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Digestibility of Corn-Mungbean Diet in Pre Starter Broiler as Affected by Multi-Enzymes

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Abstract: Feed enzymes were developed to improve nutrient utilization in monogastric. This research was conducted to study several commercial multi-enzymes and our own multi-enzymes products to determine their effect on dry matter digestibility and performance of pre-starter broiler fed corn-mungbean base diet. An *in vitro* test to determine the dry matter digestibility of the diet was also carried out. Eighty Lohman MB-202 day old chicks were randomly assigned into eight treatment diets i.e., Dc (control basal diet); D_{EA1} [basal diet+A1 (protease 7500 HUT and cellulase 44 CMCU/gram)]; D_{EA2} [basal diet+A2 (protease 7500 HUT and cellulase 44 CMCU/gram)]; D_{EB1} [basal diet+enzyme B1 (endo-1,4-β-xylanase 5500 visco units and endo-1,3/4-β-glucanase 500 AGL units/ml)]; D_{EB2} [basal diet+B2 (endo-1,4-β-xylanase 5500 visco units dan endo-1,3/4-β-glucanase 500 AGL units/ml)]; D_{EC1} [basal diet+enzyme C1 (amylase 2,5 U/ml, protease 77 U/ml and xylanase 91 U/ml)]; D_{EC2} [basal diet+enzyme C2 (amylase 2,5 U/ml, protease 77 U/ml and xylanase 91 U/ml)]. The results showed that enzymes addition could not improve *in vivo* dry matter digestibility (DMD). However feed intake and feed conversion ratio was lowest in D_{EC1} with no difference in body weight. *In vivo* digestibility did not correlate to *in vitro* digestibility. Multienzymes did not improve performance and dry matter digestibility of corn-mungbean base diet in prestarter broilers.

Key words: Digestibility, corn-mungbean, multi-enzyme, *in vitro* test, broilers, pre-starter

INTRODUCTION

Within poultry industry in the world, broiler is ranked at the top position as meat producer. The success of the industry depends on feed ingredients as it accounts 60-70% of total cost production. Corn is the major and strategic feed ingredients for energy source for poultry. Other energy sources are potentially and diversely available and there have been studies regarding corn substitution (Amerah, 2015). We have studied previously sorghum as substitute to corn (Murwani, 2008; Murwani *et al*., 2012). Soybean meal as vegetable protein source contributes up to more than 30% of broiler diet. This is a difficult feed ingredient to be substituted as it is not only high in protein but also contributes significantly as the main component of vegetable protein in poultry diet. An attempt to reduce or substitute soybean meal had been studied utilizing lupin and soybean or mungbean or combined mungbean-whole soybean (Brenes *et al*., 1993; Iji *et al*., 2001; Murwani, 2008; Murwani and Murtini, 2009; Murwani *et al*., 2011a,b). Such substitution put a great challenge as beans posses some antinutrients. These include (but not limited to) antitrypsin, phytate and Non Starch Polysaccharides (NSP). Pretreatment of the feed ingredients can reduce partly the antinutrient content. In regard to NSP it is more difficult to address as polysaccharides are the natural major nutrients in beans. In addition the amount of beans as vegetable protein source to be used in poultry diet is high amounting to more than 35% and hence may contain more NSP (Murwani, 2009, 2010a,b). In order to assist and improve the bioavailability of nutrients of bean containing diet, we studied the effect of multi-enzymes addition on the dry matter digestibility of corn-mung bean diet in pre-starter broiler.

MATERIALS AND METHODS

Diets and broilers: This study was conducted following good animal husbandry practice. Corn, sorghum, soybean and mungbean with approximately 11-12% moisture content were obtained from local producer. Each ingredient were ground separately. Prior to grinding, mungbean and soybean were pretreated by soaking followed by heating to deactivate antinutrient content. Other feed ingredients i.e., sorghum, rice bran, fish meal (54% protein), palm oil, vitamin and mineral mix (Medion Indonesia), dicalcium phosphate (PT.

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Pancaran Niaga), methionine, lysine and choline were used to complete basal diet and to meet nutrient requirement of broilers as recommended by SNI (2006) (Table 1).

