The Characteristics of Phytase Enzyme from Beef Cattle Rumen Liquor Obtained from Abattoir

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ABSTRACT

The aim of this experiment is to utilize the liquor of rumen cattle from abattoir as feed additive, as source of phytase enzyme, to increase the quality of broiler ration based on local feed materials. This experiment was conducted in two stages. First stage was to identify enzymes in rumen liquor of cattle. The second stage was to characterize and stabilize enzyme which include determination of optimum temperature and pH of enzyme, examination enzyme resistance to heating at optimum temperature, and studying effect of metal ions and chemical substances. The results of the experiment show 1) optimum precipitation of rumen liquor enzymes from local cattle is reached at the concentration level of 60 % of ammonium sulphate, meanwhile from imported cattle is obtained at the level of 70 % of ammonium sulphate; 2) general characteristics of phytase enzyme from rumen liquor of local cattle differs from that of imported cattle; those from the rumen liquor of local cattle has optimum temperature at 50 °C, optimum pH at 6, resistance to heating up to 180 minutes at optimum temperature, and needs mostly methal ions as activator; on the other hand, the enzyme from the rumen liquor of imported cattle has optimum temperature at 39 °C, optimum pH at 6, resistance to heating up to 180 minutes at optimum temperature; the phytase enzyme needs mostly methal ions as activator, some compounds inhibiting the enzyme activity are EDTA and β-mercaptoethanol; and 3) activity of the phytase enzyme from the rumen liquor of local cattle is higher than that of imported cattle.

Key words: phytase enzymes, local and imported cattle, rumen liquor, abattoir

INTRODUCTION

The experiment of rumen cattle liquor utilization as poultry feed in solid material still limited, however, the liquor of rumen cattle is never utilized as feed additive and feed supplement in poultry ration based on local feed. Based on Statistical Data of Animal Husbandry 2007, the numbers of slaughtered cattle every year are not less than 1.75 million heads and it is about 1.5 million heads come from local cattle with average of 300 kg and the rest is from imported cattle. The weight of rumen content is about 14.3 percent from body weight (Hungate, 1968), and a cattle can produce 42.9 kg of rumen content. Thus, the potency of rumen content from slaughtered cattle can reach 75.075 thousand tons per year. The liquor portion of rumen content reaches 31 liters per head (Priego *et al*., 1977), so that, the potency of rumen liquor reach 52.7 million litre per year. Huge amount of rumen liquor will become a potential pollutant for the environment if there is no good management in utilization.

This experiment was conducted with the objective to utilize the cattle rumen liquor from abattoir as feed additive, source of phytase enzyme, to increase the quality of broiler ration based on local feed materials. The experiment studying phytase enzyme from rumen liquor may have benefit as feed additive to increase the nutrient digestibility and the value of low quality local feed in increasing the productivity of poultry.

MATERIALS AND METHODS

Rumen enzyme preparation

Rumen content from local and imported cattle were taken from cattle slaughtered from the abattoir in Bogor. Sampling of rumen content from local and imported cattle was taken in two replications and each was taken from 3 – 5 cattles.

Rumen liquor was taken from rumen content by filtration in cold condition. The liquid part of filtration was centrifuged at 10 000 g (4 ^{o}C) for 10 minutes to separate supernatant from microbial cells (Lee *et al*., 2002). Supernatant was then taken as a source of crude enzyme.

Enzyme purification using ammonium sulphate

Supernatant containing enzymes was then reacted with different levels of ammonium sulphate and stirred by magnetic stirrer for 1 hour and kept one night at 4 ^{o}C. The levels of ammonium sulphate used were 40, 50, 60, 70 and 80 %. Supernatants were then centrifuged again at 10 000 g (4 ^{o}C) for 15 minutes. The filtrate was taken and added with phosphate buffer pH 7 at the ratio of 10 : 1 (100 ml supernatant of rumen liquor were dissolved with 10 ml of phosphate buffer pH 7). The precipitates (enzyme source) in phosphate buffer were then kept in freezer for enzyme assay.

Phytase enzyme assay

Determination of phytase enzyme activity was measured with Heinones and Lahti (1981) and Greiner *et al.* (1997) methods.

