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Pakistan Journal of Nutrition 12 (1): 45-49, 2013 ISSN 1680-5194 2013 The Effect of Supplementation of Phytase on Broiler Rations to the Retention of Phosphor, Calcium and Nitrogen Gita Ciptaan, Yetti Marlida, Periadnadi and Yose Rizal Department of Nutrition and Feed Technology, Faculty of Animal Husbandry, Andalas University, 25163 Padang, Indonesia Abstract: The research was conducted to determine the optimum dose of phytase supplementation of Fusarium verticillioides on Phosphor-deficient rations. This study used 24 broiler chickens (4 weeks). This research used Completely

Randomized Design (CRD) with five ration treatments with 4 replications. Ration treatment was supplemented phytase with different doses: R1: 0 U/kg, R2: 250 U/ kg, R3: 500 U/kg, R4: 750 U/kg and R5: 1000 U/kg. Ration was based on iso protein (20%) and iso energy (2882 kcal/kg). The variables measured were retention of phosphor, calcium and nitrogen. The results showed that supplementation of phytase on broiler rations which Phosphor-deficiency was influenced the retention of Phosphor, calcium and nitrogen significantly ($P<0.01$). The optimum phytase supplementation of Fusarium verticillioides on broiler rations which phosphor-deficient was 750 U/kg ratio, it is seen from retention of phosphor (71.38%), calcium (75.65%) and nitrogen (67.61%). Key words: Phytase, supplementation, Fusarium verticillioides, retention, broiler INTRODUCTION Feed is the major factor that determines the success of the animal farm business. On managing poultry farm intensively feed should always be available and adequate. The main feed ingredients of poultry ration formulation largely derived from plant sources (80-85%) for the source of energy, protein and minerals, especially phosphor minerals. The availability of phosphor in plant as a raw material of monogastric livestock feed mainly poultry is limited by the presence of phytic acid anti- nutrients that binds P-elements and other elements, hence, they can not be utilized. Phosphor that is bound to phytic acid can not be digested and absorbed by monogastric livestock, especially poultry. Phytase was not produced in the digestive tract. The unavailability of phytase in digestive tract of poultry, will make phosphor be excreted with the excreta (Mallin, 2000). In the normal physiological conditions phytic acid will bind essential minerals such as Ca, Mg, Fe and Zn. Beside it can bind the amino acid and protein and inhibit digestion by digestive enzymes (Pallauf and Rimbach, 1996) which to be lower of availability and digestive power, so that phytic acid is considered as anti-nutrients in the feed. Hence, an enzyme was given to hydrolyse it. One of the enzymes that can decrease phytic acid anti- nutrients is by suplementing phytase on the ration. Phytase can hydrolyze phytic acid to inorganic phosphor and myo-inositol phosphate derivate, thus increasing the availability of Phosphor, digestibility and utilization of feed protein and amino acids. Phytase enzyme is widespread in nature because it can be found in microorganisms (Shieh and Ware, 1968), so it can to be applied in the feed. Some of the phytase of microorganisms have been characterized as phytase from Escherichia coli, Bacillus subtilis and Aspergillus. Supplementation of phytase can increase the availability of phosphor and calcium. Phytase supplementation in broiler rations which low phosphor can increase the availability of nutrients, weight gain and feed conversion and increase phosphor and calcium retention significantly (Mondal et al., 2007). Further, addition of 1000 U phytase/kg in broiler rations is capable to increasing the availability of Arginine 3.7% and Nitrogen 4.2% and Calcium 16.6% (Lan et al., 2002). In this study the phytase produced by Fusarium verticillioides Phosphor is expected to hydrolyze phosphor that bound in phytic acid without losing its activity. Yetti et al. (2010) reported, the phytase from Fusarium verticilliodes was thermostable and acid stable with broad substrate specificity and high specific activity for animal nutrition purposes. Further according to Vats and Banerjee (2006) that the highly thermostable enzyme and acid stable with wide specificity and high specific activity is an enzyme that potentially to be a co- enzyme and applied in poultry feed. Those it need to biological testing on the feed that phytase enzyme supplemented by Fusarium verticillioides. MATERIALS AND METHODS Materials used in this study were: (1) 24 male broiler chickens of 4 weeks old. (2) 24 units cage sized 40 x 30 x 30 cm, equipped with incandescent lamps and Corresponding Author: Gita Ciptaan, Department of Nutrition and Feed Technology, Faculty of Animal Husbandry, Andalas University, 25163 Padang, Indonesia 45 Table 1: Ration composition and feed nutrient (%) and metabolism energy (Kcal/kg) of the treatment