Treatment diets consisted of Dc (control basal diet); D_{EA1} (basal diet+0.5% enzyme A (protease 7500 HUT and cellulase 44 CMCU/gram)); D_{EA2} (basal diet+1% enzyme A (protease 7500 HUT and cellulase 44 CMCU/gram); D_{EB1} (basal diet+0.5% enzyme B (endo-1,4-β-xylanase 5500 visco units and endo-1,3/4-β-glucanase 500 AGL units/ml); D_{EB2} (basal diet+1% enzyme B (endo-1,4-β-xylanase 5500 visco units dan endo-1,3/4-β-glucanase 500 AGL units/ml); D_{EC1} (basal diet+enzyme C (amylase 0,125 U/ml, protease 3.85 U/ml and xylanase 4.55 U/ml); D_{EC2} (basal diet+enzyme C (amylase 0.25 U/ml, protease 7.7 U/ml and xylanase 9.1 U/ml). Enzyme A was mixed with basal diet, B and C were added via drinking water. The amount of commercial enzyme being used in this study was determined on the basis of previous experiments *in vivo* and *in vitro* using corn-mungbean base diet.

The basal diet was mixed, pelleted, air dried (ambient temperature) and crumbled and each treatment diet was kept in separated and labeled clean plastic drums for feeding. The protein level of the diets were approximately 22.65 % with ME of 3200 Kcal/kg. A total of 80 Lohman MB-202 one day old unsexed broilers with average initial uniform body weight of 43-44 g were allocated randomly into 8 treatment diets with 5 replicates and each replicate consisted of 3 chicks. The chicks were kept in a warm brooder and given *ad libitum* access to the diet and drinking water during 7 days experiment. We study the effect of the diets during the first week/pre-starter period only as it affect the performance of subsequent period. During the first week, broilers growth reach more than four times its initial body weight (Murwani, 2010a). Chicks were vaccinated by ND La Sota vaccine (PT. Medion Indonesia) on day-4 via eye drop.

Feed intake, feed conversion ratio (FCR) and body weight: Feed intake of each replicate chicks was calculated by the difference between daily feed given and residual feed. Feed conversion ratio was calculated from feed intake divided by final body weight. Each replicate chicks was weighed weekly on 5 kg scale.

Dry matter digestibility: Dry matter digestibility was calculated from the following equation:

$$\frac{\left[\begin{array}{c}\text{Feed intake x}\\ \text{dry matter of diet}\end{array}\right]\times\left[\begin{array}{c}\text{Weight of excreta x}\\ \text{dry matter of excreta}\end{array}\right]}{\text{(Feed intake x dry matter of diet)}}\times 100\%$$

Dry matter of feed and excreta was analyzed by drying samples at 105°C until constant weight was obtained.

***In vitro* digestibility determination:** *In vitro* digestibility method was developed and optimized according to published papers and modified to upscale the amount of sample diet to be tested (Zyla *et al*., 2000; Wu *et al*., 2004). In short, 1 g of each sample diet was incubated in distilled water pH 5 at 40°C for 60 min. Three mg of pepsin and 1.5 M HCl solution (1-1.5 ml) were added into the test tube to reach pH 2 and incubated for 3 h. Subsequently 1 ml of 1 M $NaHCO_3$ was added to make the pH of the mixture 7.5 followed by addition of 4 mg pancreatin and incubated further for 18 h. The mixture was finally centrifuged at 4,000 rpm and supernatant was collected. The final residue was dried at 60°C for 4 h and kept in desiccator and weighed. Dry matter digestibility was calculated from the following equation:

$$\frac{\left[\begin{array}{c}\text{Initial dry}\\ \text{matter of diet}\end{array}\right]\times\left[\begin{array}{c}\text{Dry matter of diet after}\\ \textit{in vitro}\text{ digestion}\end{array}\right]}{\text{Initial dry matter of diet}}\times 100\%$$

Dry matter of diet was analyzed by drying samples at 105°C until constant weight was obtained.

