The prebiotic production by using cassava peels with the addition of both K\(^+\) and Mg\(^{2+}\) metal ions as an activator and their potential to enhance broiler quality

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Abstract. Prebiotic is a nutrient for probiotic bacteria in the digestive tract of broilers. Prebiotics are used as a substitute for antibiotics in stimulating safer broiler growth. This study aims to determine the effect of prebiotics on broiler weight gain, the effect of prebiotics on the addition of metal ion combinations, and the effect of antibiotics when compared with controls. Prebiotic production is carried out using cassava peels as a substrate and \textit{Lactobacillus plantarum} as an enzyme producer that acts as a catalyst. The cofactor used is a combination of metal ions K\(^+\) and Mg\(^{2+}\). Prebiotic administration was carried out for 23 days with a volume of 1 mL/L as well as the determination of blood cholesterol levels in broilers. The results showed that the weight of broiler s given prebiotics was 1230.75 grams with blood cholesterol levels 143 mg/dL. At the control of 1079.5 grams with a blood cholesterol level of 255.5 mg/dL. Antibiotics amounted to 1120.75 grams with blood cholesterol levels of 178 mg/dL. In prebiotics, metal ions of 0.4 mM are added; 0.6 mM; and 0.8 mM in a row of 1254.25 grams; 1192 grams; and 1167.25 grams with blood cholesterol levels 131 mg/dL; 132 mg/dL; and 145.5 mg/dL. This study showed that prebiotic administration affected broiler weight gain and blood cholesterol levels.

1. Introduction

Prebiotics are food (carbohydrates) that are undigested and beneficial to the intestinal microflora by stimulating bacterial growth found in the large intestine [1]. In the digestive tract, prebiotics act as a source of nutrition for the growth of probiotic, maintain the health of the digestive tract, keep the immune system and broiler growth faster [2].

Food which is categorized as a prebiotic is if it cannot be absorbed by the digestive tract of the small intestine so that it will reach the large intestine and then it will be degraded by intestinal bacteria. This will stimulate the growth of lactic acid bacteria which is beneficial for digestion and can suppress pathogenic bacteria growth [3]. The most potential prebiotics consists of carbohydrates [4]. One source of carbohydrates that has the potential as a prebiotic is cassava peels.

Cassava peels are one of the solid wastes of cassava (\textit{Manihot esculenta} Crantz) which is obtained from agro-industrial waste. The chemical content of cassava peels consists of 30.87% starch, crude protein 4.63%, crude fiber 13.04% and crude fat 1.99% [5].

\textit{Lactobacillus plantarum} is a type of BAL homofermentative [6] which forms a typical lactic acid used in starch fermentation in yams. \textit{Lactobacillus plantarum} has also been reported to be able to...
produce amylase enzymes to degrade starch [7]. Lactic acid bacteria produce the enzyme bile salt hydrolase (BSH) which can reduce cholesterol levels and lipase enzymes that can reduce triglycerides because of their ability to decide long-chain fatty acids into medium-chain chains and short chains so that they are easily absorbed in the intestine [8].

Enzymes generally require other compounds to carry out their catalytic functions such as cofactors. Metal ions have a role in the process of catalysis and structural preparation of enzymes. Most activators are inorganic ions, especially metal ions such as Na⁺, K⁺, Rb⁺, Cs⁺, Mg²⁺, Zn²⁺, Fe²⁺, Co²⁺, Ni²⁺ and Al³⁺ [9].

Research on the production of prebiotic compounds from sago through Lactobacillus casei fermentation with the addition of metal ions Ca²⁺ has been reported to show the best results on the addition of metal ions Ca²⁺ at concentrations of 0.1% and 0.15% to increase broiler weight [10].

2. Material and Methods

2.1. Material
The materials used in this study are cassava peels, distilled water, Mann Shape Broth (MRSB), peptone, KH₂PO₄, meat extract, NaNO₃, FeSO₄.7H₂O, MgSO₄.7H₂O, MgCl₂, KCl, Lactobacillus plantarum, CaCO₃, glucose, starch, acetate buffer, 3.5-Di Nitro Salisilic Acid (DNS), NaOH, Na₂SO₃, BSA, Bradford reagent, Na₂CO₃, CuSO₄.5H₂O, potassium sodium tartrate, Whatman 41 filter paper, and pH indicator paper.