ration Treatment ---------- No. Feed ingredient R1 R2 R3 R4 R5 1. 2. 3. 4. 5. Yellow corn Rice brain Soybean meal Fish meal Coconut oil Total 44 25 12 16.5 2.5 100 44 25 12 16.5 2.5 100 44 25 12 16.5 2.5 100 44 25 12 16.5 2.5 100 44 25 12 16.5 2.5 100 6. Phytase (U/kg) 0 250 500 750 100 Nutrient (%) Crude protein 20.03 20.03 20.03 20.03 20.03 Crude fat 5.59 5.59 5.59 5.59 5.59 Crude fiber 5.81 5.81 5.81 5.81 5.81 Calcium 0.98 0.98 0.98 0.98 0.98 Total phosphor 0.53 0.53 0.53 0.53 0.53 Available phosphor 0.31 0.31 0.31 0.31 0.31 Methionine 0.42 0.42 0.42 0.42 0.42 Lysin 1.26 1.26 1.26 1.26 1.26 Tryptophan 0.27 0.27 0.27 0.27 0.27 Metabolism energy (Kcal/kg) 2882.3 2882.3 2882.3 2882.3 2882.3 drinking places. Each experiment unit was occupied by one chicken. Feed ingredients used to formulate rations for treatment was consists of yellow corn, rice bran, soybean meal, fish meal and coconut oil and phytase. Ration composition and food nutrient and metabolism energy of the treatment ration can be seen in Table 1. This study used Completely Randomized Design (CRD) with five ration treatments with 4 replications. The treatment were phytase supplementation with dose of: R1: 0 U/kg, R2: 250 U/kg, R3: 500 U/kg, R4: 750 U/kg and R5: 1000 U /kg of ration. Variable measured were retention of phosphor, calcium and nitrogen. The differences between treatments were tested with Duncan's multiple range test (Steel and Torrie, 1991). RESULTS AND DISCUSSION The retention of phosphor, calcium and nitrogen are presented in Table 2. Statistical analysis showed that the phytase of Fusarium verticillioides supplementation was influenced significantly ($P<0.01$) on the retention of phosphor, calcium and nitrogen in each treatment during the study. Retention of phosphorous: DMRT test (Table 1) showed that the retention of phosphor in treatment R1 was highly significant ($P<0.01$) lower than treatment R2, R3, R4 and R5. But treatment R4 and R5 provides different effects were not significant ($P>0.05$). It indicated that the higher phytase enzyme addition will be followed by the higher of phosphor retention. The increasing addition of phytase enzyme on treatment R2, R3 and R4 cause the increase of phosphor retention. This is caused by the supplementation of phytase into the rations which phosphor-deficiency can be improve the utilization of phosphor hydrolysis, because the phytase will degradation the phosphor which bound in phytic acid. So the phosphor that initially was bound will be utilized. Similar to the research of Rezaei et al. (2007) that the supplementation of phytase in poultry rations will increase the availability of phosphor and calcium in broiler. Akyurek et al. (2005) stated that phytase supplementation improve the retention of phosphor and calcium in broiler chickens significantly. Furthermore Farrell and Martin (1998) stated the addition of phytase to duck rations containing rice bran has increased significantly of dry matter, nitrogen and phosphor. Treatments R4 and R5 was not significant. It was indicated that phytase supplementation by 1000 U/kg on treatment R5 did not change the retention of phosphor, because on treatment R4 phytase supplemented up to 750 U/kg of substrate was the optimum substrate hydrolysis by the enzyme and all sides had been occupied by enzyme. So that it became saturated causing phosphor bound with phytic acid could not be hydrolyzed because enzyme could not able to break the phytic acid ties. In the opinion of Zyla et al. (2000) that supplementation of 750 U/kg in the ration that wheat was the main ingredients can be increase of weight gain, feed consumption and retention of phosphor and calcium in broiler. Pourreza and Classen (2001) stated that the addition of 500 phytase U/kg can increase the digestibility of protein, phosphor and calcium significantly but the supplementation of phytase up to

1000 U/kg have no effect. Retention of phosphor on treatment R1 was lower than all treatments because R1 was control, there were not supplemented of phytase. So phosphor which bound to phytic acid could not be hydrolyzed and absorbed by poultry.