Experimental design and statistical analysis: A complete randomized design with 8 treatments and 5 replicates was employed. Each replicate consisted of 3 chicks. All data were analyzed by ANOVA and Duncan's multiple range test was used when means were significantly different ($p<0.05$). To determine the correlation between *in vivo* and *in vitro* DMD the data were analyzed by SPSS partial correlation test.

RESULTS

Feed intake, BW gain, FCR, *in vivo* digestibility: The results on Table 2 showed that multi-enzymes addition had no effect on feed intake, BW gain and FCR ($p>0.05$) of all groups. Enzymes addition also did not improve in vivo digestibility of dry matter. Enzymes addition in D_{EB2} (basal diet+1% enzyme B (endo-1,4-β-xylanase 5500 visco units dan endo-1,3/4-β-glucanase 500 AGL units/ml)) reduced digestibility of DM.

***In vitro* digestibility test:** In contrast to *in vivo*, *in vitro* dry matter digestibility for basal diet plus multi-enzymes were higher than basal diet (D_C) except in D_{EA1} and D_{EA2}. D_{EB1} and D_{EC2} were highest among treatments. There was no correlation between *in vivo* and *in vitro* dry matter digestibility, except that in D_{EA2} it had a negative correlation (Table 3).

DISCUSSION

Alternative feed ingredients for broilers such as in our study with corn-mungbean based diet provide flexibility according to geographic potential for developing broiler diet to empower diversity and local resources. In regard

Table 1: Composition and nutrient contents of experimental diets

Feed ingredients	Dc (%)
Corn	33.8
Rice bran	3.15
Mungbean	38.2
Soybean	4
Sorghum	5
Fish meal	12
Palm oil	0.75
Vitamins, minerals and prebiotic	1.7522
Lysine, Methionine and Choline	1.35
Total	100
Nutritive values	
[a]ME (Kcal/kg)	3132.24
Crude protein (%)	22.65*
Crude lipid (%)	4.55*
Crude fiber (%)	3.72[b]
[c]Total methionine (%)	0.70
[c]Total lysine (%)	2.16
[c]Total choline (%)	0.12
[d]Total Ca (%)	1.21
[d]Total P (%)	0.87

** : Dc = control (basal) diet

* : Proximate analyses

[a] : Calculated value by Balton formula

[b] : Calculated based on local feed composition table (Hartadi *et al*., 1986) or proximate analysis

[c] : Calculated based on feed composition table and known supplemented lysine, methionine and choline

[d] : Total calcium and phosphor in the diet were analyzed by AAS and Spectrophotometer respectively (AOAC, 1984)

to our studies of corn-mungbean base broiler diet, technological assessment to improve the availability of nutrients especially for broiler remains important and necessary to improve the performance. In this study we tested biotechnology products i.e., feed enzymes to improve digestibility. We used commercial multi-enzymes (A and B) and our own multi-enzyme product (C) to determine their effectiveness to improve dry matter digestibility of corn-mungbean base diet.

Our results showed that the performance of pre starter broilers (feed intake, body weight gain and FCR) on basal diet with multi-enzymes addition were similar to basal diet without enzymes. Our results were similar to other research which found that enzyme supplementation of corn-based diet did not alter feed intake and weight gain until day 7, as well as day 35 compared to commercial control diet (Baurhoo *et al*., 2011). Our findings were also in agreement with other reports that enzyme supplementation in corn-based diets did not alter average daily gain and feed conversion (Aftab, 2009; Madrid *et al*., 2010). In contrast, several reports showed significant improvement in body weight and feed conversion due to enzyme supplementation in corn-based diets (Zanella *et al*., 1999). Different results might indicate inconsistent effect of enzyme supplementation in corn-based diets on broiler performance. The differences could be attributed to various factors such as the types and activity of enzymes studied, feed ingredients and formulations, chicks age, management practices (Baurhoo *et al*., 2011), or their interaction.

