The addition of both Ca$^{2+}$ and Na$^{+}$ metal ions to prebiotic productions from cassava peels to enhance the quality of broilers

A S Agustina$^1$, S Wahyuni$^1$, H Natsir$^2$ and I Raya$^2$

$^1$Postgraduate student in the Department of Chemistry, Hasanuddin University, Jl. Perintis Kemerdekaan KM 10, Makassar 90245, Indonesia.

$^2$Department of Chemistry, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10 Tamalanrea, Makassar 90245, Indonesia

E-mail: indahraya05@gmail.com

Abstract. prebiotics which is made from cassava peel is one of the functional foods that cannot be digested by the intestine, functioning as a supplement to encourage the growth of good microorganisms, namely Lactobacillus plantarum in the digestive system. This study aims to determine the effect of adding Ca$^{2+}$ and Na$^{+}$ metal ions to prebiotics from cassava peels to enhance the broiler quality. Stages of research include fermentation, glucoamylase enzyme analysis using dinitro salicylate acid (DNS) and prebiotic application of broiler by observing two variables, there are broiler weight and blood cholesterol levels. The treatment given to broilers first is without any precautions namely control (P0), second where the positive control with addition of antibiotics (P1), third with the addition of prebiotics (P2), fourth is the addition of both prebiotics and metal ions 0.002 Molarity (P3), fifth is the addition of both prebiotics and metal ions 0.004 Molarity (P4), sixth is the addition of both prebiotics and metal ions 0.006 Molarity (P5). The results showed that glucoamylase enzyme activity in P2, P3, P4, and P5 were 0.0099 U / mL, 0.0117 U / mL, 0.0133 U / mL, and 0.0183 U / mL, respectively. The results for the application of chicken weights at P0, P1, P2, P3, P4, and P5 respectively were 1061 g, 1094.8 g, 1159.2 g, 1222.7 g, 1225.8 g, and 1227 g, while the results of blood cholesterol in broilers in P0, P1, P2, P3, P4, and P5 are 255.5 mg /dL, 177 mg /dL, 139 mg /dL, 118 mg /dL, 103 mg /dL, and 120.5 mg /dL respectively. The results showed that the used prebiotics and the addition of metal ion concentrations Ca$^{2+}$ and Na$^{+}$ 0.002 Molarity, 0.004 Molarity, and 0.006 Molarity were increased the broiler weight, while reducing broiler blood cholesterol levels.

1. Introduction
Rapid population growth, especially in Indonesia has triggered an increase in the need for animal protein. chicken is one of the alternative providers of animal protein that is much needed, this makes the chicken breeders do a variety of formulations on animal feed ingredients, intending to fulfill the nutrients needed for these chickens, in addition to the effects for consumers.

Antibiotics are used by farmers to increase the weight of the chicken (growth promoter). Antibiotic residues have many negative health effects, namely allergic reactions, toxicity, affect intestinal flora, immune response, and resistance to microorganisms. addition antibiotic residues can also affect the environment [1].

Functional food that has now be expanded is prebiotics. Prebiotics are foods that are not digested by digestive enzymes and can stimulate proliferation and probiotic activity in the large intestine and inhibit the proliferation of pathogens. Some prebiotics that is often added to food is inulin, fructooligosaccharide (FOS) and galactooligosaccharides (GOS) [2].
The starch content in cassava peel ranges from 59%. Cassava peel is used as a prebiotic raw material because the levels of carbohydrates contained in cassava skin are high. Starch or starch are complex carbohydrates that are not soluble in water, in the form of white, fresh and odorless powder. Cassava peel contains starch and crude fiber [3].

Probiotics are defined as microorganisms that are consumed by humans or animals in sufficient quantities, can live and pass through the condition of the stomach and digestive tract and are beneficial to the host cell by improving the health of the host. Lactobacillus plantarum is a species of bacteria belonging to probiotics [4].

The prebiotic compounds produced through fermentation can enhance the activity and stability of the enzyme by adding additives of metal ions Ca$^{2+}$ and Na$^+$ [5]. Like Rahma (2011), his prebiotic compound (sago pulp) was added with Ca$^{2+}$ metal ions having a difference in chicken weight of 386 grams compared to prebiotics without the addition of metal ions. In this research, the mixing of Ca$^{2+}$ and Na$^+$ metal ions will be carried out. The goal is to determine the performance of prebiotics and their application to chickens when a metal ion mixture is used.

