The effect of using earthworm (*Lumbricus rubellus*) meal additives as growth promoters on protein digestibility and performance of intestinal villi

Hardi Julendra,* Zuprizal,† and Supadmo†

*Researcher at Research Unit for ProcessesDevelopment and Chemical Engineering (BPPTK)-The Indonesian Institute of Sciences (LIPI); and †Lecturer at the Faculty of Animal Sciences, Universitas Gadjah Mada, Yogyakarta, Indonesia

ABSTRACT: The objective of this research was to study the effects of earthworm meal (EWM) addition in the diets on digested protein and intestinal villi, using 100-day old broilers of Cobb strain (CP 707) for 35 days. There were four treatments namely R0 : without EWM addition, R1 : 0.5% EWM, R2 : 1% EWM, R3 : 1.5% EWM with three replications, of five chickens each. Digested proteins test was obtained by means of excreta and ileum collection methods using one-way completely randomized design (CRD) then continued by *Duncan Multiple Range Test* (DMRT). Performance test of intestinal villi was obtained by means of *scanning electron microscopy* (SEM). The results showed that protein digestibility from R3 (91.69%) was similar to ($P>0.05$) with R1 (90.45%), however it was significantly different ($P<0.05$) from R0 (81.75%) and R2 (85.99%). The illustration of intestinal villi form showed that the R1, R2 and R3 were better than the control (R0). The addition of earthworm meal to diet as an additive in broilers as growth promoters improved digested proteins enhancement and increased absorption of nutrients through intestinal villi performance.

Key words: additives, growth promoters, earthworm meal, digested proteins, intestinal villi performance

INTRODUCTION

The use of antibiotics as additives antibiotics growth promoters on broiler is aimed at reducing the population of pathogenic bacteria in the intestinal tract of broiler. Antibiotics have been able to improve immunogenic (Boogard and Stobberingh, 1999), feed efficiency (Wahyu, 2004) and body weight of broilers up to 100 grams at the age of 6 weeks (Sundu, 2007).

Some theories have discussed about the mechanism of antibiotics growth promoter's (AGP's): Nutrient in the gut is maintained by antibiotics from destruction of pathogenic bacteria, absorption of foods is enhanced by antibiotics because antibiotics make a barrier on the wall of the intestine to become thin, the production of toxins from bacteria digestive tract is reduced by antibiotics that can reduce the occurrence of digestive tract clinical infection (Feihgner and Dashkevics, 1987), and they can improve the performance of intestinal villi (Rofiq, 2003), resulting in the increased of feed efficiency due to high food absorption in the intestine (Wahyu, 2004). From these reviews , it can be concluded that the antibiotic growth promoter's (AGP's) can reduce the number of pathogenic bacteria in the digestive tract in poultry, can maximize the absorption of nutrients and improve the growth to be more efficient on feed consumption.

The problem is that AGP's used today are semi-synthetic products that have been banned in several countries because of antibiotic residues in food are harmful for livestock products such as meat, eggs and milk which ultimately negatively impact human who consume them(Hakim, 2005). Antibiotic added to animal feed is often not purely derived from microbes, but from chemically synthesized antimicrobial (Cook et al., 1997). Thus, if it used in a long time it will cause the resistance effects on targeted pathogen bacteria. The use of streptomycin, sulfadiazine and tetracycline as feed supplement prove resistant to Escherichia coli (Solomons, 1978; Khachatryan et al., 2006). Antibiotics are found in products such as eggs and poultry meat caused the spread of resistant Escherichia coli from cattle to humans (Boogard and Stobberingh, 1999).

Therefore WHO recommended that all countries conduct oversight of antibiotic resistance in humans and animals (Castanon, 2007). Regulation in using of antibiotics in poultry feed by WHO has made the pharmaceutical industry to develop a natural material as a substitute for antibiotics (Sundu, 2007) and opened up opportunities for research on alternative sources of antibiotics from natural ingredients. One of these natural ingredients is earthworm meal (TCT) because it contains anti-bacterial substances called *lumbricine* (Cho et al., 1998). Other kinds of worm containing antibacterial are *Eisinia foetida* (Lange et al., 1999), *Theromyzon tessulatum* (Tasiemski et al., 2004). TCT is known to give effect to increase the immunity of cattle and has been able to stimulate the immune system (Liu et al., 2004). This research evaluated the earthworm meal used as feed additives on protein digestibility, and the performance of intestinal villi.