Our study showed that addition of different exogenous feed enzymes could not improve yet dry matter digestibility of corn-mungbean diet in pre-starter broiler. There were several possibilities for these results. The first possibility might be due to pre-treatment of the mungbean and soybean (soaking and heating) which could already reduce the amount of antinutrients (Egounlety and Aworh, 2003; Mubarak, 2005). Pre-treatment of mungbean could also reduce NSP content by their diffusion into the water (Mubarak, 2005). Another possibility might be due to the different specificity of each multi enzyme product. The enzymes may not contain enough activity and specificity to the available substrates in corn-mungbean base diet to give improvement in digestibility. In addition, enzymes combination as well as the dosage may bring different effect on the available substrates (Slominski, 2011). Our finding were in line with other report that found no significant effect of commercial enzyme addition on ileal digestible energy, protein and amino acids digestibility in broiler chickens fed corn-based starter diet (Yegani and Korver, 2013). Another possibility could be due to the age of broilers which were still in early phase of growth. The presence of NSP did not suit with the gastrointestinal tract development phase of pre-starter broilers.

In contrast to *in vivo*, *in vitro* dry matter digestibility for basal diet plus multi-enzymes was higher than that without enzymes (Dc). These results were also similar with other *in vitro* studies which reported that a combination of xylanase and cellulase increased total sugar released and reducing the relative viscosity of broiler starter diet (Malathi and Devegowda, 2001). The combination of carbohydrase could also improve *in vitro* digestibility of protein and dry matter of soybean meal, while protease inhibited the activity of carbohydrase (Saleh *et al*., 2003). In addition, when soybean meal was included at higher levels in feed, a multicarbohydrase consisting of xylanase+cellulase+pectinase+β-glucanase was better than xylanase+cellulase alone (Malathi and Devegowda, 2001). On the contrary, another *in vitro* study showed that any single enzyme and their combination did not have significant effects on dry matter digestibility of corn soybean meal mixture, although significantly increasing the digestibility of protein (Saleh *et al*., 2004). It appeared that enzymes mixture did not automatically work synergistically. Correlation analyses between in vivo and *in vitro* dry matter digestibility in this study showed no correlation. This is in contrast with other study which reported that the *in vitro* assay accurately predicted the *in vivo* and ileal digestibility of dry matter and intestinal viscosity in broilers (Bedford and Classen, 1993; de Coca-Sinova *et al*., 2008). The difference could be due to the *in vitro* system which we

Table 2: Feed intake, body weight gain, feed conversion ratio and dietary digestibility of mungbean diet with or without multi-enzymes

Performance	D_C	D_{EA1}	D_{EA2}	D_{EB1}	D_{EB2}	D_{EC1}	D_{EC2}
Feed intake (g)	100.93±9.42	102.53±11.07	91.50±10.65	97.23±6.76	95.90±14.15	86.60±7.25	101.33±11.91
Body weight gain (g)	128.87±7.91	128.13±6.96	122.70±11.00	127.73±6.62	137.10±17.17	127.00±5.51	133.70±13.91
Feed conversion ratio (%)	0.78±0.06	0.80±0.10	0.75±0.06	0.76±0.04	0.70±0.07	0.68±0.05	0.76±0.05
In vivo dry matter digestibility (%)	78.78±2.56^{a}	78.81±2.25^{a}	76.17±1.99^{ab}	77.59±3.39^{a}	73.37±1.71^{b}	76.94±3.78^{ab}	77.57±0.92^{a}
In vitro dry matter digestibility (%)	42.29±1.78^{c}	44.14±1.32^{c}	43.01±1.05^{c}	56.73±0.88^{a}	52.27±3.70^{b}	51.70±1.40^{b}	57.53±1.18^{a}

Table 3: Correlation between *in vitro* and *in vivo* DMD

---------- Correlation ----------	
In vivo DMD	*In vitro* DMD
D_C	non sig.
D_{EA1}	non sig.
D_{EA2}	sig. (-)
D_{EB1}	non sig.
D_{EB2}	non sig.
D_{EC1}	non sig.
D_{EC2}	non sig.

Non sig: Non significant, sig (-): Significant with negative correlation

employed which did not simulate the effect of diet passage rate, viscosity, microbial fermentation, continuous supply of enzyme as digestion feed back system and removal of digestion (absorption) product from the system (Weurding *et al*., 2001). Further study of multi-enzymes to elucidate their effectiveness as feed additive to improve corn-bean base diet is underway.

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