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Research

Effectivity and Safety of Endo-1,4 -xylanase (Xylanase), Endo-1,3(4) beta-glucanase (Beta-glucanase), Endo-1,4 beta-glucanase (Cellulase), and α Amylase as A Growth Promoter in Broiler Chickens

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ABSTRACT

This study evaluates the effectivity and safety of Endo-1,4 -xylanase (Xylanase), Endo-1,3(4) beta-glucanase (Beta-glucanase), Endo-1,4 beta-glucanase (Cellulase), and α Amylase that packed in Ronozyme-AX ct (MGHSPHYT+) as an alternative to antibiotic growth promoter. A total of 64 chickens were used, divided into four groups, with each group divided into two replicates, 8 chickens per replicate. The groups were divided into treatment group, and groups administered with the different dosage of supplementation, namely 184, 230, and 276 gram/ton feed for 28 days, consecutively. Parameters such as weekly weight gain, final weight, feed conversion ratio (FCR), and carcass weight were observed to determine growth performance. Meanwhile, parameters such as mortality, blood hematology and biochemistry were observed to determine the safety of the supplementation. Based on the results, there was a significant difference on weekly weight gain, particularly in the second week, and in the final weight, with the group administered with 184 gram/ton feed showing the highest result. Meanwhile, other parameters such as FCR and carcass weight did not show any significant difference. This may be due to the enzymes not working directly to increase feed utilization but work with improving nutrient digestibility and utilization in the body. The safety parameters showed that the supplementation was relatively safe as no significant difference was observed in mortality, blood constituents and biochemistry.

Keywords: Broiler chickens, Growth promotor, Ronozyme AX ct

ABSTRAK

Penelitian ini mengevaluasi efektivitas dan keamanan Endo-1,4 -xylanase (Xylanase), Endo-1,3(4) beta-glucanase (Beta-glucanase), Endo-1,4 beta-glucanase (Cellulase), and α Amylase yang dikemas dalam Ronozyme-AX ct (MGHSPHYT+) sebagai alternatif antibiotik growth promoter. Sebanyak 64 ekor ayam digunakan, dibagi menjadi empat kelompok, dengan masing-masing kelompok dibagi menjadi dua ulangan, 8 ekor ayam per ulangan. Kelompok dibagi menjadi kelompok perlakuan, dan kelompok yang diberi dosis suplementasi yang berbeda, yaitu 184, 230, dan 276 gram/ton pakan selama 28 hari, secara berturut-turut. Parameter seperti pertambahan berat badan mingguan, berat akhir, rasio konversi pakan (FCR), dan berat karkas diamati untuk mengetahui kinerja pertumbuhan. Sementara itu, parameter seperti mortalitas, hematologi darah dan biokimia diamati untuk mengetahui keamanan suplementasi. Berdasarkan hasil, terdapat perbedaan yang signifikan pada pertambahan berat badan mingguan, terutama pada minggu kedua, dan pada berat akhir, dengan kelompok yang diberi pakan 184 gram/ton menunjukkan hasil tertinggi. Sementara itu, parameter lain seperti FCR dan berat karkas tidak menunjukkan perbedaan yang signifikan. Hal ini mungkin disebabkan oleh enzim yang tidak bekerja secara langsung untuk meningkatkan pemanfaatan pakan tetapi bekerja dengan meningkatkan daya cerna dan pemanfaatan nutrisi dalam tubuh. Parameter keamanan menunjukkan bahwa suplementasi tersebut relatif aman karena tidak ditemukan perbedaan signifikan dalam mortalitas, komponen darah, dan biokimia.

Kata kunci: broiler, growth promotor, ronozyme Ax ct

INTRODUCTION

The poultry industry has experienced significant growth thoughout the years, playing a crucial role in supporting the economy, particularly in developing regions. In order to enhance and accelerate production, the use of growth promoters in the industry has been increasing, including in broiler chicken farming. Growth promoter is defined as a substance given to farm animals to improve their growth for the same amount of feed consumed in a specific period of time (Peng *et al*. 2014). In the past, antibiotics were widely used as growth promoters (AGP), as chcickens administered with antibiotics showed higher growth rates than chickens that were not (Philips *et al*. 2004). However, the use of antibiotic growth promoters have become an urgent issue in the recent time, as it poses the risk of causing antimicrobial resistance and residue in animal products (Hafez *et al*. 2021). This calls for an alternative for AGP, such as acidifiers, prebiotics, probiotics, and enzymes (Morgan *et al*. 2017).

Enzymes is mostly used in poultry industry to reduce digestive diseases and improving performance by helping the digestion of non-starch polysaccharides (NSP) as a soluble fiber in poulty feed, maximizing nutrient availability and lowering excretion of important and usable nutrients used by the chickens (Alshemani *et al*. 2021; Alshemani *et al*. 2016). There are a lot of enzymes used in the poultry industry, with various uses. Ronozyme AX ct (MGHSPHYT+) is an enzyme feed additive containing phytase, endo-1,4-beta-xylanase, endo-1,3-beta-glucanase, endo-1,4-beta-glucanase and α-amylase.

