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Potential of Phytase Enzymes as Biocatalysts for Improved Nutritional Value of Rice Bran for Broiler Feed

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ABSTRACT

Phytase is one of the enzymes belonging to the group phosphatase capable of hydrolyzing phytic compounds in the form of myo- inositol (1.2.3.4.5.6) hexsa into myo-inositol phosphatase and organic phosphate . In the digestive tract nonruminant livestock (poultry) there is no phytaseenzymes , it causes the content of the rice bran phytate compounds are difficult to digest because of the strong chelating properties , so it wasted phytate with feces. Restrictions on the use of rice bran in the diet because the fiber content and high phyticacid . One alternative to reduce the phytate content of the feed is to use phytase enzyme produced from Actinobacillus *sp* and *Bacillus pumilus*) is expected to hydrolyze phytic acid (myo-inositol 1.2.3.4.5.6- hexakisphosphate) for rice bran orthophosphate in organic produce and a series of lower phosphoric into myo -inositol -free , so all minerals such as P, Cawhich is an important mineral to be released and used for the growth of broiler chickens. The results obtained adding the enzyme phytase can improve the nutritional quality of rice bran content is increasing crude protein, crude fiber and increasing decline in the availability of minerals calcium and phosphorus.
KEY WORDS:phyticacid, Actinobacillus *sp*, *Bacillus pumilus*, rice bran, enzymesphytase

INTRODUCTION

Constraints on the use of rice bran as poultry feed in the high phytic acid content phytic acid will form insoluble salts when the phytic acid binds with phosphorus and other minerals so that these minerals cannot be absorbed by the digestive tract of poultry. Phytic acid has a negative charge at low pH, pH neutral and high pH. This causes phytic acid can bind to metal ions such as P, Ca , Mg , Zn and positive protein as a terminal amino group at pH below iso-electric point. With the formation of phytate-mineral compounds or phytate insoluble protein can cause a decrease in the availability of minerals and nutritional value of the protein [1].Phytateis the major form of phosphorus, are found in cereal grains, legumes and oilseed meal used in poultry diets monogastric animals such as poultry, but cannot take advantage of phosphorus sources due to lack of endogenous phytase enzymes. To meet the requirements of phosphorus for poultry, inorganic phosphate is added to poultry feed, which causes most of the problems of environmental pollution of phosphorus excreted in feces. Microbial phytase can be used as an alternative to this , which has a beneficial effect on growth performance, feed efficiency , protein digestibility, energy utilization, mineral retention, and bone growth of broiler chickens as a direct hydrolytic effect on phytate [2,3]. Phytase is one of the enzymes belonging to the group phosphatase capable of hydrolyzing phytic compounds such as myo-inositol(1.2.3.4.5.6) hexsa into myo-inositol phosphatase and organic phosphate. Livestock monogastric digestive system cannot digest or metabolize phyticacid. In physiological conditions, phytic acid can form *chelating* the minerals Ca, Mg, Fe and Zn to inhibit the use of these metals in the body [4]. Phytic acid can also form complexes with amino acids, proteins, thereby inhibiting the biochemical function of the proteins and amino acids. In addition, phytic acid is an inhibitor of the digestive enzymes.Phytic acid is not metabolized in the digestive systems of farm animals, also cause environmental problems because of the phytic acid will be excreted through the feces of farm animals. Accumulated phosphate groups cause pollution to the environment [5]. The absence of enzymes that can degrade phytic acid in monogastric livestock systems , should lead to the addition of inorganic phosphate minerals in the animal feed to meet the need for phosphorus, this led to increased production

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costs. Above problems can be overcome by using the enzyme phytase as *Feed Supplement* in animal feed. Degradation of phytic acid is a disconnection between the bonding process groups inositol and myo-phosphoric acid groups of the enzymesphytase produced by rumen microbes [6]. Phosphate is released will be used as a source of phosphorus minerals for livestock [7], and the beneficial effects of exogenous phytase in poultry rations is a direct hydrolytic effect on the increase in the availability of phytate and minerals, amino acids, and energy [8]. Phytase enzyme is composed of two types, namely 6-phytase which is found in plants, and 3-phytase produced by fungi [6].