Enzyme characterization

Phytase was characterized based on optimum temperature and pH, effect of methal ions and enzyme stabilized based on resistance to heating at optimum temperature.

Determination of optimum pH was done by measuring the enzyme activity in universal buffer at pH 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0. Determination of optimum temperature was carried out by measuring the enzyme activity at 29, 39, 50, 60, 70 and 80 ^{o}C in 0.05 M phosphate buffer pH 7. Examination of stabilization enzyme resistance to heating was done by heating the enzyme at optimum temperature for 0, 30, 60, 90, 120, 150 and 180 minutes before adding substrate.

The examination of methal ion effect was conducted by measuring the enzyme activity after treating with some methal ion at 1 mM concentration of β-mercaptoethanol, EDTA, $FeCl_2$, $MgCl_2$, $ZnCl_2$, $MnCl_2$, $CoCl_2$, $CuCl_2$ and $CaCl_2$. The examination was done by incubating 100 µl of enzyme with 100 µl methal ions for 10 minutes at room temperature.

Data analysis

Data obtained were analyzed by descriptive analysis (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Enzyme precipitation with ammonium sulphate

Enzyme isolation from cattle rumen liquor was done by salting out method, in which the enzyme was precipitated by adding neutral salt in high concentration (ammonium sulphate) a little by a little. Ammonium sulphate was used because it dissolved faster than other chemicals, did not cause toxic and could stabilize the enzyme (Schwzimmer and Pardee, 1957). Enzyme precipitation by ammonium sulphate was carried out because it has the same polarity as water. Addition of ammonium sulphate in protein solution will attract water molecules around the protein surface, so that, the protein is not protected by water molecules, consequently inter protein molecules are aggregated and precipated (Scope, 1987). Ammonium sulphate salt will destroy water mantel arround the protein and thus protein could be precipitated (Schwzimmer and Pardee, 1957). Choosing of optimum ammonium sulphate concentration was obtained by measuring the highest enzyme activity.

Results of the present experiment of phytase enzyme precipitation by ammonium sulphate shows that, in local cattle rumen liquor, the maximum phytase enzyme activity has been reached at 60% ammonium sulphate concentration with the value of 1.0561 µmol/ml/minute, but in imported cattle rumen liquor the maximum activity was reached at 70% ammonium sulphate concentration with the value of 0.9432 µmol/ml/minute. Before the addition of ammonium sulphate, phytase enzyme activity in local cattle rumen liquor was 0.3559 µmol/ml/minute and imported cattle rumen liquor was 0.2130 µmol/ml/minute. The increase of phytase enzyme activity after the treatment with ammonium sulphate in local cattle rumen liquor was 2.97 times, and in imported cattle rumen liquor was 4.43 times. Figure 1 shows the increase in phytase enzyme activity in rumen liquor from abattoir after precipitation with ammonium sulphate. Phytase enzyme activity of local cattle rumen liquor was nearly the same as that of imported cattle rumen liquor, but the

activity in local cattle tended to have higher value than that in imported cattle. Phytase enzyme is a phytate digesting enzyme. Phytate is antinutrition that present in many feedstuffs, mainly in cereals and roughages from leaf of plants.

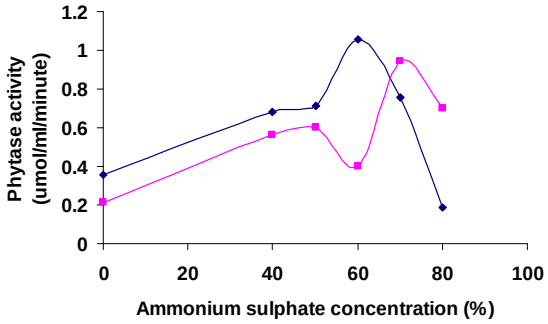

Figure 1. Effect of Ammonium Sulphate Concentration in Enzyme Precipitation on Phytase Enzyme Activity from Local Cattle (—♦—) and Imported Cattle (—■—) Rumen Liquor

Enzyme characterization

The aim of enzyme characterization was to determine the optimum condition of enzyme activity, so that, the use of enzyme could be adjusted to the optimum condition. Samples of phytase enzyme used for characterization were taken from enzyme that was precipitated with 60% ammonium sulphate concentration for local cattle rumen liquor, and 70% ammonium sulphate concentration for imported cattle rumen liquor.