MATERIALS AND METHODS

Location and Study Period

The research was conducted in the henhouse at the Biochemistry Laboratory, UGM Faculty of Animal Science, University of Gadjah Mada for 40 days followed by Chemical Analysis in the laboratory within one month, starting from September to November 2009.

Materials

The materials used were broilers, feed additives fomulated containing earthworm meal and filler. One hundred DOC Cobb strain (CP 707) were used in this experiment. Rations was in compliance with Nutrient Requirements of Broilers. The drinking water was given ad libitum. Using a pen-sized cage 1x 1 x 0.6 m, each cage provided feeder and drinker, excreta colector and analysis of proteins and digested proteins units.

Methods

Feeding Trials. A hundred broilers of the DOC were raised until the age of 35 days by means of a pen-sized cage 1x 1 x 0.6 m, wire ram floor and is equipped with 60-watt lamp. Feed were provided in the morning and afternoon, drinking water was provided ad libitum. They were given ND vaccination to prevent Newcastle Disease, the first vaccination was given at the age of 1 day and the second was at the age of 18 days.

The trademark of the basal was "Immuno-chick". It was the production of -LIPI BPPTK Yogyakarta. The basal rations used were in accordance with SNI No. 01-3931-1995 as shown in Table 1.

Experimental Design

The design used was one-way Completely Randomized Design (CRD) to compare the effect of increasing levels of earthworm meal (TCT), 0.5, 1.0 and 1.5%. Ration treatment consisted of R0 (filler 1.5% + 0% TCT), R1 (filler 1.0% + 0.5% TCT), R2 (filler 0.5% + 1.0% TCT) and R3 (filler 0% + 1.5% TCT). Each treatment used three replications, each replication uses five broilers.

Excreta and Digesta Collection

Protein digestibility test was carried out at the age of 33 to 35 days consisting of four treatments (R0, R1, R2 and R3) with three replications. Six broilers were abstaining from food to determine the metabolic digestibility for three days. The excreta collection techniques referred to that of Lee *et al* (2004) as modified by Mulyono, (2008).

Excreta were sun - dried for 3 days , weighed , ground and sub-sampled of 40 g. They were kept at a temperature of 40 ^{0}C. At the age of 35 days all the broilers were slaughtered to obtain ileal digesta. Digesta collected was weighed, dried in the oven then the analysis of proteins was performed. Ileal

digesta protein multiplied by the dry ingredients excreta is called protein excreta. The difference in protein intake with a protein excreta called is digested protein.

Chemical analyses to determine the protein digestion conducted base on dry matter (DM) includes crude protein (AOAC, 1990) from excreta, digesta, and feed intake . Digested protein formula refered to Lee et al (2004) and Mulyono (2008), by the formula writen bellow.

$$TPD = \frac{CP.fi - (DMe \times CP.ile - DM.em \times CP.ile\text{-}m)}{CP.fi} \times 100\%$$

- CP *fi* = crude protein feed intake (%DM)
- DM*e* = dry matter eksreta (%DM)
- CP *ile* = crude protein ileum (%DM)
- DM *em* = dry matter excreta metabolic digestion (%DM)
- CP.*ile-m* = crude protein ileum metabolic digestion (%DM)
- TPD = true protein digestibility

Tabel 1. Ration Composition (% Dry Matter)

Feedstuffs	R0	R1	R2	R3
Corn, %	63	63	63	63
Rice bran, %	7	7	7	7
Soybean cake meal, %	15	15	15	15
Poultry Meat Meal, %	12.5	12.5	12.5	12.5
Decalsiumphospat, %	0.5	0.5	0.5	0.5
Coconut oil, %	0.5	0.5	0.5	0.5
Filler, %	1.5	1	0.5	0
Earthworm Meal , %	0	0.5	1	1,5
Total, %	100	100	100	100
Chemical composition of treatment				
ME, kcal/kg	3200	3200	3200	3200
CP, %	19.8	20.2	20.5	20.8
Fat, %	4.5	4.5	4.6	4.7
Crude Fiber, %	3.8	3.8	3.8	3.8
Ca, %	1.0	1.0	1.0	1.0
P, %	0.7	0.7	0.7	0.7
Lumbricine, µg/g	0	0,0005	0.001	0.0015

