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Effect of Prebiotic and Immunowall® as Feed Additive in Enzyme Activity, Intestinal Characteristic, and Broiler Performance

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Abstract

The research purpose was to determine the prebiotic and immunowall® effect as the feed additive on enzyme activity, intestinal characteristic, and broiler performance. The research method was used completely randomized design with 3 treatments and 8 replicates. The materials used for this research were 720 unsex day old chicks with Ross 308 strain with average body weight 43.34±1.20 g/head. The treatments used for research were dietary with T_0 (basal feed), T_1 (basal feed + 0.05% prebiotic at 1-13 days) and (basal feed + 0.05% immunowall® at 15-32 days), T_2 (basal feed + 0.1% prebiotic at 1-13 days) and (basal feed + 0.05% immunowall® at 15-32 days) The parameters observed were enzyme activities (protease and amylase), intestinal characteristic (villus total and villus height), and performance (feed intake, body weight, feed conversion ratio, mortality, and production index broiler). The data analysis was the analysis of variance (ANOVA) and continued by Duncan Multiple Range Test. The results showed that using the prebiotic and immunowall® effect as feed additive has significant difference ($P < 0.05$) on enzyme activities (protease and amylase), intestinal characteristic (villus total and villus height), performance (final body weight and mortality) and significantly different ($P < 0.01$) (feed conversion, index production, and IOFC). The addition of 0.05% prebiotic and 0.05% immunowall® gave the best effect on enzyme activity, intestinal characteristic, and broiler performance.

Keywords

Prebiotic; Enzyme Activity; Intestinal Characteristic; Performance; Broiler

1. Introduction

Sources of protein can be obtained from livestock products e.g. meat. There are several commodities that produce meat product e.g. chicken, cattle, and goat. However, broiler are given biggest sources of protein resources from white meat products. The quality of broiler carcass mostly from several factors. The feed is the effect to the quality of broiler. The using feed additive is one method to improve the quality of feed. The antibiotics are given as growth promoter but antibiotics cause bacterial resistance and residue in the carcass (Sjofjan, 2003).

Nowadays, prebiotics use as the substitute from antibiotics because it's safer. The uses Saccharomyces cerevisiae is the most in the prebiotics. The Saccharomyces cerevisiae is the most lactic acid bacteria in the intestinal. The Saccharomyces cerevisiae produce an essential enzyme that increased quality in the digestive tract of broiler (Sjofjan *et al.*, 2015). The prebiotics can increase the absorption nutrient in the broiler that will affect the internal organs. The indicator performance (feed intake, body weight, feed conversion ratio, mortality, and production index broiler) and enzyme activities (protease and amylase), intestinal characteristic (villus total and villus height).

According to Sjofjan *et al.* (2015) stated the Saccharomyces cerevisiae are an organism that produce amylase enzyme. The Saccharomyces cerevisiae are source vitamin, carbohydrate, and protein that increased total protein absorb in intestinal. Sjofjan *et al.* (2012) stated used of prebiotics increased immunity of the broiler starter periods. The prebiotics consist enzyme that has biocatalisator for metabolism in intestinal of the broiler.

The small intestinal of broiler consist villus that has a function to absorb nutrient content. The villus are indicator the nutrient can absorb in the intestinal of broiler. The use prebiotics as feed additive are help the villus absorb nutrient. In otherwise the research used prebiotics as feed additive need to be develop in addition of intestinal characteristic (villus total and villus height). The performance indicator use prebiotics are help from MOS. Man nan oligosaccharides are yeast that produce endogenous enzyme that will hydrolysis in the intestinal wall of broiler (Sjofjan *et al.*, 2012).

2. Materials and Methods

2.1. Materials

The principal equipment for the research are litter, lighting, analytic scale, the postal cage with closed house, feed bunk, and drink bunk. The principal materials are 720 unsex day old chicks with Ross 308 strain with average body weight 43.34 ± 1.20 g/head produced by Cibadak Indah Sari Farm. The prebiotic given 1-13 days and immunowall® has given 15-32 days.

2.2. Methods

The research method was used completely randomized design with 3 treatments and 8 replicates. The replicates consist 30 heads of broiler with total (720heads). The level of dietary and prebiotics were:

T_0 = (basal feed + 0% probiotic)

T_1 = (basal feed + 0.05% prebiotic at 1-13 days) and (basal feed + 0.05% immunowall® at 15-32 days)

T_2 = (basal feed + 0.1% prebiotic at 1-13 days) and (basal feed + 0.1% immunowall® at 15-32 days)

2.3. Variables

The variables observed were enzyme activities (protease and amylase), intestinal characteristic (villus total and villus height), and performance (feed intake, body weight, feed conversion ratio, mortality, and production index broiler).