Phytic acid can be classified as anti-nutrientscomponents in the feed, so that necessary enzyme phytase producing bacteria capable hydrolysisphytic acid. Ruminant origin rumen bacteria (Actinobacillus *sp* and *Bacillus pumilus*) is expected to produce the enzyme phytase as *Feed Supplement* to produce quality animal feed ingredients in terms of the availability of protein and minerals P, Mg, Mn, Fe, Zn, Ca were high that in addition to meet the nutritional needs for broiler chickens are also environmentally friendly. Local feed ingredients that could potentially be used as poultry feed them is rice bran, palm kernel cake, palm oil sludge, coconut cake, other agricultural and industrial waste. Rice bran has been widely used as an animal feed ingredient for poultry. If the rice bran can be used more in the ration will be able to lower production costs because the price is relatively cheaper rice bran. Restrictions on the use of rice bran in the diet because the fiber content and high phytic acid. Poultry does not produce the enzyme phytase producing bacteria that should be added to the ration.

Enzymes have been used for more than 20 years in the feed industry , mostly to increase the use of energy in the grains on non-starch-polysaccharides (NSP), which is soluble such as wheat, barley, oats and rye [2]. Feed efficiency in broiler chickens by enzyme supplementation in the starter phase better than the grower phase . The efficiency of the addition of exogenous enzymes in rations vary according to the period of growth [9] .Based on the background above proficiency level, then conducted research with the addition of phytase enzyme in the degradation of rice bran formula for the preparation of poultry feed ingredients.

MATERIALS AND METHODS

Materials used in this study were obtained from rice bran Surabaya . Fermented rice bran using enzymes phytase derived from bacteria lignocellulolytic (Actinobacillus *sp* and *Bacillus pumilus*) production of beef cattle rumen. Inoculum used was stock Forage Laboratory Faculty of Veterinary Medicine, University of Airlangga.

Production of crude extract obtained from a single colony isolates of rumen bacteria Actinobacillus *sp* and Bacillus pumilus were grown in 5 mL Luria Bertani medium (LB) liquid temperature of 40° C, with agitation using a shaker incubator at 150 rpm for ± 16-18 hours. Furthermore, as much as 1 % liquid cultures were inoculated in 100 mL phytase screening medium, temperature 40° C, with agitation using a shaker incubator at 150 rpm for 16-18 hours. Suspension was centrifuged at 3500 rpm 4° C for 15 minutes. Supernatant obtained a crude extract phytase used to degrade rice bran. Determination of phytase activity performed each 0.2mL of1% phyticacid substrate (dissolved in acetate bufferpH4) plus0.2mL of the enzyme, the enzyme was incubated at the optimum temperature for 30minutes.Then added 0.4 mL of 15 % TCA (Trichloroacetic). Then centrifuged at 10.000 rpm for 5 minutes. 0.05 mL of the supernatant was added to 0.45 mL of distilled water, then treated with 0.5 mL of vanadate-molybdate reagent and absorbance was measured using a UV spectrophotometer at a wavelength of 392 nm.

This study used a completely randomized design with five treatments and five replications. As much as ten kg of rice bran which is divided into twenty study samples. This study used a Ccompletely Randomized Design(CRD) . Five treatments used in this study are : DPF0 = Rice Bran (RB) (control) ; DPF1 = RB + 5 % EF ; DPF2 =RB + 10 % EF ; DPF3 = RB+ 5 + 5 % EF % BF ; DPF4 = RB+ 5 + 10 % EF % BF . EL is Phytase Enzymes and BL is the bacterium Actinobacillus *sp* and Bacillus pumilus. Rice bran weighed each weighing fourhundredgrams. Fermented rice bran performed for each treatment using the appropriate dose of phytase enzyme treatment. Rice bran put in a plastic bag labeled experimental and facultative *anaerobic* fermentation of rice bran is seven days. After an incubation period of seven days is complete, the sample was opened and analyzed content of nutrients. Observation parameters such as dry matter content (DM), organic matter (OM) , crude protein (CP) , crude fiber (CF) ,

phosphorus, calcium [10]. The results of the study were analyzed using analysis of variance, followed by Duncan 's Multiple Range Test [11] .

RESULTSANDDISCUSSION

Average nutrient content of fermented rice bran using phytase enzymes are presented in Table1.

