The effect of giving mannan extract from palm kernel cake to pathology and histopathology liver and intestine chicken which infected by *Salmonella thypimurium*

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Abstract. *Salmonella thypimurium* is an enteric disease in poultry and still a major problem in the livestock industry and human health because it is zoonotic. Mannan Oligosaccharide was originated from palm kernel cake which expected to replace the role of antibiotics. The purpose of this study was to find out the effect of giving mannan extract to relative weight of liver and intestine, pathology and histopathology changes on liver and intestines from broiler chicken. This test was used ninety six broiler chicken of 1 day age Cobb strain infected orally with $10^5$ CFU *S. thypimurium* on the third day. Levels of the mannan extract given were 0%, 0.1%, 0.2%, 0.3%, 0.4% and antibiotic as controls. The results showed that giving of mannan extract to relative weight of liver and intestine was not significant (P> 0.05). On the observation the effect of giving mannan extract to pathology and histopathology showed that it was not significant (P> 0.05) It is concluded that mannan extract is safe to use in livestock because it was not affected to the intestine and liver organ.

1. Introduction

Palm oil is the main plantation commodity that has developed very extensively. In 2016 the total area was more than 11.67 million/hectare with oil palm production reaching 33 million tons, which spread across all major islands in Indonesia such as Sumatra, Kalimantan, Sulawesi, Papua and Java [2]. With the title as the world palm oil producer, the availability of palm kernel cake is abundant. PCK (palm kernel cake) is widely used as animal feed because of its high protein, crude fibre and fat content. The use of BIS as a potential feed has been widely reported in ruminants [6] and livestock [8]. [7] reported that Mannose PCK content reached 68.9% and availability was guaranteed. High mannan content as well as limiting factors can also be considered as a potential to get prebiotic feed additives that will improve livestock health. Suspect that there are similarities between palm kernel meal with mannan oligosaccharides (MOS) which will improve the health and immune system of poultry.

Enteric disease can be caused by infection with pathogenic bacteria. One of the bacteria that often attacks and contaminates chickens is *Salmonella thypimurium* bacteria which can cause interference or
infection in the chicken's digestive tract. Salmonella itself is an infectious disease that is zoonotic and includes food borne disease [3].

Considering the small number of researchers who examined the effect of mannan extract from BIS on anatomical pathology and histopathology, the authors were interested in further investigating the study of the use of mannan extract from palm kernel cake as controllers of Salmonella thypimurium bacteria by looking at the potential use of mannan extracts approaching mannan-oligosaccharides from structural changes. Palm kernel cake in inhibiting and preventing the attachment or colonization of bacteria in the intestine and the level of tissue damage to the liver caused by pathogenic bacteria *Salmonella thypimurium* in broiler chickens

2. Materials and methods

2.1. Characterization of palm kernel cake (PCK) results of acetate and enzyme acid extraction.

In this first stage, a process study and characterization of palm kernel cake (PCK) extracts were carried out through the application of technology using acetic acid and enzymes. The treatment combination will also be applied to obtain the most effective and efficient process in overhauling the structure of palm kernel cake (PCK) into a simpler material in the form of residues (solids) as poultry feed ingredients and supernatant (liquid) which is a mannan extract from PCK to be made feed additives for broiler chickens, which are considered capable of being anti-microbial and immune stimulator in poultry [11].

The process of palm kernel cake extraction (PCK) can be carried out by adding PCK to acetic acid (1%, 110°C, 1 hour), enzymes (100 U/L, 60°C, 72 hours) and a combination of both, in comparison 1 : 10. This process starts with the grinding and screening (screening) of PCK. Then the PCK is mixed with each solvent and enzyme, followed by heating by using autoclave. Then the separation process was carried out using centrifugation (temperature 10°C; rpm 4,200; 15 minutes). After that the supernatant (liquid) and its residue are separated. The product produced at this stage in the form of supernatant (liquid) will then be tested for its ability to control *Salmonella typimurium* bacteria in broiler chickens.

2.2. Polysaccharide mixing contains mannan from PCK

The mixing process is done by spraying supernatant (liquid) which contains polysaccharides containing mannan from PCK into rations in the form of corn, bran, soybean meal, fish meal, Dicalcium phosphate, coconut oil, premix, minerals, CaCO3 homogeneously. After that it is dried in the sun to dry the air.

2.3. Bacteria and additives

The bacteria used in this study, namely malignant type *Salmonella thypimurium*. Chicken infected with *Salmonella thypimurium* at 3 days at a dose of $10^5$ CFU/head. Infection is done orally by inserting this type of bacteria into the mouth. After the infecting process is complete, each chicken is given vita stress. In the treatment, the antibiotics used were 0.5 g-l of oxytetracycline given for 3 days through drinking water.