2.4. Data Analysis

The data analysis using analysis of variance (ANOVA) and continued by Duncan's Multiple Range Test (Steel and Torrie, 1992).

3. Results and Discussion

3.1. The Prebiotic Effect in Enzyme Activities

Table 1. Enzyme activities and intestinal characteristic

CODE	Variables			
	Protease*	**Amylase***	**Villus total***	**Villus Height****
	(μmoll tyrosine/g enzyme minute)	(μmoll glucose/g enzyme minute)	(unit/transversal cut)	(μm)
T_0	6.59 ± 0.99^{a}	62.42 ± 4.46^{b}	211.16 ± 16.33^{a}	887.25 ± 62.54^{a}
T_1	7.70 ± 1.24^{b}	63.89 ± 3.38^{a}	230.33 ± 16.50^{b}	1191.50 ± 119.44^{b}
T_2	8.22 ± 0.91^{b}	69.65 ± 6.91^{b}	234.33 ± 10.76^{b}	1246.33 ± 96.90^{b}

** Superscript showed significantly different (0.01) ; * Superscript showed significant different (0.05)

3.1.1. The Prebiotic Effect in Protease Enzyme Activities

Based on table 1 result from the research on the protease enzyme activities showing the T_2 higher are (8.22 ± 0.91) with basal feed + 0.1% prebiotic at 1-13 days) and (basal feed + 0.1% immunowall® at 15-32 days) and lower T_0 are (6.59±0.99) (basal feed + 0% prebiotic). The treatment from lower to higher be T_0, T_1, T_2. The average protease enzyme activities are significant different ($P<0.05$). The used prebiotics are impacted to the protease enzyme.

The enzyme reacts because of MOS, while the protolithic bacteria produce protease enzyme to react the absorption. According to Haryati (2011) stated the used of prebiotics using Lactobacillus and Bifid bacteria in intestinal can support micro faunas and the pathogen microorganism reduce can't react and the absorption of nutrient will react in the binding site using blocking factors. Lactobacillus are bacterial that produce protease enzyme. The Lactobacillus are using MOS to optimizing the synthesis the protease enzyme.

Microorganism in the intestinal using MOS from the feed while the Lactobacillus produce protease enzyme and transform into potential energy for growth and increased the production. The using prebiotic that produce enzyme more efficiency than basal feed. According to Sjofjan *et al.* (2015) stated the function of the protease enzyme are for catalysator to hydrolyzed protein. Protease enzymes is needs to break down the peptide in protein feed and breakdown the amino acid in the broiler body. The prebiotics in the T_1 and T_1 showed that protease enzyme activity is increased because the composition was given. The nucleotide in the feed are affected to broiler body that produces the enzyme. Nucleotide are the semi essential nutrient substance that produce from broiler body. The nucleotide in the broiler body are substance from growth and given in the brooding period will increase optimum absorb nutrient. The activities of the protease enzyme are increased due to the acid condition in the intestinal of broiler. The several factors affect are genetic, feed composition, and feed intake. Protease enzyme will breakdown the protein into amino acids that will absorb by broiler into body weight (Wahyu, 2004).

3.1.2. The Prebiotic Effect in Amylase Enzyme Activities

Based on table 1 result from the research on the amylase enzyme activities showing the T_2 higher are (69.65±6.91) with basal feed + 0.1% prebiotic at 1-13 days) and (basal feed + 0.1% immunowall® at 15-32 days) and lower T_0 are (62.42±4.46) (basal feed + 0% prebiotic). The treatment from lower to higher be T_0, T_1, T_2. The average amylase enzyme activities are significant different ($P<0.05$). The used prebiotics are impacted to the amylase enzyme.

The enzyme react because of MOS, while the amyl lithic bacteria produce amylase enzyme. According to Oyeleke and Oduwele (2009) stated MOS consist high amylin that breakdown the starch into molecule e.g. glucose, maltose, dextrose as the carbon source to produce amylase enzyme that has a potential than basal feed. The differences enzyme activities in the each level prebiotics provide feed additive to bacteria growth for produce amylase enzyme to optimum phase. Amylase enzyme cycle IS breakdown the glycoside in the amylin. The enzyme activity is increased due to pH that changes enzyme intermolecular structure and enzyme become denaturation (Sjofjan *et al.*, 2012).

