ACTA VETERINARIA INDONESIANA
P-ISSN 2337-3202, E-ISSN 2337-4373
Vol. 13, No. 1: 16-21, Maret 2025

Research

The Effect of Endo-1,4-β-xylanase as a Feed Additive on the Growth and Overall Health of Broiler Chickens

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Submitted 16 November 2024, Accepted 15 January 2025

ABSTRACT

Enzyme has been used widely as a feed additive to complement poultry needs of nutrition. Endo-1,4 beta-xylanase as its main component, which is effective in digesting non-starch polysaccharides (NSPs), hence improving the digestive system. This research aims to observe the efficacy of Endo-1,4-β-xylanase as a growth promotor and its safety. A total of 96 chickens were used in this research, divided into four groups. Administration depending on each group was conducted for 28 days, consecutively. Growth parameters that were observed includes body weight gain and feed conversion ratio, meanwhile for safety assessment, the parameters used were mortality rate, and blood profile. Based on the results, the enzyme was proven to enhance growth performance through the increased body weight gain and final weight. This is due to the enzyme's mechanism which improves nutrient utilization. Based on the safety assessment, Endo-1,4-β-xylanase was also proven to be safe, not causing any inflammation responses in the body nor organ damage based on the blood assessment.

Keywords: broiler chickens, growth performance, Endo-1,4-β-xylanase, safety

ABSTRAK

Enzim telah digunakan secara luas sebagai aditif pakan untuk melengkapi kebutuhan nutrisi unggas. Endo-1,4-β-xilanase sebagai aditif pakan mengandung efektif dalam mencerna polisakarida non-pati (NSP), sehingga meningkatkan sistem pencernaan. Penelitian ini bertujuan untuk mengamati efikasi Endo-1,4-β-xilanase sebagai pemacu pertumbuhan dan keamanannya. Sebanyak 96 ekor ayam digunakan dalam penelitian ini, dibagi menjadi empat kelompok. Pemberian tergantung pada masing-masing kelompok dilakukan selama 28 hari, secara berturut-turut. Parameter pertumbuhan yang diamati meliputi pertambahan berat badan dan rasio konversi pakan, sedangkan untuk penilaian keamanan, parameter yang digunakan adalah tingkat mortalitas dan profil darah. Berdasarkan hasil, enzim terbukti meningkatkan kinerja pertumbuhan melalui peningkatan pertambahan berat badan dan berat akhir. Hal ini disebabkan oleh mekanisme enzim yang meningkatkan pemanfaatan nutrisi. Berdasarkan penilaian keamanan, Endo-1,4-β-xylanase juga terbukti aman dengan tidak menyebabkan respons peradangan dalam tubuh maupun kerusakan organ berdasarkan hasil dari gambaran darah.

Keywords: ayam broiler, performa pertumbuhan, Endo-1,4-β-xylanase, keamanan

INTRODUCTION

The poultry industry is growing rapidly in many countries, particularly in developing ones, driven by increasing demand of affordable protein sources. However, this rapid growth is followed by a number of challenges, one of the most significant ones being the rising expense of feed ingredients that are crucial for supporting the growth and productivity of poultry. Currently, feed expense accounts for up to 75% of the total cost in poultry production (Alagawany 2018), making it the largest operational cost for poultry farmers.

In response to these challenges, the poultry industry has turned to the use of feed additives, particularly enzymes, as one of the strategies to reduce feed costs while maintaining or even enhancing poultry productivity. Enzymes are used to substitute the function of some feed ingredities such as soybean meal (SBM) and yellow corn (Alshelmani 2021), which are commonly used as feed ingredients in poultry but are becoming more expensive due to market fluctuations and global supply challenges. While poultry animals produce enzyme naturally, they do not typically produce them in sufficient quantities to break down complex feed ingredients efficiently. Supplementing poultry diets with specific enzymes can help address the problem, increasing growth rate and feed efficiency (Kalantar et al., 2015) by improving digestion system, maximizing the use of nutrient and lowering excretion of important nutrients (Abd El-Hack et al., 2017).