In addition, the absence of phytase produced in the digestive tract of

monogastric livestock, especially Table 2: Retention of phosphorus (%), calcium (%) and nitrogen (%) of treatment rations Treatment rations Retention of phosphor Retention of calcium Retention of nitrogen R1 41.53a 56.69a R2 49.95b 58.53b R3 56.85c 67.98c R4 71.38d 75.65d R5 71.40d 76.98d Mean 58.22 67.17 SE 0.65 0.46 55.72a 64.99b 66.65c 67.61d 68.44d 64.68 0.52 Note : Means on the same row with different superscripts are significantly (P<0.01) different. SE : Standard error. poultry. According to Mallin (2000), that monogastric livestock, especially poultry do not produce the phytase enzyme in the digestive tract, so phosphor will be excreted with excreta. The results also indicate that the amount of phosphor excreta in treatment R1 was the highest. Calcium retention: Statistical analysis (Table 2) showed that the retention of calcium in the treatment R1 decreased significant (P<0.01) than the

treatment of R2, R3, R4 and R5. The treatment of R4 and R5 were not

significant (P>0.05). Calcium retention of treatments R1 was lower than all treatments because treatment R1 was not supplemented by phytase, so that calcium bound with phytic acid could not be hydrolyzed and absorbed by poultry. According to Pallauf and Rimbach (1996) phytic acid can bind to essential minerals such as Ca, Mg, Fe and Zn. Phytic acid can also bind to amino acids and proteins and inhibit digestion by digestive enzymes, so the enzyme phytase can not be utilized by the body and will be wasted with excreta. This is caused on treatment R1 did not supplemented with phytase, so calcium was bound with phytate could not be hydrolyzed which results in calcium retention of broiler was low. Phytic acid would interfere with mineral absorption valency 2 to Cu, Zn, Mg and Ca, hence, careful measures should be taken for usage in poultry liver (Cullison, 1978). The increasing addition of phytase enzyme on treatment R2, R3, R4 has increased the calcium retention. This is caused that phytase supplemented into ration with phosphor-deficiency will be improve the utilization of calcium, where phytase will be break down phytase calcium bound to phytic acid that initially bound calcium will be utilized. Similar to the research of Rezaei et al. (2007) which states, that supplementation of phytase in poultry rations will increase the availability of mineral phosphor and calcium for broilers. As stated by Akyurek et al. (2005) that phytase supplementation can improve significantly the retention of Phosphor and calcium in broiler chickens. Phytase supplementation

of 750 U/kg (R4) and 1000 U/kg (R5) were not increased calcium retention because supplementation of phytase up to 750 U/kg was optimum. In this case all the substat side had occupied by enzyme, hence, it became saturated. If the addition of phytase increased further to 1000 U/kg the result was not better. Similar results were also obtained by Zyla et al. (2000) suggest that supplementation of 750 U phytase/kg in the ration where the wheat is the main ingredients can be increase weight gain, consumption of ration and retention of phosphor and calcium in broiler. Pourreza and

Classen (2001) stated that the addition of 500 U phytase/kg can increase

the digestibility of protein, phosphor and calcium significantly but the

supplementation of 1000 U phytase/kg had no effect further because equaling with phytase supplementation 750 U phytase/kg. Nitrogen retention: DMRT test (Table 2) showed that the retention of nitrogen in the ration R1 was lower significant