The used prebiotics in the T_2 are increased T_0 and T_1. The content of nucleotide in the each treatments are affect to produce enzyme from the broiler body. Nucleotide are essential substance that needed to be growth for broiler and cell replicates. The used nucleotide in the brooding periods give growth and cell replicate for broiler performance especially in the intestinal (Sjofjan *et al.*, 2015).

3.1.3. The Prebiotic Effect in Villus Total

Based on table 1 result from the research on the protease enzyme activities showing the T_2 higher are (234.33±10.76) with basal feed + 0.1% prebiotic at 1-13 days) and (basal feed + 0.1% immunowall® at 15-32 days) and lower T_0 are (211.16±16.33) (basal feed + 0% prebiotic). The treatment from lower to higher be T_0, T_1, T_2. The average villus total are significant different ($P<0.05$). The used prebiotics are impact to the metabolism in the broiler that has positive physiology intestinal condition. The average of villus total (ileum) are 211.16-233.33. The average are higher to Sjofjan *et al.* (2015) that the average of the villus total are 180.50-216.00 that used probiotic in the drinking water laying hens.

According to Ningrum *et al.* (2016) stated that the used prebiotics in the Broiler strain Ross 308 (35 aged) are 218.33 – 251.75. The optimum level is T_2 that used by the microorganism for optimum nutrient absorption. The decreasing absorption affects to the amount total and length of villus broiler. The several factors affect the absorption are the feed, disease, total pathogen and non-pathogen bacteria in the broiler intestinal.

The prebiotics effect in the T_2 and T_1 is better than T_0. The result showed that villus total is absorption nutrient are well and affect to the internal organ and metabolism increased while final body weight approached. According to Hamsah

(2013) stated increased total villus activities due to microbial and enzyme work to absorb the nutrient from feed. The villus total decreased due to age factors. Ermalia *et al.* (2016) stated the villus total decrease started at 14 days and 21 days. The several factors impact the decreased of villus total are feed, disease, and balancing between the total pathogen and non-pathogen bacteria in the broiler intestinal.

3.1.4. The Prebiotic Effect in Villus Length

Based on table 1 result from the research on the protease enzyme activities showing the T_2 higher are (1246.33±96.90) with (basal feed + 0.1% prebiotic at 1-13 days) and (basal feed + 0.1% immunowall® at 15-32 days) and lower T_0 are (887.25±62.54) (basal feed + 0% prebiotic). The treatment from lower to higher be T_0, T_1, T_2. The average villus length is significantly different (P<0.01). The used prebiotics are increased the villus length due to beta-glycan and Man nan oligosaccharides (MOS) that use by microbial that increased intestinal absorption. Beta-glycan has functioned as anti-septic, anti-oxidant, anti-aging, immune activator, radiation factors, anti-inflammation, anti-cholesterol, and anti-diabetes. Beta-glycan are extracted from yeast has positive impact to the absorption nutrient (Sjofjan *et al.*, 2015).

The villus length average is 887.25 µm to 1246.33 µm. the result showed the used prebiotics are better than the basal feed to villus length the result better Beski and Saldary (2015) that 558.3 – 883.3 µm and Ningrum *et al.* (2016) that the used prebiotics in the Broiler strain Ross 308 (35 aged) are 700.50 – 1072.76 µm. According Rofiq (2003) villus are the important part to absorb the nutrient from the feed. MOS function are help endo β-mannose enzyme and galactosidase. The high nutrient content in the MOS are affected to the palatability while feed intake increase digestibility enzyme.

3.2. The Prebiotic Effect in Performance

Table 2. Probiotic effect in performance

CODE	Variables			
	Feed intake	Final BW*	Feed Conversion**	Mortality*
	(g/head)	(g/head)		(%)
T_0	3186.93 ± 83.29	2040.73 ± 63.62 ^{a}	1.56 ± 0.05 ^{b}	5.00 ± 1.78 ^{b}
T_1	3083.98 ± 106.39	2124.32 ± 70.06 ^{b}	1.45 ± 0.06 ^{a}	2.08 ± 2.48 ^{a}
T_2	3086.26 ± 82.06	2128.34 ± 57.02 ^{b}	1.45 ± 0.06 ^{a}	1.67 ± 2.52 ^{a}