2.2. Method

2.2.1. Preparations of Cassava Flour
The outermost cassava peels are separated from the inner cassava peels. Then take the inner cassava peels (epidermis). After that boiled for 1 hour. After boiling, then the cassava peels is soaked for 3x24 hours in water added with salt (NaCl) with a concentration of 5%. After that, the cassava peels are dried under the sun. After that, roast using a blender until it becomes flour. Then sifted with a 40 mesh sieve.

2.2.2. Media Inoculum Preparations
MRSB weighed 13 grams and 2.5 grams of cassava peels flour. Then the mixture is dissolved in 250 mL of distilled water. Then sterilized at 121°C for 15 minutes. After that 5 mL of active inoculum was pipetted to the sterilized inoculum media. Then incubated in the water bath shaker incubator for 1x24 hours at 37°C at a speed of 150 rpm.

2.2.3. Production Media Preparations
Pepton is weighed as much as 2.8 grams; KH₂PO₄ 1.4 grams; 7 grams of NaNO₃; 0.024 gram FeSO₄.7H₂O; MgSO₄.7H₂O 0.035 gram. 2.8 grams of meat extract dissolved to a volume of 1400 mL. Then all media mixtures were dissolved in 1400 mL of meat extract solution. 350 mL of media added KCl and MgCl₂ with variations in concentration of 0.4 mM; 0.6 mM; and 0.8 mM. 350 mL of media were not given the addition of KCl and MgCl₂ (control). After that, it was sterilized in an autoclave for 20 minutes at 121°C.

2.2.4. Prebiotic Production
After the production media was sterilized, the active inoculum was piped 52.5 mL in 350 mL of production media. Then added CaCO₃ as much as 0.7 grams. After that homogenized. Then incubated in a shaker incubator for 72 hours at 37°C at 150 rpm.
2.2.5. Test For Glucoamylase Enzyme Activity

The crude enzyme extract was piped as much as 1 mL and 1 mL of starch was added and then 1 mL of acetate buffer 0.2 M pH 4.97 was added. After that, it was incubated at 37°C for 20 minutes. Then 1 mL DNS was added and heated at 100°C for 5 minutes. After that, it is cooled in cold water. Then the absorbance is measured at λ 500 nm.

The activity of glucoamylase enzymes is calculated based on relative glucose level data as mg glucose produced per mL of enzyme filtrate per unit time using the following formula:

\[
AE = \frac{MG \times 1000}{BMg \times MI}
\]

Information:
AE = Enzyme activity (Unit / mL)
MG = Glucose weight
BMg = Glucose molecular weight
MI = Incubation period

2.2.6. Determining of chickens blood cholesterol levels after being given prebiotics using Nesco Multi Check N 01

A sample of broiler blood is taken from the pectoralis vein under the wing using a syringe. After that, the cholesterol test chip is installed on the measuring device. Then the cholesterol measuring strip is installed at the top of the measuring device. After appearing on the monitor as a sign of entering a blood sample, a broiler blood sample inside the syringe is inserted into the attached strip. A few minutes later the number of blood cholesterol measurements will be read on the monitor.

2.2.7. Apply Prebiotic to The Broilers

Prebiotics that have been made are mixed with 1 mL of broiler drinking water in 1 L of water for 23 days. Control broilers were not given prebiotics.

3. Results and Discussion

3.1. Optimum Lactobacillus Plantarum Incubation

Lactobacillus plantarum produces enzymes at 37°C with 6.0 media pH and optimum incubation time for 72 hours. As shown in Figure 1, at the 96th hour there was a decrease in absorbance which indicated a decrease in the bacterial population. This is because the nutrients in the media have been reduced, resulting in no more bacterial growth. In the exponential phase, bacteria experience rapid growth, besides the need for energy for bacteria in this phase is higher so that in the exponential phase the cells produce more metabolites needed to meet the needs for growth.

![Figure 1. Graph of growth of Lactobacillus plantarum at [S] 1%, pH 6, temperature 37°C with a speed of 150 rpm](image-url)
Figure 1 shows that at 72nd hour is the exponential phase of bacterial growth while at the 96th hour is the phase of population decline. Enzyme production occurs when the cell is in an exponential phase, namely at the 72nd hour. At 72 hours the growth of bacteria is higher than in the 96th hour. This is evidenced by the absorbance value at 72nd hour is greater than in the 96th hour.

3.2. Test for glucoamylase enzyme activity
The glucoamylase enzyme activity test was carried out in prebiotic samples and prebiotic samples by adding both of K⁺ and Mg²⁺. One characteristic of enzyme activity is that it requires a cofactor, a non-protein group of enzymes that determines its catalytic activity. The cofactor used in this study is inorganic molecules (metal ions).