(P<0.01) than the treatment of R2, R3, R4 and R5. But the treatment

of R4 and R5 were not significant (P>0.05). The increasing phytase supplementation also increased the nitrogen retention but phytase supplementation on ration R5 could not improve nitrogen retention as much as treatment R4. Nitrogen retention in treatment R 1 was lower than other treatments, it caused by treatment R1 without phytase supplemented, so the nitrogen bound to phytic acid could not be hydrolyzed and absorbed by poultry, besides the absence of phytase produced in the digestive tract. According to Mallin (2000) that monogastric livestock, especially poultry do not produce the enzyme phytase in the digestive tract, then the nitrogen was excreted along with excreta. When the nitrogen retention was low the more excreta excreted because many were absorbed by the body, resulting in low value of nitrogen retention and the more nitrogen retention, the less excreta excreted, resulting in high retention value of nitrogen. Anggorodi (1984) states the poor quality of protein in the ration will lead to lower nitrogen retention value or only a small number of proteins that can be used for growth and maintenance. The increasing addition of phytase enzyme on treatment R2, R3, R4 was followed by increasing nitrogen retention. This is caused that supplementation of phytase into ration with phosphor-deficiency can increase the utilization of nitrogen and energy, because phytase will degradation and breakdown the nitrogen that bound to phytic acid. So initially nitrogen was bound will be utilized. Phytase enzymes were able to hydrolyzed phytic acid, so that the enzyme phytase can improve the protein or amino acids and energy, as well as calcium and phosphor in feed ingredients (Shelton et al., 2004). In accordance with Selle and Ravindran (2007), that phytase supplementation not only improve the digestibility of P, Ca, Mg and Zn but also can directly increase the utilization of nitrogen and energy. Further opinion of Lim et al. (2002) stated, that the supplementation of phytase enzyme into the ration can improve significantly

digestibility of dry matter, crude fat, P, Zn, Mg and Cu as well as to increase the

retention of nitrogen, minerals, Ca, P, Mg and Zn. Furthermore Farrell and Martin (1998a) states the increase phytase supplementation on duck ration contain rice bran has influenced significantly in dry matter, nitrogen and phosphor. Treatments R4 and R5 not significant. It indicated that phytase supplementation of 1000 U/kg on the R5 did not change the retention of phosphor. It caused treatment R4 with phytase supplemented 750 U/kg had been reached the point of optimum and all the side of substrate was occupied hydrolysis by the enzyme, so that it became saturated. Phosphor bound with phytic acid could not be hydrolyzed because enzyme could no longer afford to break the phytic acid ties. In accordance to Pourreza and Classen

(2001) stated that the addition of 500 phytase U/kg can increase the digestibility of protein, Phosphor and calcium significantly but the supplementation of 1000 U/kg had no effect. Nitrogen retention in the ration showed the difference between rations nitrogen consumed with the excreted nitrogen through the excreta, so the higher the nitrogen retention the higher the nitrogen retained in the body (Scott et al., 1982). The difference of treatment A4 and A5 is not significant because phytase supplementation in treatment A4 can match phytase on treatment A5, where enzyme phytase could hydrolyzed phytic acid. Consequently the protein or amino acids and energy was improved as well as calcium and phosphor in feed ingredients (Shelton et al., 2004). Lloyd et al. (1978) states that nitrogen retention is one method for assessing protein quality of the ration by measuring the consumption and excreted nitrogen in excreta, to know the amount of nitrogen retained in the body. Retention of nitrogen in the ration showed the difference between nitrogen consumed with the nitrogen rations secreted through the excreta, the higher retention of nitrogen, the higher nitrogen retained in the body (Scott et al., 1982). Conclusion: The optimal phytase supplementation on phosphor-deficiency broiler ration was 750 U/kg ration, this can be seen from the retention of phosphor 71.38%, calcium 75,65% and nitrogen 67,61%. ACKNOWLEDGEMENTS The Author was very Grateful for the financial support

