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The Utilization of Phytase Enzymes and SEM Analysis in order to increase the Quality of Rice Bran as a Layer and Fish Feed

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Abstract. Phytase is one of the enzymes belonging to the group phosphatase, which is capable of hydrolyzing the phytic compounds in the form of myo-inositol (1.2.3.4.5.6) into myo-inositol phosphatase and organic phosphate. In the digestive tract of non-ruminant livestock (poultry) and fish, there are no phytase enzymes. This causes the content of the rice bran phytate compounds to be difficult to digest because of the strong chelating properties. Restrictions on the use of rice bran in the fish's diet are because of the fiber content and the high level of phytic acid. One alternative to reduce the phytate content of the feed is to use the phytase enzyme, which is expected to hydrolyze the phytic acid (myo-inositol 1.2.3.4.5.6) in the rice bran orthophosphate in organic produce. All of the minerals, such as P and Ca, are important minerals to release and it is used for the growth of both layers and fish. The result showed that: 1. The scanning Elektron Microscope (SEM) analysis of the surface with the addition of rice bran phytase enzymes caused the bond breaking force with the inositol phosphate acid groups. These showed changes in the structure of the phytic irregular bond and 2. The addition of the phytase enzymes to degrade phytic acid increases the availability of minerals such as phosphorus and calcium and crude protein, and decreases the crude fiber in the rice bran.

1. Introduction

The feed requirements in animal husbandry and aquaculture business activities intensively require quality feed, which requires substantial costs. Some ingredients used in pellet-laying hens and fish involve ingredients from grains, among others, soybean meal, wheat shards and rice bran, which contain a lots of phytate compounds. This compound is able to bind metals such as P, Mg, Mn, Fe, Zn, Ca and protein, which are very useful for the growth of laying hens and fish. Phytic acid (mio-inositol heksakifosfat) compounds are difficult to solve in the digestive tract of poultry and fish because of the strong chelating nature [1]. If the phytate compound problem is not solved, then the important digestive metals and proteins will be discarded in the feces. Considering the importance of the metals and proteins which are bound by the phytate compounds for the growth of laying hens and fish, an alternative is needed to optimize feed efficiency by breaking down the phytate compounds. The unavailability of phytase enzymes in the digestive tract of poultry and fish makes phytate compounds undigested; they will be wasted in the faeces and discarded into the environment with other metals and digestive proteins that are important for the growth of poultry. The addition of phytase-producing enzyme bacteria to the feed will certainly help the digestive process of the laying hens and fish. Phytase is one of the enzymes belonging to the



phosphatase group which is able to hydrolyze phytate compounds in the form of myo-inositol (1,2,3,4,5 and 6) hexa phosphatase into myo-inositol and organic phosphate. One alternative to reduce the phytate content in feed is to use phytase enzyme-producing bacteria [2].

Phytic acid can be classified as an anti-nutrient component in feed, so phytase enzyme-producing bacteria is needed, which can hydrolyze phytic acid. Local feed ingredients that are potentially used as poultry feed include rice bran, palm kernel cake, palm mud, coconut cake and other agricultural industrial waste. Rice bran has been widely used as an animal feed ingredient for poultry and fish. If the rice bran can be used more in rations, then it will be able to reduce production costs because the price of rice bran is relatively cheaper. The limitations in relation to the use of rice bran in rations are due to the high fiber content and phytic acid. Poultry does not produce phytase enzyme-producing bacteria so it must be added to the ration. The results of the study by [3] reported that the analysis of SEM (Scanning Electron Microscope) showed that there was damage done to the surface structure of rice straw and water hyacinths after being treated with recombinant L-L-arabinofuranosidase after 8 hours of elubation. Based on the above considerations, it is necessary to conduct research in order to determine the effect of the addition of phytase enzymes in rice bran to changes in the surface structure using SEM analysis and the improvement of the nutritional quality in order to feed laying hens and cultivated fish.

2. Materials and method

2.1 Materials

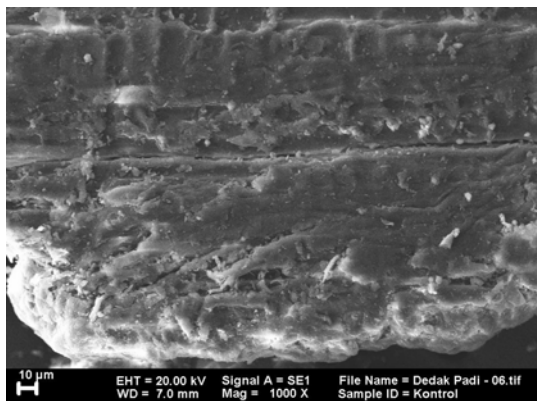
The material used in this study consisted of rice bran obtained from Surabaya. The rice bran was fermented using phytase enzymes derived from *Actino bacillus sp* and *Bacillus pumilus* bacteria. The inoculum used was the stock of the Animal Food Laboratory in the Faculty of Veterinary Medicine, Airlangga University, Surabaya. The production of the crude extract was obtained from a single colony isolated from the rumen of the *Actino bacillus sp* and *Bacillus pumilus* bacteria grown in 5 mL of liquid LB media at a temperature of 40 ° C, with shaking using a shaker incubator at a speed of 150 rpm for ± 16-18 hours. Furthermore, as much as 1% of the liquid culture was inoculated to 100 mL of phytase screening media at a temperature of 40 ° C, with shaking using a shaker incubator at a speed of 150 rpm for 16-18 hours. The suspension was centrifuged at a rate of 3500 rpm at 4 ° C for 15 minutes. The supernatant obtained was a crude phytase extract that was used for degrading rice bran. The treatment in this study was to determine the nutrition of the rice bran: P0 as a control and P1 with 4% phytase enzymes added to the rice bran. Proximate analysis was carried out.

