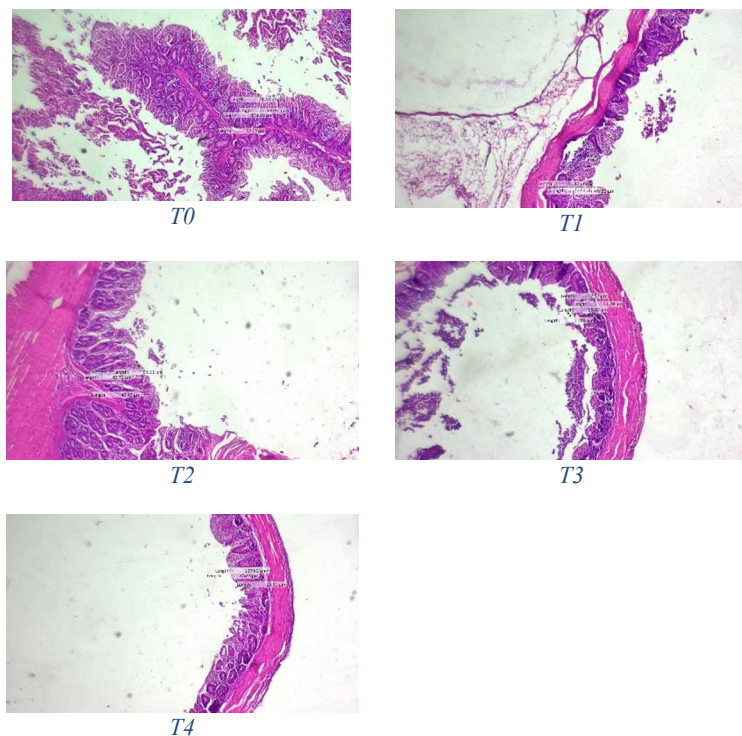


Fig. 2. Histological images of broiler caecum under different treatments on day 35. Scale bar = 100 μm .

3.7 Basal Width of Vili

The basal villi width test values are in Table 2. Based on the study, the results of data analysis showed that the administration of xylanase enzymes and prebiotics in feed had a very significant effect ($P < 0.05$) with the results of T0 (52.18 ± 4.52), T1 (50.71 ± 4.83), T2 (47.90 ± 7.91), T3 (34.23 ± 4.21), T4 (42.48 ± 7.03). This shows that the research that has been done with the level of administration of xylanase enzymes and inulin prebiotics has a good effect on the basal villi width with an average of $45.5 \mu\text{m}$. The basal diet provided already contains ingredients to maintain optimal cecal health, thus minimizing the potential for further increase through additional supplementation. In addition, villi width is more closely related to epithelial cell turnover and regeneration, which may not be significantly affected by mild prebiotic or enzymatic stimulation in a healthy caecal environment.

3.8 Apical Width of Vili

The value of the apical width test of the caecum villi is shown in Table 2. Based on the study, the results of data analysis showed that the administration of xylanase enzymes

and inulin prebiotics in the feed had no significant effect ($P>0.05$) with an average crypt depth from the lowest to the highest being T3 (33.04 ± 3.61), T0 (37.80 ± 3.22), T4 (38.56 ± 4.58), T1 (39.94 ± 13.72), T2 (40.35 ± 6.43). The highest value of the apical width of the cecum villi was 40.35 in T2 with the addition of 0.01% xylanase + 0.4% inulin, while the lowest value was 33.04 in T3 with the addition of 0.02% xylanase + inulin 0.2%. The duration of the study (approximately 20 days post-incubation) also affected how long the feed additives could have an effect. Changes in villi width are usually caused by ongoing changes in cecal physiology, which may require a longer intervention period, so the duration of the study still did not affect cecal villi width. Previous research has shown that combining xylanase with XOS (Xylooligosaccharides) can improve digestive health [38], in contrast to the combination of xylanase and inulin which has less effect on the digestive health of chickens, especially in the cecum.

3.9 Surface Area of Villi

The test value of villi surface area is shown in Table 2. Based on the study, the results of data analysis showed that the administration of xylanase enzyme and prebiotic inulin in feed did not have a significant effect ($P>0.05$) on the surface area of the villi. The absence of a significant effect is suspected because there is no significant difference in the height of the villi. The surface area of the villi will increase if the height of the villi increases. It is estimated that the level of administration of xylanase enzyme and prebiotic inulin is still relatively low so that it does not have an effect on the surface area of the cecum villi. The surface area of the villi has a positive correlation with the height and width of the intestine [39], which is also in line with [40] which states that an increase in the height of the villi and the width of the villi is associated with a wider surface area of the villi for absorption of nutrients into the bloodstream. The more and wider the surface of the intestinal villi, the more efficient the absorption of nutrients, this is influenced by hormonal, nerve, and digestive gland factors in the digestive tract.

4 Conclusion

The conclusion of the addition of xylanase and inulin did not affect on histology in the caecum broiler. It was recommended to use higher levels of xylanase and in combination with probiotics.

Disclosure of Interests. The authors have no competing interests to declare that are relevant to the content of this article.

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