Specimen Preparation for Intestinal Villi Obeservation by Means of Scanning Electron Microscopy (SEM)

At the age of 32 days four broilers were slaughtered, the duodenum was taken and cut. The sample was then inserted into the duodenum 70% alcohol solution for preparation in zoology laboratory of Biology Research Centre- LIPI Cibinong- Bogor.

Sample Preparation. The sample was cleaned by caccodylat buffer using ultrasonic agitation sonicator. It was left for a while, after that fixation was applied by using several reagents. The next stage was dehydrating sample with 50% alcohol to absolute alcohol and finally it was put in the freeze dryer. Before being inserted,it was coated a coating machine.

The Sample Study. The sample study was carried out after the saturation was optimally reached on the monitor. Zooming to view a sample image can be performed.

Observed Parameters

Digestibility protein data were analyzed by means of one-way variance Complete Random Design (RAL) and the analysis used by Duncan multiple range test (DMRT) according to Steel and Torrie (1993). Observations of intestinal villi were also performed

RESULTS AND DISCUSSIONS

Protein Digestibility

The feed is said to be qualified if all the ingredients of their food are digested properly. Feed quality is determined by the nutrient content of feedstuffs in accordance with the maintenance and production of the animal (McDonald , 2002).

Earthworm (*Lumbricus rubellus*) are animals that can be used as a source of feed because it contains protein (Resnawati, 2002). Crude protein content of earthworm meal is 63% dry material (Damayanti *et al.,* 2008). *Lumbricus rubellus* nutrient content is higher compared to *Lumbricus terestris* and *Perionyx excavatus* (Damayanti *et al*., 2008). Edwards (1985) reported that *Lumbricus rubellus* can be used to feed chickens the extent of the use of 72 to 215 g / kg., Istiqomah *et al* (2009) stated that the amino acid content of earthworm meal was quite balanced, the amino acid indices (essential amino acids index) earthworm meal was 58.67%, close to the amino acid index of egg, earthworm meal so that expected can improve the quality of chicken feed. The influence of the addition of additives containing earthworms meal to the digestion of protein in broiler chickens are presented in Table 2.

Table 2. The influence of the addition of feed additives on the digestion of proteins

	Treatment			
Protein digestibility	R0 (0%)	R1 (0.5%)	R2 (1 %)	R3 (1.5 %)
true digestibility	81,75[a]	90,48[bc]	85,99[ab]	91,69[c]

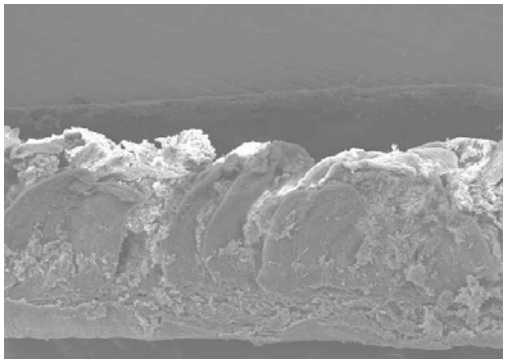
Description: [abc] indicates significantly (P <0.05)