The improvement of digestion system by enzyme works with breaking down non-starch polysaccharide (NSP) enzymes. NSPs, such as xylan, arabinoxylan, and cellulose are found in some feed ingredients, and are able to bind water in the digestive system, causing fluid viscosity that affects the digestion of carbohydrate, protein, and fat. High viscosity also causes sticky dropping amounts (Bieniek and Buksa 2023; Walters et al., 2018). Supplementing poultry diets with specific enzymes can address this challenge.

Endo-1,4-β-xylanase as a feed additive contains endo-1,4 beta-xylanase as its main active component. Endo-1,4 beta-xylanase is an enzyme produced by a vast array of organisms and this enzyme is crucial in xylan saccharification, which is the breakdown of xylan, a major component of hemicellulose in cell walls, thus causing hydrolysis (Mendonça et al., 2023). The enzyme has been widely used in the poultry industry, particularly in the countries where feed costs are a significant concern, due to its potential to enhance feed efficiency by breaking down NSPS such as xylan in plant-based feed ingredients (Saleh *et al.* 2024).

Considering the increasing need of feed additive such as enzyme and its potential benefits, this study aimed to evaluate the effects of Endo-1,4-β-xylanase on the growth performance through observation of feed conversion ratio (FCR) and body weight gain.

MATERIALS AND METHODS

Ethical approval

Ethical approval had been obtained prior to the research from the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University.

Feed Ingredients

The feed additive material used in this study is Ronzyme-AX. One kilogram of Ronozyme-AX (feed ingredient 80%) contains 400 grams of Endo-1,4-β-xylanase as the active compound, followed with 300 grams each of rice hulls and granulated limestones. 100 gram of Ronozyme-AX (feed ingredient 80%) was supplemented in a ton of feed.

Management of Experimental Animals and Research Design

A total of 96 ross broiler chickens, aged 2-3 weeks old were used for this research. The chickens were acclimatized for seven days prior to the research conduction, given vaccination and anthelmintics to ensure they were free from any disease that might affect the research. Subjects were housed in the Laboratory Management Unit with temperature of 26.8°C and humidity ranging from 65% to 80% (Okonkwo and Akubuo, 2007).

The research was conducted using completely randomized design (CRD). The chickens were divided into four groups, with 16 chickens per treatment. Each treatment group was divided into two replicates, with 8 chickens per replicate. The groups were dividied into negative control group and treatment groups administered with 80 gram/ton (P80), 100 gram/ton (P100) and 120 gram/ton (P120) Ronozyme-AX (feed ingredient 80%).

Administration according to each group was done with mixing Ronozyme-AX (feed ingredient

80%) with the chicken feed for 28 days, consecutively.

Growth Performance

Body Weight Gain

Body weight gain was measured each week, starting from the first day of the research. Body weight gain was calculated by substracting weight on the recent week and the previous week (BWG = RW – PW).

Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) was calculated to determine the effect of the amount of feed consumed to the weight gain of the chickens. FCR was measured following the formula: feed intake (g) / total weight gain (g).

Safety Assessment

Mortality Rate

Mortality rate was measured to determine the number of dead chickens during the 28-day-study. The chickens were checked for mortality everyday prior to the administration of feed additive. At the end of the day, the mortality rate would be calculated using the formula: number of moralities / total number of chickens.

Blood Count and Biochemistry Panel

On the last day, blood was collected from the chickens from brachialis vein using a 3 mL syringe, with approximately 3 mL of blood drawn. Blood was collected into ethylene diamine tetra-acetat (EDTA) tubes. Blood parameters that were observed include red blood cells (RBC), monocytes, lymphocytes, eosinophils and heterophils. Blood chemistry was also analyzed, with parameters such as SGPT, SGOT, creatinine, and ureum.

RESULTS

Based on the results on the weekly body weight gain, although there was no significant difference ($p<0.05$) observed between the administered groups. a significant difference could be seen on the first and third week between the control group and the groups administered with Ronozyme-AX (feed ingredient 80%), with the groups administered with 80 gram/ton Ronozyme-AX (feed ingredient 80%) showed the highest average body weight gain of 485.0 ± 97.7 grams (Table 1). This shows that the administration of Ronozyme-AX (feed ingredient 80%) had a positive effect on body weight gain of the chickens in the 3-weeks study period.

The results for the overall growth performance, including the initial body weight and final body weight, feed conversion rate (FCR) and carcass weight are presented in Table 2. Based on the results, there was a significant difference observed between the final weight of negative control group and groups administered with Ronozyme-AX (feed ingredient 80%). The groups administered with the enzyme had a higher final weight. However, FCR and carcass weight showed no significant difference.