Reaction of enzyme catalytic was affected by temperature. Palmer (1991) indicates that increasing the temperature would increase energy kinetic of enzyme and would increase the vibration, translation and rotation movement of enzyme and substrate, so that probability of enzyme and substrate to have contact would be increased. Therefore, enzyme activity increased up to certain temperature, but decreased if optimum temperature has passed over. Thus, increasing of temperature up to certain border line would increase enzyme catalytic activity, but would destroy the enzyme because heating. Each enzyme has a range of optimum pH, where the enzyme showed maximum activity and stability. Lehninger (1995) shows that characteristic of enzyme pH could be indicated by the pH when proton receiver and donor on the enzyme catalytic side were in the required ionization level. Some enzymes need methal ions (cations) as activator for its activity and some chemical substances could decrease enzyme activity. Some of methal ions and chemical substances were examined in the experiment were $FeCl_2$, $MgCl_2$, $ZnCl_2$, $MnCl_2$, $CuCl_2$, $CoCl_2$, $CaCl_2$, β-mercaptoethanol and EDTA. Results of enzyme characterization are shown in Table 1.

The present results showed that optimum temperature to reach maximum enzyme activity in local cattle was at 50 ^oC and in imported cattle was at 39 ^oC. Phytase enzyme from local cattle rumen liquor was more resistance to the change of temperature than that from imported cattle rumen liquor. Figure 2 shows phytase enzyme activity in different incubation temperature.

The result also indicated that the optimum pH to obtain maximum enzyme activity in local cattle rumen liquor has been reached at pH 6, and in imported rumen liquor at pH 7. Figure 3 shows that phytase enzyme activity in different pH. Both enzymes were more stabile in range of pH 6 up to pH 7.

Sariyska *et al.* (2005) demonstrated that optimum temperature of phytase enzyme isolated from *Aspergillus niger* was 55 – 58 ^oC, and optimum pH at 5.05.

Table. 1. Characteristic of phytase enzyme of cattle rumen liquor from abbatoir

Variables	Local cattle	Imported cattle
Optimum temperature (^oC)	50	39
Optimum pH	6	7
Resistance to heating (minutes)	180	180
Cations / substances positive effect	Fe++, Mg++, Mn++, Zn++, Cu++, Co++, Ca++, EDTA and β-Mercapto-ethanol	Fe++, Mg++, Mn++, Zn++, Cu++, Co++ and Ca++
Cations / substances negative effect	None	EDTA and β-Mercapto-ethanol
Cations / substances little effect	None	None

However, Greiner and Farouk (2007) that investigated phytase enzyme isolated from wastewater bacteria in Malaysia, obtained optimum temperature at 65 ^{o}C and optimum pH at 4.5. Powar and Jagannathan (1982) showed that optimum pH of phytase enzyme isolated from bacteria *Bacillus subtilis* was at pH 7.5.

Cao *et al.* (2007) indicated that phytase enzyme was very sensitive to temperature, high pressure and heat treatment at 100 ^{o}C for 10 minutes had caused loss of enzyme activity. Most of phytase enzyme had optimum temperature at the range of 45 – 60 ^{o}C and optimum pH at the range of 4.5 – 6.0, but phytase enzyme from bacteria such as *Bacillus amylolequefaciens* and *Bacillus subtilis* had optimum temperature at 55 – 70 ^{o}C and optimum pH at 6.5 – 8.0 (Cao *et al.*, 2007). Cheng *et al.* (1999) showed that phytase enzyme from rumen microbe, *Selenomonas ruminantium*, had active temperature at the range of 20 ^{o}C – 55 ^{o}C and pH from 3 – 6 depending on the buffer used, and the optimum pH were 4.0 – 5.5 in sodium asetate buffer. On the other hand, Puhl (2006) demonstrated that phytase enzyme from *Selonomonas ruminantium* and its cloning had optimum pH at the range of 4.5 – 6.0, and optimum temperature 55 – 60 ^{o}C. This has shown that temperature and pH of phytase enzyme depends on types of microbes.