The influence of earthworm meal addition of additives R0 (81,75) was significant (P< 0.01) with R1 (90,48) and R3 (91,69), R0 was non- significant with R2 (85,99) and R1 (90,48) was non-significant with R2 (85,99) but R2 (85,99) was significance (P<0.01) with R3. It can be learned that the quality of feed additives with the addition of earthworms was better than that which was not added. Mulyono (2008) stated that the poor quality feed will decrease digestibility. Besides digestibility feed quality is also influenced by the absorption of nutrients in the intestinal mucosa (Cheeke, 2005) and the thickness of the coating on the chicken intestine (Wahyu, 2004). Feed protein quality affects the digestibility poultry protein, a protein having the reforms by hydrolytic enzymes (Wahyu, 2004). Protein digested in the first proventrikulus glandular stomach where the existence of a secrete pepsinogen and HCl to break the tertiary structure of protein feed (Yuwanta, 2004). Having begun by pepsin proteolysis in the small intestine, then in catalysis by the enzyme trypsin, trypsin and elastase (Wahyu, 2004). The completion of digestion of proteins by erepsin (proteolytic enzyme) will produces amino acids and then continued with the absorption process (Zuprizal, 2006). Nutrient absorption is also influenced by microbial pathogens in the gut because pathogenic bacteria can cause intestinal wall thick (Wahyu, 2004). Increased protein feed with the addition digestibility earthworms meal occurred due to the depletion of intestinal mucosa causing increased absorption of food. Addition of antibiotic additives to attenuate the intestinal wall (Wiyana, 2005) that can improve the digestion. Low levels of antibiotics in feed can increase the absorption of amino acids (Zuprizal, 2006). Low digestibility values of feed causes the feed conversion tended to increase (Martawidjaya *et al*., 1999).

Performance Intestinal Villi

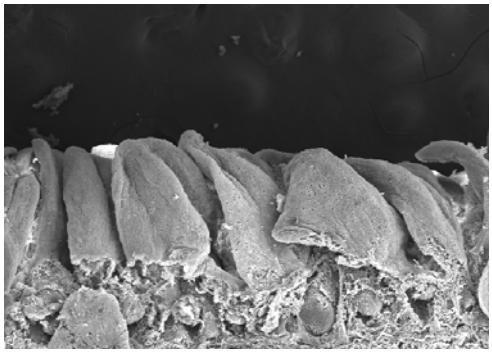
Villi intestine is an organ attached to the intestinal mucosa cylindrical with a height between 0.5 to 1.5 µm (Larbier and Leclercq, 1992) plays a role in nutrient absorption from the gastrointestinal tract into the blood vessels (Larbier and Leclercq, 1992). Nutrient absorption in the villi is influenced by the quality of feed and digestibility (McDonald, 2002), absorption will be high if villi is not distorted(Cheeke, 2005). Villi distortion occurs because of anti-nutrients in feed (Ortiz *et al*., 1994), such as crude fiber content is high (Rahim et al,. 2007) and populations of many pathogenic bacteria

in the digestive tract (Rofiq 2003). Earthworm meal contain complete amino acids (Istiqomah et al., 2009) and has the active ingredient of anti microbial pathogens (Damayanti *et al*, 2008), so the possibility can improve the performance of villi intestine. The influence of the addition of additives feed containing flour earthworms to the performance of villi intestine can be seen in Figure 1 below.



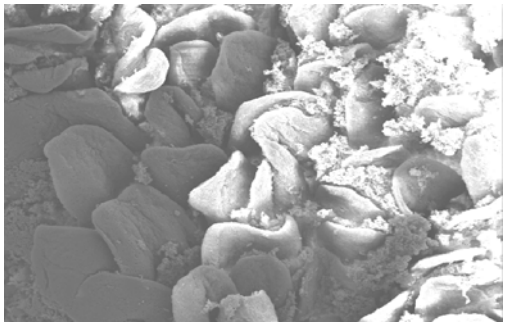
R0

Performance of the villi in the treatment without TCT



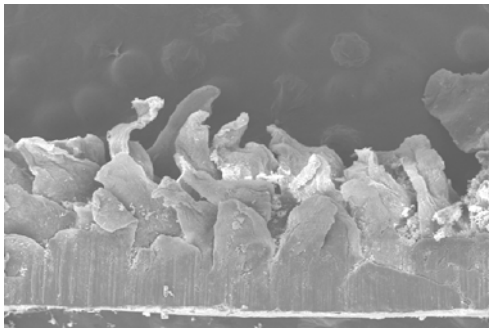
R1

performance of the villi with the addition of 0.5 % TCT



R2

performance of the villi with the addition of 1.0 % TCT



R3

performance of the villi with the addition of 1.5 % TCT

Figure 1. Intestinal villi (*using SEM : JSM- 5000, MAG X 100 000*)