The safety assessments showed that no chickens died during the studies, and the blood count and biochemistry showed no significant difference compared to the control group, further supporting the safety of Ronozyme-AX (feed ingredient 80%) in chickens (Table 3).

Table 1. Effects of Ronozyme-AX (feed ingredient 80%) on the weekly weight gain of ross broiler chickens

Periods	Negative control (g)	P-80 (g)	P-100 (g)	P-120 (g)
Week-1	394.9 ± 24.0^{b}	515.9 ± 44.0^{a}	462.6 ± 51.9^{a}	489.6 ± 69.0^{a}
Week-2	508.0 ± 96.9^{a}	586.1 ± 47.8^{a}	529.0 ± 69.0^{a}	563.9 ± 49.6^{a}
Week-3	299.3 ± 69.9^{b}	352.9 ± 54.3^{a}	366.6 ± 44.8^{a}	378.1 ± 44.5^{a}
AVERAGE	400.8 ± 85.3	485.0 ± 97.7	452.8 ± 66.7	477.2 ± 76.4

The same superscript letter shows no significant difference (P<0.05)

Table 2. Effects of Ronozyme-AX (feed ingredient 80%) on the overall growth performance of ross broiler chickens

Parameters	Negative control (g)	P-80 (g)	P-100 (g)	P-120 (g)
Initial weight (g)	40.0 ± 0^{a}	40.0 ± 0^{a}	40 ± 0^{a}	40.0 ± 0^{a}
Final weight (g)	1459.81 ± 177.4^{b}	1825.38 ± 157^{a}	1685.56 ± 179.9^{a}	1766.56 ± 177^{a}
FCR	1.317 ± 0.217^{a}	1.037 ± 0.081^{a}	1.146 ± 0.102^{a}	1.077 ± 0.09^{a}
Carcass weight (g)	1330 ± 49.5^{a}	1731 ± 284.3^{a}	1598.5 ± 9.192^{a}	1591 ± 29.7^{a}

The same superscript letter shows no significant difference (P<0.05)

Table 3. Safety evaluation of Ronozyme-AX (feed ingredient 80%) on ross broiler chickens

Parameters	Negative control (g)	P-80 (g)	P-100 (g)	P-120 (g)
Mortality				
Number of mortality	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Blood count				
RBC (10^6/µl)	0.74 ± 0.8[a]	1.28 ± 0.42[a]	1.44 ± 0.82[a]	0.94 ± 0.62[a]
Monocytes (%)	20.4 ± 22.6[a]	2.2 ± 5[a]	0 ± 0[a]	1.3 ± 2.9[a]
Lymphocytes (%)	79.6 ± 22.6[a]	84.1 ± 17.9[a]	96.3 ± 5.2[a]	98.7 ± 2.9[a]
Eosinophiles (%)	0 ± 0[a]	2.2 ± 5[a]	3.7 ± 5.2[a]	0 ± 0[a]
Heterophiles (%)	0 ± 0[a]	11.4 ± 18.6[a]	0 ± 0[a]	0 ± 0[a]
Blood biochemistry				
SGPT	6.8 ± 0.6[a]	4.0 ± 2.3[a]	6.7 ± 1.1[a]	8.8 ± 3.4[a]
SGOT	3.5 ± 0.5[a]	70.0 ± 102.1[a]	61.8 ± 70.2[a]	3.6 ± 0.8[a]
Creatinine	0.3 ± 0.0[a]	0.4 ± 0.0[a]	0.3 ± 0.1[a]	0.3 ± 0.0[a]
Ureum	3.5 ± 0.4[a]	5.0 ± 1.8[a]	4.5 ± 3.5[a]	6.8 ± 2.0[a]

The same superscript letter shows no significant difference ($P<0.05$)

DISCUSSION

The results showed that Ronozyme-AX (feed ingredient 80%) had a positive effect on the body weight gain in chickens, with the 80 g/ton dose showing the most notable increase in average weight gain during the four weeks study period, particularly on the first and third week. This is mainly due to the active compound of the enzyme, which is endo-1,4- beta xylanase, as xylanase is one of the most crucial NSPases, affecting soluble, insoluble, and fermentable NSPs (Masey-O'Neill *et al.* 2014). Most of chicken feed, which comes from processed plants, contain cell wall that has NSPs, that can work with encapsulating nutrient, preventing enzymes to penetrate nutrients and ability to produce short-chain fatty acids. This caused reduced absorption of nutrients through gastrointestinal wall, and increased water consumption with excreta moisture content (Morgan *et al.* 2018).