Yanke *et al.* (1998) had screened the rumen bacteria having phytase enzyme activity which were *Provotella ruminicolla*, *Ruminobacter amylophylus*, *Selenomonas ruminantium* and *Streptococcus bovis*. Further examination on the rumen bacteria showed that the bacterium having high phytase enzyme activity were *Selonomonas ruminantium*, followed by *Megasphaera elsdenii*. Other bacteria had also shown phytase enzyme activity, but the phytate enzyme activity could not be detected. Phytase enzyme activity was higher in precipitated bacteria than that in supernatant of rumen liquor and depended on types of feeds. Feeds from high cereal barley (90% barley) had higher phytase enzyme activity than feed containing lower amount of cereal barley (55% barley).

The experimental results in local cattle rumen liquor showed that no methal ions and other chemical substance β-mercaptoethanol and EDTA could decrease phytase enzyme activity. Most of methal ions and chemical substances examined could be used as activator or were needed to increase phytase enzyme activity. The highest phytase enzyme activity in local cattle rumen liquor was obtained when ion Ca++ (150.78%) was added and followed by Mg++ (156,24%), Fe++ (155,73%), Zn++ (150.13%), Mn++ (139.69%), Co++ (139.23%), EDTA (135.95%), Ca++ (122.50%) and β-mercaptoethanol (112.27%) respectively. In contrast, in imported cattle rumen liquor, all methal ions did not show to decrease or inhibit phytase enzyme activity, but β-mercaptoethanol and EDTA decreased and inhibited phytase enzyme activity. The highest phytase enzyme activity was obtained when ion Zn++ (164.10%) was used and followed by ions Cu++ (150.19%), Ca++ (142.15%), Fe++ (141.61%), Mn (130.59%), Co++ (115.16%), Mg++ (111.34%), EDTA (82.86%) and Mercaptoethanol (63.47%). Figure 4 shows the effect of methal ions and chemical substances on phytase enzyme activity of catlle rumen liquor from abbatoir.

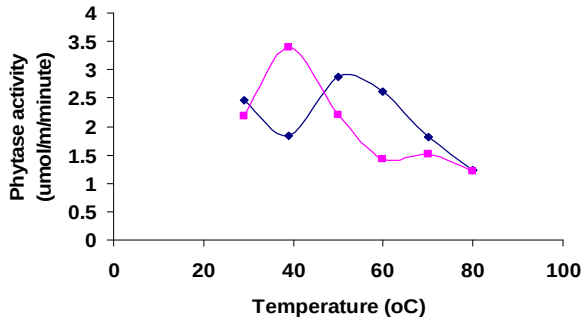

Figure 2. Effect of Temperature on Phytase Enzyme Activity in Local Cattle (—♦—) and Imported Cattle (—■—) Rumen Liquor

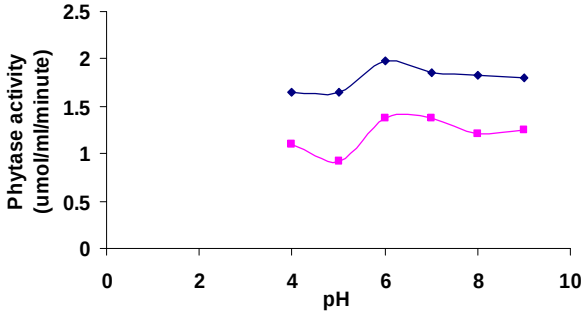

Figure 3. Effect of pH on Phytase Enzyme Activity from Local Cattle (—♦—) and Imported Cattle (—■—) Rumen Liquor

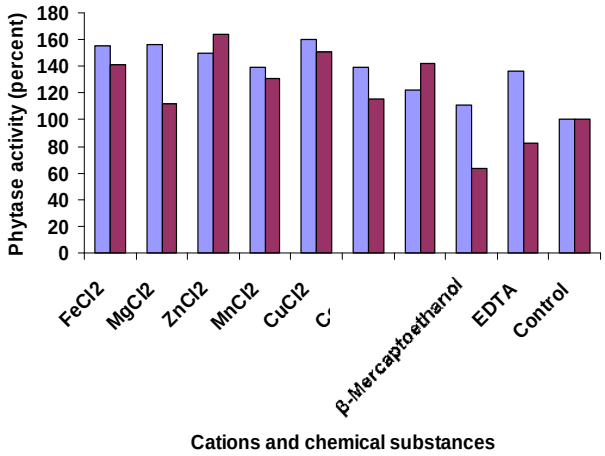

Figure 4. Effect of Cations and Chemical Substances on Phytase Enzyme Activity in Local Cattle (■) and Imported Cattle (■) Rumen Liquor