Figure 1 shows that broilers that do not use additives of earthworm meal (R0) are different compared to the villi of chicken intestine using earthworm meal additives (R1, R2, R3). This is caused by the influence of microbial populations in the gut (Larbier and Leclercq, 1992), intestinal microbial pathogens that are satisfied by the structure will damage the villi (Rofiq, 2003). Intestinal villi damage caused by bacterial pathogens that attach to the mucosal layer fold, consequently inhibited nutrients reaching the cells so that the absorption was disturbed (Larbier and Leclercq, 1992). Inhibition of absorption can be overcome by giving the low levels of antibiotics in animal feed (Sundu, 2007). Nutrient absorption is enhanced by antibiotics because antibiotics can make the barrier on the wall to become thin, small intestine and production of bacterial toxins can be lowered so that it can reduce the occurrence of digestive tract infection subklinik (Feihgner and Dashkevics, 1987). Earthworm meal can replace the role of antibiotics in low doses, the use of low-dose antibiotics in feed can increase nutrient absorption capacity (Zuprizal, 2006), as shown in Figure 1 above .The structure of the villi in R0 look distorted due to the piling food substances around the different villus . R1, R2 and R3 look better.

CONCLUSIONS

Protein digestibility and performance of intestinal villi has been enhanced with the addition of additives from earthworm meal up to 1.5% in the feeding of broiler chickens. Anti-bacterial (*lumbricin*) in earthworm meal (TCT) is potentially used as feed additives to the extent of 1.5% of total ration

LITERATURE CITED

Bogaard, Van, De., and E.E Stobberingh. 1999. Antibiotic Usage in Animals: Impact on Bacterial Resistance and Public Health. *Drugs*. 58(4):589-607.

Castanon, J. I. R. 2007. History of the Use of Antibiotic as Growth Promotors in European Poultry Feeds. *Poultry Science,J 86: 2466-2471*

Cheeke, P. R. 2005. Applied Animal Nutrition, Feeds and Feeding. 3rded, Pearson Prentice Hall, New Jersey.

Cho, J.H., C.B. Park, Y.G. Yoon and S.C. Kim. 1998. Lumbricin I, a novel proline-rich antimicrobial peptide from the earthworm: purification, cDNA cloning and molecular characterization. Biochim. Biophys. Acta. 1408 (1): 67-76.

Cook, D., D. Brata.,Z. Szep., J. Dougan and D. Nepert., 1997. Effects of Antibiotics on Animal Feed. http://www.udel.edu/chem/C465/senior/fall97/feed/present.html [tanggal akses : 25 April 2008]

Damayanti, E., H. Julendra and A. Sofyan. 2008. Antibacterial Activity of Earthworms meal (*Lumbricus rubellus*) with Different Manufacturing Methods for *Escherichia coli*. Proceeding of National Seminar on Food. Yogyakarta, January 17, 2008. pp. MK 54-60.

Edwards, C. A. 1985. Production of Feed Protein from Animal Waste by Earthworms. Phil. Trans. R. Soc. Lond. B 310: 299 – 307.

Feighner, S. D., and M. P. Dashkevicz. 1987. Subtherapeutic levels of antibiotics in poultry feeds and their effects on weight gain, feed efficiency, and bacterial cholyltaurine hydrolase activity. *Appl. Environ. Microbiol*. 53:331-336

Hakim, R. S. 2005. Prospect of probiotics on broilers. CP-ed Service Bulletin, No. December 72/VI.

Istiqomah, L., A. Sofyan., E. Damayanti and H. Julendra. 2009. Amino Acid Profile of Earthworm and Earthworm Meal (*Lumbricus rubellus*) for Animal Feedstuff. J. Indo. Tropical anim. Agri. 34 (4), December 2009. 253-257.