2.2 Analysis of the changes in the surface structure of the rice bran using SEM

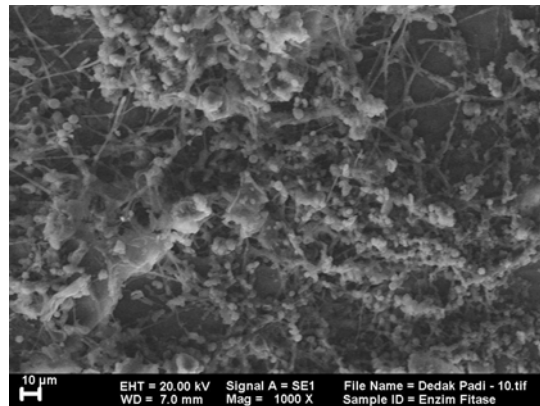
The rice straw and rice bran that had been hydrolyzed using enzymes was dried to a constant weight. The rice straw was cut to ± 2-3 mm in size, and then the rice straw and rice bran was attached to the holder. The sample was coated with gold-palladium and then the researcher observed changes in the surface structure of the rice bran using SEM. The rice bran that was not treated with enzymes was also observed using SEM, which was used as a control to determine any changes in the surface structure of the rice straw and rice bran respectively.

3. Results

The results of the SEM on the rice bran treated with phytase enzymes (1000x magnification) can be seen in Figure 1.



Control (-)



Control (+)

Figure 1. The results of the SEM rice bran with added phytase enzyme.

The results showed that the addition of phytase enzyme had good results on the crude protein, crude fiber, phosphorus and calcium. Table 1 shows the pattern of the phosphorus reduction in the rice bran. This was caused by the phytase enzyme, as it can hydrolyze phosphate bonds from phytic acid. The phytic acid can bond to metal ions, thus increasing the bioavailability of the P and Ca minerals.

Table 1. Feed Treatment of the Rice Bran with Phytase Enzymes

Nutrition (%)	Treatments	
	P0 (control)	P1 (add phytase)
Dry matter	90.19	89.85
Ash	16.18	16.92
Crude protein	18.19	18.70
Extract ether	5.40	6.75
Crude fiber	5.17	5.07
Organic matter	74.01	72.94
NFE	45.26	40.25
Calcium	5.78	6.33
Phosphorus	0.50	0.65
EM (kkal/kg)	2671.79	2596.72

4. Discussion

The Scanning Electron Microscope (SEM) is one type of electron microscope that uses electron beams to draw a surface profile of objects; this was used to study the texture, topography and surface of the objects. The requirements for SEM to produce a sharp surface are that the surface of the object must be an electron reflector or that it could release electrons when fired at with an electron beam. Materials that have such properties include metals. If the metal surface was observed under SEM, then the surface profile was clearly visible. For non-metallic materials, it needs to be coated with metal before being observed under SEM. This was done so that then the surface profile of the non-metallic materials could be clearly

observed. The phytic acid degradation is a termination process between the bonds of the myoinositol group and the phosphoric acid groups through the phytase enzymes produced by the rumen microbes [4]. This is further explained that the phosphate released would be used as a source of phosphorus (P) minerals for livestock [5]. The breaking of the phytate bonds occurred randomly in irregular bonds, causing the digestibility value to be high and the released nutrients could therefore be immediately utilized by the livestock's body. The results showed that the addition of phytase enzymes from the *Actino bacillus sp* and *Bacillus pumilus* bacteria bonded to the myoinositol group and phosphoric acid groups so then the phosphate group would be compared to the control - (without treatment) and control + (addition of phytase). With the fermentation process, it causes the phytate-mineral or phytate-starch and phytate-protein breaking by bonding the phytase enzymes in the bran during the fermentation process (Figure 1). This enzyme degrades the phytate into inositol and phosphoric acid, thereby increasing the availability of phosphorus available for the livestock's body. The phytase enzymes were actually present in the intestinal mucosa of the poultry, but at a low level. Phytic acid has properties as a chelating agent, especially against two valence ions [6] in the biological availability of the minerals in low poultry cattle. In monogastric cattle, phytic acid could still be used as a source of phosphorus and inositol, but the materials containing phytic acid need to be treated or there needs to be phytase enzyme supplements.

Phytic acid or its salt form was the main form of the phosphorus deposits found in the outer layer (aleurone) of the cereal grains. This compound was very difficult to digest, meaning that the form of phosphorus phytate could not be used by the body. Phosphorus and other minerals in the phytate complex can only be used when the phosphate group is degraded by the phytase enzyme. This enzyme can reduce the phytic acid into inositol and phosphate, which can increase the availability of phosphorus and casium in animal bodies. Phytic acid as a chelating agent has properties, especially related to divalent ions [6], which means that the bioavailability of minerals in poultry and fish is low. In monogastric animals, phytic acid can still be used as a source of phosphorus and inositol, but it contains ingredients that must be treated with phytic acid or phytase enzyme supplements. The degradation of phytic acid or phytase by the enzyme phytase causes the minerals P, Ca and Mg to be bound to the phytate that is freely available so then it can be absorbed into the gastrointestinal wall [7].

5. References

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