2.4. Method

The research method used was experimental using a completely randomized design (CRD) with 6 treatments and 4 replications, each replication consisting of 4 chickens. The treatment given in the study:

- R0A = S thypimurium + ration infection supplemented with 0% mannan extract from PCK
- R0B = S thypimurium + ration supplement supplemented with 0% Mannan extract from PCK + antibiotics
- R1 = S thypimurium + Ration Infection supplemented with 0.1% Mannan Extract from PCK
R3  = S. thypimurium + ration infection supplemented with 0.2% Mannan Extract from PCK  
R4  = S. thypimurium + ration infection supplemented with 0.3% Mannan extract from PCK  
R5  = S. thypimurium + ration infection supplemented with 0.4% Mannan extract from PCK

2.5. Changes in anatomical pathology (PA)  
The lesion score is determined by changes in PA and histology in the intestinal organs and the liver in the form of degrees of damage from the infected organ. According to the method namely by giving a score of 0 to 4 as follows:

2.5.1. Intestinal organ. Changes observed in macroscopic examination of intestinal organs were carried out by giving a score of 0-4, i.e. 0 = no change, 1 = hyperaemia, 2 = enteritis cataract, 3 = haemorrhagic enteritis, and 4 = necrotic. The assessment of lesions microscopically carried out based on the degree of change of 0-5 namely, 0 = no change, 1 = hyperaemia, 2 = oedema, 3 = bleeding, 4 = inflammatory cell infiltration and 5 = desquamation of epithelium.

2.5.2. Liver organ. Changes observed in macroscopic examination of the liver were done by giving a score of 0-4, which is 0 = no change, 1 = hyperaemia characterized by striped colour, 2 = enlarged and pale, 3 = fragile, 4 = bleeding, and 5 = necrotic. Microscopic assessment of lesions was carried out based on the degree of change 0-4, i.e. 0 = no change, 1 = hyperaemia, 2 = hepatocyte degeneration, 3 = inflammatory cell infiltration, 4 = necrotic hepatocyte.

2.6. Histopathology analysis  
After the maintenance and cutting process is complete, followed by collecting samples and making histopathological preparations. Collection of samples of small intestine organs, liver is done after post infection when broilers are 5 days old and 15 days in the observations are processed descriptively.

3. Results and discussion

3.1. Effect of mannan extract of PCK against relative weights relative weights intestinal and liver.

3.1.1 Relative Intestinal Weight. The results of observation on days 5 and 15 post-infection on the relative weight of the intestine treated with mannan extract from PCK with different concentrations can be seen in Table 1.

The results of statistical analysis using ANOVA showed that the administration of mannan extract from PCK on each treatment give no significant different effect (P> 0.05). The highest relative intestinal weight was shown in the treatment given ration with the content of mannan extract from PCK (0.4%) and 7.18 g kg⁻¹ of live weight and antibiotic control of 7.18 g kg⁻¹ and the lowest weight in control without mannan extract. 6.90 g kg⁻¹ of live weight, while on the 15th day after infection the relative weight of chicken intestine given control treatment with antibiotics had a higher weight of 21.55 g kg⁻¹ of live weight while in the treatment of mannan extract of PCK 0.1% showed relative weight of intestine 21.06 g kg⁻¹ of live weight and the smallest relative weight of intestine was found in the ration treatment with extract concentration of 0.3% PCK, which was 20.53 g kg⁻¹ of live weight.

This indicates that the mannan extract treatment from 0.1% PCK does not interfere with chicken palatability and is also able to overcome the adverse effects that occur due to the infection of pathogenic bacteria (Salmonella thypimurium), one of them through histopathology examination (microscopic) where there is a change with decreased intestinal epithelial damage. [1] reported that increasingly severe and long intestinal changes were followed by the number of intestinal villi and the ability of secretion
of digestive enzymes. According to research by [5] administration of microbial enzymes can increase intestinal surface area, especially in the jejunum and ileum where the process of nutrient absorption takes place.

**Table 1.** Effect of using mannan extract from BIS on the relative weight of intestine (g/kg live weight)

| Treatment | 5<sup>th</sup> Time (day of post infection) | 15<sup>th</sup> Time (day of post infection) |
|-----------|--------------------------------------------|---------------------------------------------|
| R0A       | 6.90 ± 0.09                                | 20.72 ± 0.27                                |
| R0B       | 7.18 ± 0.21                                | 21.55 ± 0.41                                |
| R1        | 7.05 ± 0.22                                | 21.06 ± 0.43                                |
| R2        | 7.11 ± 0.30                                | 20.71 ± 0.19                                |
| R3        | 7.14 ± 0.52                                | 20.53 ± 0.87                                |
| R4        | 7.18 ± 0.42                                | 20.81 ± 0.56                                |

3.1.2. **Relative weight of heart.** The difference in the relative weight of chickens in each treatment can be influenced by the weight of the chicken. States that heavy and large hearts can be influenced by many factors including the type of animal, body size, genetics and feed given. The magnitude of the heart is caused by the increasingly heavy work of the liver on the detoxification process so that liver swelling occurs. Relative relative weight gain can be seen from changes in anatomical (macroscopic) pathology in the form of congestion, hyperemia, swelling, colour and bleeding in all treatments and this can lead to an increase in liver volume this is in line with [12] study which states the mean value - The average percentage of chicken liver infected by Salmonella thypimurium with the addition of polysaccharides containing mannan from BIS is still above the normal average weight range.