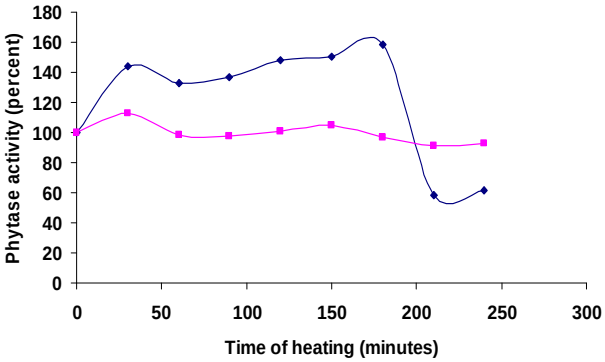

Figure 5. Effect of Heating Time on Phytase Enzyme Activity in Local Cattle (—♦—) and Imported Cattle (—■—) Rumen Liquor

Powar and Jagannathan (1982) indicated that phytase enzyme from *Bacillus subtilis* needed methal ion Ca++, and it was the same as phytase enzyme in cattle rumen liquor from abbatoir in this experiment; however, the enzyme activity was inhibited by Ba++, Sr++, Hg++, Cd++, and borate. Saryska *et al.* (2005) showed that phytase enzyme from *Aspergillus niger* needed ions Ca++ and B++; the enzyme also had interesting phenomena because it needs ions Pb++ and Ag++ too, but the activity was inhibited by the presence of methal ions Hg++, Cu++, Zn++, Fe++ and Al++. Greiner and Farouk (2007) reported the same finding that, phytase enzyme activity was inhibited significantly by methal ions Zn++, Fe++, Fe+++, Mo++ and phosphate. Cheng *et al.* (1999) indicated that phytase enzyme from rumen microorganisms was stimulated by methal ions Ca++, Na++, K++, and Mg++, but inhibited by methal ions Fe++, Zn++, Mn++ and was not affected by ions Co++ and Ni++.

Effect of heating on phytase enzyme activity showed that in local cattle rumen liquor, heating at 50 ^{o}C temperature (optimum incubation temperature) for 180 minute did not decrease phytase enzyme activity. Phytase enzyme activity was decreased after heat treatment for 210 minutes with the activity was only 58.04%, but it was 61.87% after heat treatment for 240 minutes. The enzyme needs heat stimulation to work effectively. Heating for 30 up to 180 minutes increased phytase enzyme activity. In imported rumen liquor, heat treatment at 39 ^{o}C temperature (optimum incubation temperature) for 180 minutes increased phytase enzyme activity, but heat treatment for 210 minutes has slightly decreased the enzyme activity with the value was 91.32%. The value of enzyme activity after heating for 240 minutes was 92.92%. Both phytase enzymes from local and imported cattle rumen liquor were resistance to heat treatment. Figure 5 shows the effect of heating on phytase enzyme activity from cattle rumen liquor.

CONCLUSIONS

Optimum precipitation of phytase enzyme from rumen liquor is obtained at 60% ammonium sulphate concentration for local cattle and at 70% ammonium sulphate concentration for imported cattle.

General characteristics of phytate enzyme from the rumen liquor of local cattle differ from those from the rumen liquor of imported cattle. Phytase enzyme from local cattle rumen liquor has optimum temperature at 50 ^{o}C, optimum pH at 6, resistance to heat treatment up to 180 minutes, needs most of methal ions as activator, but no chemical substances, such as EDTA and β-mercaptoethanol, inhibit enzyme activity. On the other hand, phytase enzyme from imported cattle rumen liquor has optimum temperature at 39 ^{o}C, optimum pH at 7, resistance to heat treatment up to 180 minutes, needs most of methal ions as activator; however, chemical substances, such as EDTA and B-mercaptoethanol, inhibit phytase enzyme activity. The activity of the phytase enzyme from rumen liquor of local cattle is higher than that of imported cattle.

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