** Superscript showed significantly different (0.01) ; * Superscript showed significant different (0.05)

3.2.1. The Prebiotic Effect in Feed Intake

Based on table 2 result from the research on the protease enzyme activities showing the T_0 higher are (3186.93±83.29) with basal feed + 0.1% prebiotic at 1-13 days) and (basal feed + 0.1% immunowall® at 15-32 days) and lower T_1 are (3083.98±106.39) (basal feed + 0.05% prebiotic). The treatment from lower to higher be T_0, T_2, T_1. The average feed intake is not significant different (P>0.05). The used prebiotics are not significant different due to feed and the nutrient content of feed each other is same. The protein and energy are given different to the amount feed consumption, due to energy is inhibit factor.

Warisah (2015) stated that the used prebiotics as feed additive is not significant different to the feed intake. According to Daud (2005) are the feed and the nutrient content of feed each other is same. The feed intake level does not depend on absorption in the intestinal. The several factors affected is body weight, strain, production levels, temperature levels, broiler activities, and humidity. The capacities of the gizzard effect to the feed intake. The other factors affect to the feed intake are palatability (color, taste, odor, and texture).

3.2.2. The Prebiotic Effect in Final Body Weight

Based on table 2 result from the research on the final body weight showing the T_2 higher are (2128.34±57.02) with (basal feed + 0.1% prebiotic at 1-13 days) and (basal feed + 0.1% immunowall® at 15-32 days) and lower T_0 are (2040.73±63.62) (basal feed + 0% prebiotic). The treatment from lower to higher be T_0, T_2, T_1. The average feed intake is significant different (P<0.05). The used prebiotics are not significant different due to feed and the nutrient content of feed each other is same. The protein and energy are given different to the amount feed consumption, due to energy is inhibit factor. The final body weight has correlation positive after given prebiotics. The final body weight is increased due to the nucleotide content in the prebiotics.

The used of prebiotics as feed additive because the nucleotide can absorb by enterocyte in the intestinal and proliferation cycle was at the optimum point. The absorption of the nutrient content affects the final body weight are optimum in the broiler. According to Daud (2005) stated that increased the broiler body weight due to metabolism from the microbial in the prebiotic, the enzyme that absorbs nutrient content combine with metabolism to produce and grow the organs in the broiler. The prebiotic increase microbial population and immunity. The final body weight has the correlation with the number of MOS that provide the substrate for lactic acid bacteria.

3.2.3. The Prebiotic Effect in Feed Conversion

Based on table 2 result from the research on the protease enzyme activities showing the T_0 higher are (1.56±0.05) with basal feed + 0.1% prebiotic at 1-13 days) and (basal feed + 0.1% immunowall® at 15-32 days) and lower T_1 are (1.45±0.06) (basal feed + 0.05% prebiotic). The treatment from lower to higher be T_0, T_2, T_1. The average feed intake is significantly different ($P<0.01$). The low feed intake and the final body weight are increased converted into high feed conversion in the broiler. The feed conversion lowers the feed efficiency are increased.

According Lesson and Summer (2000) feed conversion are to determine the productivity and define the ratio and feed conversion with the relative time of the final body weight. The used probiotics in the treatment are due to microbial that can do absorb nutrient content in the intestinal and effect to the final body weight of the broiler. The used prebiotics and probiotics increased digestibility of a nutrient that feeds conversion in the optimum levels. The used MOS as feed additive is given significantly different in the intestinal broiler performance. The prebiotics function is for sources energy, the nutrient for mucosa, and substrate for fermentation that providing vitamin and anti-oxidant (Ghiyasi *et al.*, 2007). According to James (2004) stated that feed conversion depends on the genetic, feed, feed additive, management, and environment. MOS in the prebiotics has the mechanism to increase the beneficial microbial population, while the MOS can work in the lecithin e.g. Salmonella and E. coli and throughout intestinal in the broiler.

3.2.4. The Prebiotic Effect in Mortality

Based on table 2 result from the research on the mortality showing the T_0 higher are (5.00±1.78) with basal feed + 0.1% prebiotic at 1-13 days) and (basal feed + 0.1% immunowall® at 15-32 days) and lower T_1 are (1.67±2.52) (basal feed + 0.05% prebiotic). The treatment from lower to higher be T_0, T_2, T_1. The average mortality is significant different ($P<0.05$). The mortality is determined by the number of mortality divide the amount of the early population times 100%.