Feed supplementations, such as xylanase, works with breaking down these NSPs with several ways, such as reducing the viscosity of the gastro intestinal tract and breaking down the cell walls of feedstuff, directly accelerating the process of fermentation (Ceylan *et al.* 2023).

Xylanase cleave the internal glycosidic linkages in xylan and arabinoxylan, producing short-chain xylo-oligosaccharides (XOS) and arabinose-substituted xylo-oligosaccharides (AXOS) (Jommuengbout *et al.* 2009).

These produce short chain fatty acids (SFCA) that could be reduced by NSPS, which induce a positive and balanced effect on the overall composition and activity of gastrointestinal microbiota, thus increasing the performance of broiler (De Maescchalck *et al.* 2015; Bautil *et al.* 2020). This is due to SCFAs role in cellular differentiation, gastrointestinal tract integrity, and the balance of gut microbiota (Gonzalez-Ortiz *et al.* 2019; Van Hoeck *et al.* 2021).

This explains how the administration of Ronozyme-AX (feed ingredient 80%) could significantly increased the body weight gain of the administered groups, compared to the control, clearly suggesting that the enzyme has a beneficial and sustained impact on growth performance, through enhancing digestive efficiency in better utilization of feed ingredients.

While significant differences were found in final body weight between the control and enzyme-related groups, there were no observed differences in feed conversion rate (FCR) or carcass weight. This lack of difference is actually intriguing, as xylanase, the active compound of Ronozyme-AX (feed ingredient 80%) are often expected to increase feed efficiency through improving nutrient digestibility. This might be explained due to the short duration of the study, as three weeks might not be long enough to see

significant impact on FCR and carcass weight. Additionally, this also may be due to how the effects of the enzyme are more focused to improving the availability of specific nutrients, such as proteins, amino acids, or fiber, rather than a general increase in feed efficiency. It is also possible that the enzyme might primiarily enhance the digestibility of certain nutrients that do not directly impact FCR or carcass weight, such as fibers (Rybicka *et al.* 2024).

Based on the safety assessments, no chickens died during the three weeks period, showing that the enzyme is relatively safe to use. The results of blood count also showed no significant difference compared to normal. Blood count is vital and highly related with the safety of substance administered and its reaction within the system. White blood count (WBC) such as monocytes, lymphocytes, eosinophiles and heterophiles are important, as they signal the responses of the body towards various stimulus, such as infection caused by microorganisms and inflammation (Angelina *et al.* 2019). Heterophils, a unique white blood cells in chickens, which is similar to neutrophils in mamals, hold a certain role to circulate as phagocytic cells in early response of inflammation (Mirantika *et al.* 2021). No significant difference in these parameters suggest that Ronozyme-AX (feed ingredient 80%) did not cause any inflammation response in the body that triggers the immune system.

Blood chemistry parameters, such as SGPT, SGOT, creatinine and ureum suggest the overall function of excretion organs, which are the kidneys and the liver. No significant difference between administered groups and control group showed that the liver cells were not damaged, as impaired liver results from damaged hepatocytes, causing impairment in transport and membrane permeability, increasing the release of SGPT and SGOT in the cytoplasm into blood circulation (Saputro *et al.* 2022). Meanwhile, creatinine and ureum indicate whether there is a dysfunction in the kidneys, which was not shown in this study (Kamal 2014).

Ronozyme-AX (feed ingredient 80%) was proven to improve body weight gain in broiler chickens, through enhancing nutrient digestibility due to its active compound, which is endo-1,4-beta xylanase. While no significant difference was observed in FCR and carcass weight, the enzyme's positive impact on final weight suggests it improves nutrient utilization. Safety assessments showed no negative effects on blood parameters or organ function, showing that Ronozyme-AX (feed ingredient 80%) is safe to use in boriler chickens.

"The author states that there is no conflict of interest with any of the parties involved in this research".

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