**Table 2.** Use of mannan extract from BIS on relative liver weight (g/kg of live weight)

| Treatment | 5<sup>th</sup> Time (day of post infection) | 15<sup>th</sup> Time (day of post infection) |
|-----------|--------------------------------------------|---------------------------------------------|
| R0A       | 31.16 ± 6.44                              | 30.23 ± 3.20                               |
| R0B       | 31.43 ± 5.34                              | 30.72 ± 3.23                               |
| R1        | 31.02 ± 3.57                              | 28.49 ± 4.21                               |
| R2        | 31.16 ± 6.86                              | 28.13 ± 0.48                               |
| R3        | 31.69 ± 3.24                              | 30.57 ± 2.81                               |
| R4        | 28.28 ± 9.12                              | 31.73 ± 4.09                               |
3.2. The effect of giving mannan extract from PCK on changes in anatomical (macroscopic) pathology of the intestine and liver

3.2.1 Changes in anatomical (macroscopic) pathology of the intestine. Examination of intestinal anatomical pathology on days 5 and 15 after infection with the control group is by infecting *S. thypimurium* bacteria without giving 0% mannan extract from PCK and infecting *S. thypimurium* bacteria with addition of antibiotics as well as mannan extract content from PCK by 0.1%, 0.2%, 0.3% and 0.4% did not show any changes in the chicken intestine in the form of hyperaemia, cataract enteritis even though after infection occurred diarrhoea but macroscopically did not show any changes in the intestine. In this case it was concluded that bacterial infection with a dose of $10^5$ CFU did not show macroscopic changes in the intestine on days 5 and 15 after infection but in the study of [8] reported that infected chickens, with a dose of $10^8$ cfu can be detected in the intestine and feces without showing typical clinical symptoms.

3.2.2. Changes in anatomical (macroscopic) pathology in the liver. In the macroscopic observation on the 5th day we can see in the treatment using mannan extract content from PCK, the lesion level is relatively low, but on the 15th day there began to be an improvement in the condition of the liver and fragile. Hyperaemia characterized by striped colour indicates excessive blood in the blood vessels, so the liver cells undergo degeneration or necrose. Histopathologically there is dilatation of veins and capillaries which are full of blood, while swelling (inflammation) is a protection mechanism whereby the body attempts to neutralize and eradicate dangerous agents at the site of injury and to prepare conditions for tissue repair.

Table 3. Percentage score of anatomical pathology of broiler chicken liver on days 5 and 15 after infection

| Treatment | 5th Day (Post Infection) | 15th Day (Post Infection) |
|-----------|--------------------------|---------------------------|
| R0A       | 1.88 ± 0.25              | 1.63 ± 0.25               |
| R0B       | 1.63 ± 0.25              | 1.50 ± 0.00               |
| R1        | 1.75 ± 0.50              | 1.63 ± 0.25               |
| R2        | 2.13 ± 0.75              | 1.63 ± 0.48               |
| R3        | 2.50 ± 0.00              | 2.00 ± 0.00               |
| R4        | 3.25 ± 0.50              | 2.75 ± 0.96               |

3.3. Effect of mannan extracts from PCK on histopathological (microscopic) changes in the intestine and liver

3.3.1. Histopathological (microscopic) changes in the intestine. Histopathologically there is no change in the organ with the concentration of mannan extract from PCK of 0.4% intestinal histopathological observation (duodenum) there was the highest score of 4 (inflammatory cell infiltration) in jejunum organs with a concentration of mannan extract from BIS of 0.1%. Inflammatory cells are an immunological response due to the presence of an antigen. The presence of inflammatory cells due to *Salmonella thypimurium* bacteria that infects the intestine causes intestinal epithelial damage. Epithelial
cell damage that occurs is degeneration of the superficial epithelium resulting in shortening of the villi and the production of exudates in the intestinal lumen [8, 9].

3.3.2. Histopathological changes (microscopic) liver organ. On histopathological observations found on the 15th day there was a decrease in lesions. Lesions that occur uniformly. With found inflammatory cell infiltration in all treatments there is an increased level of inflammatory cells compared to day 5, this indicates that antibiotics cannot prevent or reduce the toxic substances that enter the liver. Allegedly because the dose and type of antibiotics given are less effective for killing or reducing S. typhimurium bacterial colonies or even can be caused by the bacteria S. typhimurium has been resistant to these antibiotics.

In the observations of the 5th and 15th days after infection after the mannan extract from PCK, it was concluded that the administration of mannan extract did not give a negative influence on the condition of the liver, even on the contrary mannan extracts can maintain good liver condition even though chicken has been infected by S. typhimurium. Decreased level of liver damage treatment with mannan extract, allegedly due to the presence of mannose active ingredients that play a role in suppressing the presence of S. typhimurium bacteria.

4. Conclusions
Treatment with mannan extract at various levels of concentration in anatomical (macroscopic) and histopathological (microscopic) pathologies showed no difference, so it can be concluded that the administration of mannan extract from PCK is safe to use in livestock.

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