B-xylanase, β-mannanase, β-glucanase, α-amylase, and phytase are the majority of enzymes used in animal farm industry nowadays (Junior *et al.* 2024). These enzymes are classified as exogenous enzymes, supplemented in the daily diet of animals to help digest complex structures of cellular components in feed through the mechanism of hydrolysis (Aderibigbe et al. 2020; Adeola *et al.* 2011). Phytase as an enzyme is responsible for catalyzing hydrolysis and release phosphor from phytic acid in plant-based feedstuffs (Lei *et al*. 2003; Cowieson *et al*. 2016). Xylanase is a unique enzyme because other than its function to increase nutrient digestibility, this enzyme also regulates immune function (Passos *et al*. 2015; He *et al*. 202). The other enzyme, β-glucanase, works in energy metabolism and intestinal cell proliferation (Karimi *et al*. 2015). Meanwhile, the last component in Ronozyme AX ct (MGHSPHYT+), α-amylase, improves nutrient and energy digestibility, and has been proven to be able to enhance growth performance in broilers (Woyengo *et al*. 2015).

Theoritically, these enzymes, used in combination and incorporated into broiler chickens feed as a feed additive, may help boost the growth of the broilers, as each enzyme works differently and targets specific components of the feed, optimizing digestion and nutrient absorption in broiler chickens. This study focuses on evaluating the efficiency of the enzyme combination in Ronozyme AX ct (MGHSPHYT+) as a growth promoter in broiler chickens. By observing parameters such as feed conversion ratio (FCR), body weight gain (BWG), and mortality rate. This study also observed the safety of the product by measuring blood constituents and histopathological staining of vital organs.

MATERIALS AND METHODS

Ethical Approval

The research has been approved by 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 *Ronozyme-Ax Ct (Mghsphyt+)*. One kilogram of Ronozyme AX ct (MGHSPHYT+) contains 256 grams of phytase, combination of Endo-1,4-xylanase, Endo-1,3-beta-glucanase, Endo-1,4-beta-glucanase and α-amylase in a total of 480 grams, and 120 grams of α-amylase. Additional compounds added were 10 grams of anticake, 0.5 grams of antioxidant, 113.5 grams of rice hulls, and 20 grams of granulated limestones. 230 grams of Ronozyme AX ct (MGHSPHYT+) was supplemented in a ton of feed.

Experimental animals

A total of 64 ross broiler chickens aged 2-3 weeks old were used for this research. The chickens were acclimatized for seven days prior to the research to ensure they were used to the new environment, and given vaccination and anthelmintics so that they were free from any parasite or disease that might affect the research. The broilers were kept in the Laboratory Animal Management Unit with temperature of 26.8°C and humidity ranging from 65% to 80% (Okonkwo *et al.* 2007).

The research followed completely randomized design (CRD) method and divided into several groups with different concenterations of Ronozyme AX ct (MGHSPHYT+). Each group was divided into

two replicates, with 8 chickens per replicate. The groups were divided into negative control group and treatment groups administered with 184 gram/ton (P-184), 230 gram/ton (P-230) and 276 gram/ton (P-276). Administration was done for 28 days, consecutively with the feed additive mixed into the chicken feed.

Body weight gain

Body weight gain was measured each week, starting from day 0. Body weight gain was compared from the recent week to the previous week by substraction, following the formula; BWG = RW – PW.

Feed conversion ratio (FCR)

Feed conversion rate (FCR) was calculated in order to observe 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).

Mortality rate

The number of death was measured daily to determine the safety of the feed additive. At the end of the day, mortality rate per group would be calculated by comparing the number of dead chickens with the total of chickens used in the research.

Complete Blood Count (CBC)

On the last day of the research, blood would be collected from the chicken through the brachialis vein using a 3 mL syringe, with approximately 3 mL of blood collected into ethylene dieamine tetra-acetat (EDTA) tubes. CBC was measured using hematology analyzer. Parameters observed include red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), thrombocytes, red blood cells (WBC), lymphocytes, monocytes and granulocytes.

Histopathological examination

After blood collection, the chickens would be necropsied for vital organ collection, including liver, kidney, innards, heart, lungs, and spleen. The samples were collected in 10% formaldehyde prior to the histopathological staining.

The organs were stained using hematoxylin-eosin (HE) staining method with a series of dehydration, paraffination and deparaffinization. Abnormalities were observed in each sample to determine the effect of the feed additive on vital organs.