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Ministry of National Education, the republic of Indonesia (No. 002/H.16/PL/HB-PSN/III/2010. Tanggal 4 Maret 2010). REFERENCES Akyurek, H., N. Senkoylu and M.L. Ozduven, 2005. Effect of microbial phytase on growth performance and nutrients digestibility in broilers. Pak. J. Nutr., 4: 22- 26. Anggorodi, R., 1984. Kemajuan Mutakhir dalam Ilmu Makanan Ternak Unggas, UI Press, Jakarta. Cullison, A.E., 1978. Freed and Feeding Animal Nutrition Practice Hall of India. Private Limite, New Delhi. Farrell, D.J. and E.A. Martin, 1998a. Strategies to inprove the nutritive value of rice bran in poultry diets.I.The Addition of Food Enzymes to Taget the non-starch Polysaccharide Fractions in Diets of Chickens and Ducks Gave no Response. Br. Poult. Sci., 39: 549- 554. Lan, G.Q.N., Abdullah S. Jalaludin and Y.W. Ho, 2002. Culture Conditions Influencing Phytase Production of Mitsuokella jalaludinii, a New Bacterial Species from the Rumen of Cattle. J. Applied Microbiol., 93: 668-674. Lim, H.S., Namkung, J.S. Um, K.R. Kang, B.S. Kim and I.K. Paik, 2002. The effects of phytase supplementation on the performance of broiler chikerns fed diet with different levels of non-phytase phosphorus. Asian-Aust. J. Anim. Sci., 14: 250-257. Lloyd, L.E., B.E. McDonald and E.W. Krempton, 1978. Fundamental of Nutrition 2th Edn., W.H. Freeman and Company. Mallin, M.A., 2000. Impacts of industrial animalproduction on rivers and estuaries. Am. Sci., 88: 26-37. Mondal, M.K., S. Panda and P. Biswas, 2007. Effect of microbial phytase in soybean meal based broiler diets containing low phosphorous. Int. J. Poult. Sci., 6: 201-206. Pallauf, J. and G. Rimbach, 1996. Nutritional significance of phytic acid and phytase. Arch. Anim. Nutr., 50: 301-319. Pourreza, J. and H.L. Classen, 2001. Effect of suplementation phytase n xylanase on phytate phosphor degradation. Illeal protein and energy digestibility of corn-soybean-wheat bran diet in broiler chiks. J. Agric. Sci. Tecnol., 3: 19-25. Rezaei, M.S., Borbor, M. Zaghari and A. Teimouri, 2007. Effect of Phytase supplementation on nutrients availability and performance of broiler chicks. Int. J. Poult. Sci., 6: 55-58. Scott, M.L., M.C. Nesheim and R.J. Young, 1982. Nutrition of chicken. 3rd Edn., Scott, M.L. (Ed.) and Associeates Publishers, Ithaca, New York. Selle, P.H. and V. Ravindran, 2007. Microbial phytase in Yetti, M., R. Deltita, P. Adnadi, N. Gusmanizar and G. poultry sciece. Anim. Feed. Sci. Technol., 135: 1-41. Ciptaan, 2010. Identification and characterization of Shelton, J.L., Southern, L. A. Gaston and A. Foster, 2004. a phytase from endophytic fungus a potential Evaluation of nutrient matrix values for phytase in commercial for feed applications. Pak. J. Nutr., 9: broilers. J. Applied Poult. Res., 13: 213-221. View Record in Scopus Cited By in Scopus. 471-474. Shieh, T.R. and J.H. Ware, 1968. Survey of Zyla, K., J. Korelski, S. Swiatkiewiccz, M. Kujawski, J. microorganisms for the production of extracellular Piironen and D.R. Ledoux, 2000. Effect of phytase. Applied Microbiol., 16: 1348-1351. phosphorylitic and cell wall-degrading enzymes on Steel, R.G.D. and J.H. Torrie, 1991. Prinsip dan the performance of growing broilers fed wheat- Prosedur Statistik. Suatu Pendekatan. PT. based diets containing different calcium levels. Gramedia Pustaka Utama. Jakarta. Poult. Sci., 79: 66-76. Vats, M.S. and U.C. Banerjee, 2006. Catalytic characterization of phytase (myo-inositolhexakisph- pshate phosphohydrolases) from A. niger van Teighem: Glycosylation pattern, kinetics and molecular properties. Enzyme Microb. Technol., 39: 596-600. Pak. J. Nutr., 12 (1): 45-49, 2013 Pak. J. Nutr., 12 (1): 45-49, 2013 Pak. J. Nutr., 12 (1): 45-49, 2013 Pak. J. Nutr., 12 (1): 45-49, 2013 46 47 48 49