2. Material and Methods

2.1. Material
The material used in this research is cassava peel, aquades, Mann Shape Rogosa Broth (MRSB), peptone, KH$_2$PO$_4$, yeast extract.

2.2 Method

2.2.1 Preparation of Cassava Peels
Cassava peel is separated from cassava, boiled for 1 hour then soaked in a salt solution (NaCl) of 3: 9 (3 kg of cassava peel: 9 liters of 5% saltwater solution) for 3 days. It is dried, then blended until smooth and sieved with a size of 60 mesh.

2.2.2 Preparation of Mann Rogosa Shape Broth (MRSB)
MRSB 5.2 grams and then dissolved in 100 mL distilled water. After that, it is heated to homogeneous. Then sterilized in an autoclave at 121°C pressure of 2 atm with a time of 15 minutes.

2.2.3 Preparation of Suspension of Lactobacillus plantarum
Lactobacillus plantarum was suspended in a 2 mL MRSB 250 mL media solution Incubated using a shaker water bath for 2x24 hours at 37°C

2.2.4 Preparation of Inoculum
4.5 g of MRSB dissolved with aquabides and homogenized. 2.5 g cassava peel flour dissolved with aquabides. The MRSB solution and cassava skin solution were mixed until the volume of 250 mL was then sterilized in the autoclave at 121°C for 25 minutes. After the solution is cold, 5 mL Lactobacillus plantarum suspension is added. Then shake it for 2x24 hours using an incubator shaker.

2.2.5 Preparation Production Media
Pepton 2.8 g; KH$_2$PO$_4$ 1.4; NaNO$_3$ 7 g; FeSO$_4$.7H$_2$O 0.015 g; MgSO$_4$.7H$_2$O 0.035 g then dissolved in 2% meat extract to a volume of 1400 mL. Each 350 mL was put in 500 mL Erlenmeyer and added CaCl$_2$ (metal ions Ca$^{2+}$) and NaCl (Na$^+$ metal ions) with each concentration of 0.002 Molarity; 0.004 Molarity; and 0.006 Molarity. Closed tightly then sterilized the autoclave at 121°C at a pressure of 2 atm for 25 minutes. Then shaking for 3 x 24 hours at 37°C using an incubator shaker.
2.2.6 Analysis of glucoamylase enzyme activity
1% 1 mL of starch; crude enzyme extract 1 mL; acetate buffer 0.2 M 1 mL (pH 5), then incubated at 37o C for 20 minutes. 1 mL of DNS was added and heated on a hotplate at 100oC for 5 minutes. After that, cooled with cold water. The next absorbance is measured at a wavelength of 500 nm (max \( \lambda \)).

2.2.7 Analysis of Broiler Blood Cholesterol Levels Using Nesco Multi Check N 01
A 35-day-old chicken blood sample was taken by injecting the wings (the blood vessels are in the wings). After that, the blood that has been taken is placed on the cholesterol kit for blood. Then wait a few seconds for the results of blood cholesterol to appear on the screen of the device.

2.2.8 Prebiotic Application of Broiler
Broilers aged 0 days to 35 days are maintained. Every 5 days a week the treatment is given as follows:
P0 : control - (without prebiotics)
P1 : control + (antibiotics)
P2 : prebiotics
P3 : prebiotics + Ca\(^{2+}\) and Na\(^+\) metal ions 0.002 M
P4 : prebiotics + Ca\(^{2+}\) and Na\(^+\) metal ions 0.004 M
P5 : prebiotic Ca\(^{2+}\) and Na\(^+\) metal ions 0.006 M

3. Results and Discussion
3.1 Analysis of glucoamylase enzyme activity.
The activity of glucoamylase enzyme produced by the Lactobacillus plantarum bacteria is done by adding inorganic compounds in the form of metal ions, namely a mixture of metal ions Ca\(^{2+}\) and Na\(^+\) in the form of salt compounds (CaCl\(_2\) and NaCl) with concentrations of 0.002 Molarity, 0.004 Molarity and 0.006 Molarity to 350 mL cassava peel substrate (as a fermentation medium). The results obtained from glucoamylase enzyme activity can be seen in Figure 1:

![Figure 1. Graph of Effect of Addition of Ca\(^{2+}\) and Na\(^+\) Metal Ions to Prebiotic Glucoamylase Enzyme Activity](image)