RESULTS

Based on the results, there was a significant difference in the weekly weight gain of the chickens, particularly in week-2, where the negative control group showed a significant difference compared to the groups administered with Ronozyme-AX ct (MGHSPHYT+), with the latter having higher weight gain. Meanwhile, no significant difference was observed on the first and third week. Group administered with 184 gram/ton feed (P-184) showed the highest average of weekly gain of 492.7 ± 77.0 (Table 1).

Table 1. Effects of Ronozyme-AX ct (MGHSPHYT+) on the weekly weight gain of ross broiler chickens

Periods	Negative control (g)	P-184 (g)	P-230 (g)	P-276 (g)
Week-1	479.3 ± 141.3^{a}	493.8 ± 54.0^{a}	527.1 ± 34.9^{a}	450.0 ± 19.4^{a}
Week-2	392.4 ± 219.8^{b}	586.4 ± 92.7^{a}	571.4 ± 50.4^{a}	573.1 ± 32.5^{a}
Week-3	399.0 ± 123.7^{a}	397.8 ± 40.6^{a}	353.7 ± 64.4^{a}	310.3 ± 35.8^{a}
AVERAGE	423.6 ± 39.5	492.7 ± 77.0	484.0 ± 93.9	444.4 ± 107.4

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

There was no significant difference observed on the initial weight, FCR, and carcass weight. However, groups administered with Ronozyme-AX ct (MGHSPHYT+) showed a significant difference compared to the negative control group on the final weight, with the negative control group showing lower final weight compared to the administered groups. P-184 showed the highest final weight of 1846.38 ± 203.2 (Table 2).

Based on the safety evaluation, no mortality was observed during the study for neither groups, and the parameters of blood count and biochemistry did not show any significant difference between the control and administered groups (Table 3), proving the safety of Ronozyme-AX ct (MGHSPHYT+) in this study.

Table 2. Effects of Ronozyme-AX ct (MGHSPHYT+) on the overall growth performance of ross broiler chickens

Parameters	Negative control (g)	P-184 (g)	P-230 (g)	P-276 (g)
Initial weight (g)	40.0 ± 0[a]	40.0 ± 0[a]	40.0 ± 0[a]	40.0 ± 0[a]
Final weight (g)	1525.06 ± 79.95[b]	1846.38 ± 203.2[a]	1786.13 ± 179.7[a]	1702.94 ± 105.4[a]
FCR	1.260 ± 0.065[a]	1.0417 ± 0.115[a]	1.0602 ± 0.107[a]	1.111 ± 0.068[a]
Carcass weight (g)	1383.5 ± 149.2[a]	1584 ± 63.64[a]	1821.5 ± 85.56[a]	1584 ± 63.64[a]

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

Table 3. Safety evaluation of Ronozyme-AX ct (MGHSPHYT+) on ross broiler chickens

Parameters	Negative control (g)	P-184 (g)	P-230 (g)	P-276 (g)
Mortality				
Number of mortality	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Blood count				
RBC (10^6/µl)	0.98 ± 0.03[a]	0.66 ± 0.3[a]	1.04 ± 0.73[a]	0.94 ± 0.62[a]
Monocytes (%)	20.3 ± 22.1[a]	9.8 ± 15.8[a]	15 ± 14.9[a]	1.3 ± 2.9[a]
Lymphocytes (%)	78.3 ± 21.8[a]	90.2 ± 15.8[a]	80 ± 12.6[a]	98.7 ± 2.9[a]
Eosinophiles (%)	1.4 ± 3.2[a]	0 ± 0[a]	5 ± 11.2[a]	0 ± 0[a]
Heterophiles (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Blood biochemistry				
SGPT	6.3 ± 0.3[a]	7.0 ± 5.6[a]	6.7 ± 1.1[a]	7.0 ± 1.1[a]
SGOT	3.6 ± 1.3[a]	1.9 ± 1.1[a]	2.6 ± 0.8[a]	2.8 ± 0.6[a]
Creatinine	0.3 ± 0.0[a]	0.3 ± 0.1[a]	0.3 ± 0.1[a]	0.3 ± 0.0[a]
Ureum	2.9.± 0.6[a]	3.8 ±0.0[a]	4.5 ± 3.5[a]	4.2 ± 2.1[a]

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

DISCUSSION

The results showed that Ronozyme-AX ct (MGHSPHYT+) has an effect in increasing weekly body weight gain and final weight, with 184 g/ton dosage showing the best growth performance. This proves that the active composition of Ronozyme-AX ct (MGHSPHYT+), including phytase, endo-1,4-beta-xylanase, endo-1,3-beta-glucanase, endo-1,4-beta-glucanase and α-amylase. These compositions work with various mechanisms in enhancing growth performance, specifically in broiler chickens.