Khachatryan, A.R., T.E. Besser, D.D. Hancock & D.R. Call. 2006. Use of a Nonmedicated Dietary Supplement Correlates with Increased Prevalence of Streptomycin-Sulfa-Tetracycline-Resistant *Escherichia coli* on a Dairy Farm, Applied and Environmental Microbiology 72 (7) :4583-4588.

Lange, S., E. Kauschke, W. Mohrig & E.L. Cooper. 1999. Biochemical characteristics of eiseniapore, a pore-forming protein in the coelomic fluid of earthworms. Eur. J. Biochem. 262: 547-556.

Larbier, M., and B. Leclercq. 1992. Nutrition'et Alimentation des Volailes. INRA (editor), Paris. *352 pp*

Liu, Y-Q., Z-J Sun., C. Wang., S-J. Li. and Y-Z. Liu. 2004. Purification of a Novel Antibacterial Short Peptide in Earthworm *Eisenia foetida*. Acta Biochimica et Biophysica Sinica *36 (4):297 – 302.*

Martawidjaja, M. B. Setiadi., And S. S. Sitorus. 1999. The influence of protein level of rations Energy Performance Against Young Goat Production. Livestock Research Center. Journal Livestock and Veterinary Science 4 (3): 161-171.

McDonald, P. 2002. Animal Nutrition. 6th edition. Prentice Hall. London

Mulyono, A. M. W. 2008. Mutant of the fungus Trichoderma sp Selulolitik to Improve Quality of Feed Materials *Onggok* For Broiler Chickens. Dissertation. Animal Science Studies Program. Postgraduate School. Gadjah Mada University.

Ortiz. L. T., C. Alzueta., J. Trevino and M. Castano. 1994. Effects of Faba Tannins n The Growth and Histological Structure of The Intestinal Tract and Liver of Chicks and Rats. British Poultry Science 35 : 743-754

Rahim, F., Sabrina., Rusmawati and M. Syibli. 2007. Broiler Small Intestine Villi Response to feeds Containing Palm Kernel Cake Which Fermented With *Rhizopus SP. J. Indonesian Tropical Anim and Agric. 32(4) Dec 2007 251-256I*

Resnawati, H. 2004. Carcass Weight and Fat Abdomen broiler fed rations containing Earthworm Meal. Proceeding of National Seminar on Animal Husbandry and Veterinary Technology. Bogor, 4-5 August 2004. pp: 473-478

Rofiq, M. Nasir. 2003. The influence of Local feedstuff Made of Performance of The Small Intestine Villi Broiler Chicken. Journal of Science and Technology of Indonesia, V5. N5, August 2003, pp. 190-194

Solomons, I. A., 1978. Antibiotics in Animals Feeds-Human and Animal Safety Issues. *Journal of Animal Science 46:1360 – 1368.*

Steel, R. G. D and J. H. Torrie. 1993. Prinsip dan Prosedur Statistika, Suatu Pendekatan Biometrik, Penerjemah Sumantri, Penerbit PT. Gramedia Putaka Sarana, Jakarta.

Sundu, B. 2007. Balance Sheet Profit and Loss of Use Antibiotics Growth Promoters (AGP) in the "Indonesian Poultry Magazine" Vol II. January 2007. P. 44-45

Tasiemski, A., D. Schikorski, F.L. Marrec-Croq, C.P-V. Camp, C. Boidin-Wichlacz & P.E. Sautiere. 2006. Hestidin: A novel antimicrobial peptide containing bromotryptophan constitutively expressed in the NK cells-like of the marine annelid, *Nereis diversicolor.* Developmental and Comparative Immunology, I (IIII) III-III. http://www.sciencedirect.com

Wahyu, J. 2004. Poultry Nutrition, 5th printing, Gadjah Mada University Press, Yogyakarta.

Wiyana, I. K. A. 2006. Influence Oxsitetrasiklin and Amoksisilin as Performance Against Animal Feed Additives, Residues in the Network and Eskreta Broiler. Thesis. Faculty of Animal Husbandry. UGM.

Yuwanta, T. 2004. Basic of Poultry Science. Publisher Kanisius.

Zuprizal, 2006. Poultry Nutrition. Faculty of Animal Husbandry- UGM, Yogjakarta.