Figure 1 shows the results of the study that the greater the concentration of the alloy of metal ions Ca\(^{2+}\) and Na\(^+\), the higher the enzyme activity of glucoamylase. The concentration of a mixture of metal ions Ca\(^{2+}\) and Na\(^+\) at 0.002 Molarity, 0.004 Molarity, and 0.006 Molarity is an activator. This is because the metal cations around the active side function to stabilize the structure of the enzyme because it is known that metal ions Ca\(^{2+}\) are divalent ions that have a major influence on the activity of the glucoamylase enzyme. This is appropriate with research did by Savchenko, et al (2000), which states that metal ions Ca\(^{2+}\) generally increase and stabilize the activity of the enzyme \( \alpha \)-amylase and glucoamylase [6].
3.2 The Effect of Prebiotics on Weighting of Broiler

The results of broiler weights obtained when raising chickens can be seen in Figure 2:

The results showed that prebiotic administration can increase broiler weight compared to without prebiotics. giving Ca²⁺ and Na⁺ metal ions at a concentration of 0.002 Molarity, 0.004 Molarity, and 0.006 Molarity can make broiler weight increase. The greatest weight of chicken is obtained when P5 (prebiotic + metal ion Ca²⁺ and Na⁺ 0.006 M). This result is directly proportional to the activity of the glucoamylase enzyme, the greater the addition of metal ions Ca²⁺ and Na⁺, the greater the performance of the enzyme in prebiotics.

Broiler weight in controls and antibiotics showed a difference. Control of broilers has the lowest weight, this is due to the lack of additional supplements given to broiler feed which results in broilers consuming less feed so that the development is not good.

When compared with previous studies using one metal Ca²⁺, the highest weight obtained was 1724 g. while in this study a mixture of Ca²⁺ and Na⁺ metal ions had the highest weight of 1703 g. addition of metal ions to prebiotics has different weights because, in previous studies using raw materials of sago flour as prebiotics, sago flour is known to have more starch content than cassava peel. The difference in starch content certainly affects the performance of the glucoamylase enzyme performance. The greater the starch contained in prebiotics, the performance of microorganisms to produce enzymes also increases [7].

3.3 Effect of Prebiotic on Broiler Blood Cholesterol

The results of broiler blood cholesterol levels obtained when raising chickens can be seen in Figure 3:

The difference in starch content certainly affects the performance of the glucoamylase enzyme performance. The greater the starch contained in prebiotics, the performance of microorganisms to produce enzymes also increases [7].
The results of the study in Figure 3 show that giving prebiotic broilers provides benefits that can reduce broiler blood cholesterol. When compared with P0 and P1, blood cholesterol levels are lower in prebiotic giving (P2). Cholesterol levels obtained by P0, P1 and P2 respectively are 255.5 mg/dL, 177 mg/dL and 146.5 mg/dL.

The use of lactic acid bacteria as a prebiotic is a potential one to reduce cholesterol. One characteristic of BAL is that it can reduce cholesterol levels. Based on previous research that by consuming products that contain lactic acid bacteria it can reduce cholesterol levels both in animals and humans [8].

Decreasing cholesterol levels by Lactobacillus can be through several mechanisms. The first mechanism is through feces, conjugated bile salts are not absorbed by the intestine, and are more easily wasted from the digestive tract compared to conjugated bile salts. The second mechanism, namely LAB, can inhibit cholesterol synthesis, thereby reducing cholesterol production. The third ability is LAB which can bind cholesterol to prevent cholesterol absorption back to the liver [9].

Giving probiotic microorganisms, namely Lactobacillus plantarum is quite optimal in increasing nutrient absorption and producing enzyme bile salt hydrolize (BSH). BSH enzyme activity has been detected in Lactobacillus, Bifidobacterium, Enterococcus, Clostridium, and Bacteroides spp. The BSH enzyme can reduce blood cholesterol levels and the lipase enzyme lowers blood triglycerides without leaving a residue.

**Conclusion**

Based on the results of research that has been done, it can be concluded that the addition of metal ions Ca\(^{2+}\) and Na\(^{+}\) 0.002 Molarity, 0.004 Molarity and 0.006 Molarity to prebiotics has a very large influence on the enhance of broilers weight and decrease in broilers blood cholesterol levels.

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