Most of poultry feed is derived from plant-based ingredients, containing 10-30% non-starch polysaccharides (Choct 2015). However, the influence of dietary NSP depends on its quantity and physio-chemical composition and structure (Raninen *et al.* 2015). Too much NSPs may have negative effects on chickens, hindering overall growth performance. However, feed additive such as xylanase has been widely used to overcome the challenges presented by the NSPs. Previous studies have shown that xylanase can reduce the intestinal viscosity by partially hydrolizing NSP, improving nutrient digestilities and growth performance of broilers (Gao *et al.* 2008; Vandeplas *et al.* 2010). Based on research conducted by Saleh *et al.* (2024), endo-1,4-beta-xylanase supplementation on chicken feed has been proven to increase gene expression related to growth and fatty acid syntesis, affecting growth performance, abdominal fat percentage, nutrient digestibility and immunity for broiler chickens.

Similarly, beta-glucanase as contained in endo-1,3-beta-glucanase and endo-1,4-beta-glucanase has been proven to increase growth in poultry (Cho *et al.* 2013), improve immune status, and maximize digestibility of feed (Ding *et al.* 2019). 1,3-beta glucanase has been proven to have significant impact to the quality of meat as it reduces oxidative stress in poultry, specifically during growing periods (Moon *et al.* 2016). However, based on previous research, studies have proven that beta glucan has no effects on growth performance, such as average daily intake and feed conversion ratio (Moralez-Lopez *et al.* 2009; Cox *et al*. 2010), which is in line with this study. This suggests that while beta-glucanase does not affect growth performance directly, it enhances meat quality and the overall health of the poultry by improving immune systems.

The third component, phytase, as an essential phosphorus mineral, accounts for up to 80% of the total phosphor (Kumar *et al.* 2012). Phytases work with catalyzing the hydrolysis and release of phosphor from phytic acid in plant-based feedstuffs (Cowieson *et al.* 2016), helping the digestibility of phytic P, influencing the microbiota and antioxidant status in non-ruminant animals and improving the integrity of intestinal morphology, thus enhancing growth in poultry (Zhang et al. 2021; Amiri *et al*. 2021) with maximizing the utilization of nutrient uptake, specifically phosphor as an essential mineral in poultry.

Amylase, as the last component, plays an important role in digesting native starch. Chickens originally has a high pancreatic secretion of amylolytic juice (Lehrner and Malacinski 1975). However, as they grow older, intestinal mass and pancreatic tissue tend to be diminished , limiting the enzyme secreted (Croom *et al.* 1999). Thus, supplementing amylase increases starch digestibility, performance, and a reduction in pancreatic mass in broiler chickens (Gracia *et al.* 2003). Based on research conducted by Abedribigbe *et al.* (2020), not only amylase improves growth performance in poultry due to the increased release of simple sugars from starch digestion, but also through changing the morphology of small intestine, which improves nutrient absorption.

Furthermore, while the study showed no significant differences in feed conversion ratio (FCR) and carcass weight, these parameters might be influenced by factors not directly measured in this study. The lack of significant difference in FCR and carcass weight could be attributed to the fact that the enzymes primarily work by improving nutrient availability, digestibility, as well as muscle composition and fat deposition, which are crucial for the quality of meat, rather than affecting the efficiency of feed utilization. This also may be due to the time of the study, which was only for four weeks. It is possible that with longer-term feeding, the supplementation may have a more noticeable impact on FCR, as it improves gut health and nutrient absorption by time.

Based on the safety evaluation, no mortality was observed during the study, further proving that the supplementation counts as relatively safe

and may enhance survival rate. No significant difference was observed in blood parameters as well. Parameters such as monocytes, lymphocytes, eosinophiles, and heterophiles show the immune response of the chickens toward the supplementation. No significant difference towards the administered groups and the negative control shows that the administration does not alter the immune response or cause any inflammation reaction (Chmielewski and Strzelec 2017).

Blood biochemistry also showed no significant difference, indicating that the supplementation does not alter vital excretory organs such as the liver and kidneys. SGPT and SGOT shows whether there are any liver dysfunction, causing elevation in these enzymes as they are released from the hepatocytes to the blood circulation (Harton and Prabowo 2018). Meaning that the lower their concentration is, the better the overall condition of the liver. Meanwhile, creatinine and urea are crucial parameters for any dysfunction of the kidneys (Kamal 2014), which were not shown in this study.

Based on the results, Ronozyme-AX ct (MGHSPHYT+) has been proven to enhance growth performance in broiler chickens as they showed significant weekly weight gain and final weight compared to the negative control group, with P-184 group showing the highest value. However, FCR and carcass weight were not affected in this study. This could be due to the enzymes working with improving digestibility and the quality of meat without directly affecting feed utilization and weight directly. Ronozyme-AX ct (MGHSPHYT+) has also been proven to be safe in broiler chickens as no significant differences in blood hematology and biochemistry were observed.

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

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