The addition of metal ions has the potential to increase enzyme work activity. The addition both K⁺ and Mg²⁺ used in this study provides an increase in enzyme activity. Metal ions K⁺ and Mg²⁺ are activators of glucoamylase enzyme activity on starch substrates.

Figure 2. Graph effect of metal ion addition on glucoamylase enzyme activity

Figure 2 shows the effect of adding metal ions on prebiotics to glucoamylase enzyme activity. The activity of glucoamylase enzymes in prebiotics without the addition of metal ions is smaller than prebiotics which is added to metal ions. The enzyme activity in prebiotics without the addition of metal ions was 0.010 U/mL while the activity of prebiotic enzymes with the addition of metal ions at a concentration of 0.4 mM; 0.6 mM; and 0.8 mM respectively 0.0160; 0.0166; and 0.0167 U/mL.

The increase in enzyme activity at each increase in concentration is not so great. This can be caused because the increase in the concentration of metal ions has reached a saturation state so that it is not able to increase the enzyme activity even greater. Also, the glucoamylase enzyme activity obtained showed relatively small enzyme activity. This can be caused by a decrease in enzyme activity during the storage period. The longer the storage time of enzyme extracts, the enzyme activity will decrease.
3.3. Prebiotic Effects on Broiler weight Growth

![Figure 3](image)

The effect of prebiotics, prebiotics by the addition combination of metal ions, antibiotics and control (without the addition of prebiotics) with a prebiotic volume of 1 mL on broiler weight gain is shown in Figure 1. On the 28th day, broiler weight Q0; Q1; Q2; Q3; Q4; and Q5 in a row amounting to 1079.5 grams; 1120.75 grams; 1230.75 grams; 1254.25 grams; 1192 grams and 1167.25 grams.

Broiler weight in controls was smaller compared to broilers given antibiotics. This is because antibiotics are growth promoters that can spur growth in broiler livestock. Nonetheless, giving prebiotics shows that broiler weight is greater than giving antibiotics. This shows that prebiotics can stimulate the growth of probiotic bacteria in the digestive tract so that probiotic bacteria in the digestive tract of broilers can improve the digestive tract in the absorption of higher nutrients. A healthy digestive tract will affect the weight of broilers. This is supported by the statement of Kompiang (2009) that probiotic bacteria can improve the digestive tract and increase the digestibility of feed by suppressing pathogenic bacteria and assisting the absorption of nutrients [11].

Based on the data shown in Figure 3 shows that prebiotic administration with the addition both of K⁺ and Mg²⁺ 0.4 mM (Q3) has a greater weight than prebiotics that is not given metal ions (Q2). Broiler weight in Q3 is greater than Q2 while Q4 is smaller than Q3. This shows that the optimum concentration of metal ions in prebiotics on the growth of *Lactobacillus plantarum* at a concentration of 0.4 mM (Q3). The optimum amount of beneficial bacteria needed by the broiler digestive tract will affect the weight gain. It can be said that in prebiotics which added metal ions 0.4 mM is the right dose for broiler growth. Just like antibiotics, prebiotics will work to function if given to broilers in the right dosage.
3.4. Blood cholesterol levels in broilers after being given prebiotics

Based on Figure 4, it shows a comparison of broiler blood cholesterol levels in controls, antibiotics, prebiotics, and prebiotics with the addition both of K⁺ and Mg²⁺ at 1 mL prebiotic volume. Blood cholesterol Q0 is greater than Q1, which is 255.5 mg/dL and 178 mg/dL respectively. Whereas in Q2 it is 143 mg/dL. This shows that broilers given prebiotics had lower cholesterol levels than those who were not given prebiotics and who were given antibiotics. Giving prebiotics to broilers can reduce blood cholesterol levels. The decrease was allegedly due to the activity of lactic acid bacteria (BAL) which produced the enzyme bile salt hydrolase (BSH) in the reabsorption of fat and salt and bile [12]. Saputri (2012) reported that LAB produced the enzyme BSH which can reduce cholesterol levels and lipase enzymes which can break long-chain fatty acids into medium-chain chains and short chains so that they are easily absorbed in the intestine [13].

4. Conclusion
Prebiotics affect broiler weight gain when compared with the weight in broilers not given prebiotics and can reduce blood cholesterol levels in broilers.

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