Table1. Average Nutritional Content of Rice Bran with Phytase EnzymeAddition

Nutrisi % DM	DPF0	DPF1	DPF2	DPF3	DPF4
CP	8.82^{d}	10.63^{bc}	10.4696^{c}	12.47^{a}	12.07a^{b}
CF	21.40^{a}	19.87^{b}	19.20^{bc}	18.29^{c}	18.06^{c}
OM	85.60^{c}	88.50^{b}	88.54^{b}	89.61a	89.13a^{b}
Ash	14.40^{a}	11.50^{b}	11.46^{b}	10.39^{bc}	10.87^{c}
Calsium	1.30^{a}	0.95^{b}	0.88^{b}	0.66^{c}	0.61^{c}
Phosphorus	1.15^{a}	0.99^{b}	0.97^{b}	0.82^{c}	0.81^{c}

Degradation of phytic acid is a disconnection between the bonding process groups inositol and myo-phosphoric acid groups of the enzyme phytase produced by rumen microbes [6]. Further explained that the phosphate is released will be used as a source of mineral phosphorus [7] , which in turn is having a beneficial effect on growth performance, feed efficiency, digestibility, energy utilization , mineral retention , and bone growth of broiler chickens as a direct hydrolytic effect on phytate [3] .Degradation of phytic acid by phytase produced from the bacteria Actinobacillus *sp* and *Bacillus pumilus* more regularly than chemical degradation of phytic acid on *anaerobic* fermentation process. Termination of phytate bonds occur randomly in irregular bond, thus causing a high value and nutrient digestibility be released soon be directly utilized by the animal body. The results showed the use of phytase enzyme from the bacterium Actinobacillus *sp* and Bacillus pumilus, it can lead to the termination of bond orphytate-mineral-phytateandphytate-protein starchbyenzymephytase that of the bran during the fermentation process.

The results showed DPF3 and DPF4 treatment gives the best results when compared to treatment DPF2, DPF1, DPF0 (Table. 1) on the content of crude protein , crude fiber, organic matter. This is due to the enzyme phytase produced from Actinobacillus *sp* and *Bacillus pumilus* lignocellulolytic bacteria also produce enzymes lignocellulase that can degrade crude fiber and crude protein rice bran. On the other hand the degradation of phytase in rice bran phytase untied acid and protein complexes. Phytic acid or salt form is the main form of phosphorus deposits found in the outer layer (aleurone) cereal grains. This compound is very difficult to digest, so in the form of phytate phosphorus cannot be utilized by the body. Thus most of the phosphorus is excreted through feces and can cause contamination [12]. Phosphorus and other minerals in phytate complex can only be used when the phosphate group is degraded by the enzyme phytase. These enzymes will degrade phytic acid into inositol and phosphate, so that can increasing the availability of phosphorus for livestock body. Phytase enzyme is actually present in the intestinal mucosa but very few birds. Phytic acid as a *chelating* agent has properties especially against divalent ions [13], so the bioavailability of minerals in poultry is low. In monogastric livestock was still phytic acid can be used as a source of phosphorus and inositol but containing material should be treated phytic acid or enzymesphytase supplementation received.

Degradation of phytic acid or salt by the enzyme phytase, causing mineral P, Ca and Mg are bound to phytate be freely available and can be absorbed so that the walls of the gastrointestinal tract [14]. Table.1 shows the pattern of reduction in phosphorus in rice bran, it caused the enzyme phytase can phosphorus bond hydrolysis of phytic acid which can ability of phytic acid to metal ions bonded with, thereby increasing the bioavailability of mineral P. In this study,phytase enzyme produced by bacteria Actinobacillus *sp* and Bacillus pumilus were able to hydrolyze phosphorus binds to phytic acid from rice bran. With the onset of the fermentation process can lead to the termination of bond or phytate-mineral and phytate-protein starch by enzyme phytase that of the bran during the fermentation process. The formation of phytate - mineral compounds or insoluble proteins can cause a decrease in the availability of minerals and nutritional value of feed protein. Minerals and protein that forms a complex with

phytatecannot be absorbed by the intestinal wall for livestock [1]. This is consistent with the research results obtained by a decrease in the content of calcium and phosphorus due to the termination of phytate-mineral bond, so that the calcium and phosphorus that can be freely utilized in the digestive tract of poultry.

CONCLUSION

The addition of phytase enzymes to degrade phytic acid that increases the availability of minerals calcium and phosphorus, increase the nutrient content of rice bran that is increasing crude protein, crude fiber lowers.

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