According to Bell and Weaver (2002) mortality is the indicator that calculated used percentage. The higher mortality are in the 3 weeks and 5 weeks. The causes of mortality are Escherichia coli (Colibacillosis). The indicate Escherichia coli are in the broiler are broiler is weakness, the feather number are not widely, feed intake is low, and look pale. The used prebiotics in the mortality variable is significant different due to MOS content and β-glycan has ability to decreasing pathogen bacterial in the broiler. The prebiotics in the MOS produces mannose that reduce Salmonella, Campylobacter, Clostridiumperfringens, and Escherichia coli.

Spearman (2004) stated Man nan oligosaccharide has a structure inhibitor and unique chain for mannose (mannose-specific type-1 fimbriae). According to El Banna (2010) the prebiotics fermentation e.g. short chain fatty acid can decreasing pH in the colon and make the environment are not suitable for pathogen bacteria. Lactic are produce by Lactobacillus, Bifid bacterium, Enterococcus, Pediococcus, Streptococcus during fermentation cycle including the MOS. The lactic acid can inhibit the Salmonella, Escherichia coli, and Clostridium with decreasing the pH (Xu *et al.*, 2006). The several factors affected the mortality are body weight, strain, breed, climate, sanitation, and disease. The higher temperature causes disturbing physiology, in otherwise the growth graphic decrease and feed intake low and cause mortality.

Table 3. Probiotic effect in production index and IOFC

CODE	Variables	
	Production Index (IP)**	**Income Over Feed Cost****
		(IDR/head)
T_0	354.94 ± 21.25 [a]	12383.87 ± 1059.36 [a]
T_1	409.86 ± 27.50 [b]	14334.32 ± 1291.23 [b]
T_2	412.96 ± 27.34 [b]	14448.30 ± 1153.90 [b]

** Superscript showed significantly different (0.01); *Superscript showed significant different (0.05)

3.2.5. The Prebiotic Effect in Production Index (IP)

Based on table 2 result from the research on the production index showing the T_2 are higher (412.96±27.34) with basal feed + 0.1% prebiotic at 1-13 days) and (basal feed + 0.1% immunowall® at 15-32 days) and lower T_0 are (354.94±21.25) (basal feed + 0% prebiotic). The treatment from lower to higher be T_0, T_1, T_2. The average production index are significantly different (P<0.01). The production index are the amount of the successful in the end period of broiler. The indicator successful of broiler management is the production index. The production determine by comparison between average body weight times live percentage of broiler divided feed conversion and times the age of broiler.

The used prebiotics in the production index are given positive correlation to the final body weight of broiler, decrease the mortality, and increased the feed conversion (T_1, and T_2). The factors affected to both treatment are the performance index e.g. body weight, feed conversion, and mortality. Bahari *et al.* (2012) stated the important component in the performance index are the amount of mortality, body weight, feed conversion ratio, and aged. The production index are depend on the final body weight, live broiler, time management, and feed conversion (Daud, 2005).

3.2.6. The Prebiotic Effect in Income Over Feed Cost (IOFC)

Based on table 2 result from the research on the production index showing the T_2 are higher (14448.30±1153.90) with basal feed + 0.1% prebiotic at 1-13 days) and (basal feed + 0.1% immunowall® at 15-32 days) and lower T_0 are (12383.87±1059.36) (basal feed + 0% prebiotic). The treatment from lower to higher be T_0, T_1, T_2. The average income over feed cost are significantly different (P<0.01). The income over feed cost the number showed the between income that gained from one period in the broiler. The income over feed cost is gained from the number selling chicken with income among the one periods broiler rearing (Sjofjan, 2008).

The used prebiotics in the broiler are significantly different due to the value of IOFC are consist the value of feed intake and final body weight of broiler. The significantly different of IOFC impact to the value of income and price of selling broiler. The lower feed consumption given the number of expenditure lower. Tantalo (2009) stated the IOFC value depend on the final body weight broiler. The IOFC value depend on the income and the expenditure during rearing of broiler (Sjofjan, 2008).

4. Conclusions

The prebiotic and immunowall® effect as feed additive has significant difference (P<0.05) on enzyme activities (protease and amylase), intestinal characteristic (villus total and villus height), performance (final body weight and mortality) and significantly different (P<0.01) (feed conversion, index production, and IOFC). The addition of 0.05% prebiotic and 0.05% immunowall® gave the best effect on enzyme activity, intestinal characteristic, and broiler performance.

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