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INTRODUCTION

In relation to poultry feed technology for higher achievement of productive efficiency, some illustration concerning the physically particle size of feed in general are provided as a comparison prior to describing protein microparticle in particular. It have been reported previously elsewhere (Amerah *et al.*, 2008; Svihus, 2011) that particle size was correlated with gizzard development and digestive activity. Large particles of mash diet promoted broiler performance (Amerah *et al.*, 2007b), but the effect of particle size on performance was controversial when pelleted diets were fed. Jacobs *et al.* (2010) reported that apparent metabolizable energy (AMEn) and weight gain of 21-day-old broilers were lowered by feeding diets containing corn with particle size more than 1387 μm . However, Amerah *et al.* (2007a) did not observe any differences of either AMEn or digesta passage rate between birds given diets formulated with medium (3 mm) or coarse (7 mm) particle size. The effect of particle size may be related to the type of feedstuff evaluated. Ganzer *et al.* (2007) observed that reduction in amino acid digestibility by 6.3% in diets formulated with finely-ground corn (0.62 mm) compared to coarsely ground corn (0.96 mm).

However, since the increase in price of dietary protein sources both fish meal and soybean meal has forced poultry nutritionist to find an alternative way to improve nutrients utilization efficiency. Reduction of particle size provides some advantages in nutrients utilization and efficiency to support productivity of growing poultry. Particle size reduction bring about the increase in both the number of particles and the surface area per unit volume allowing greater access to digestive enzymes (Huang and Stein, 2016). The interest in dietary protein particle size has increased due to the economical purposes of optimizing protein utilization and improving poultry production efficiency. Special concern in the present study was focused on the processing procedure of proteins of fish meal and soybean meal. Fish meal and soybean meal are the two

common protein sources in poultry feeding. Fish meal as animal protein source is produced from different types and varieties of fish and may caused much variation of protein and amino acid contents. When fish meal was not properly stored, its protein content can be broken down and resulting biogenic amines such as histamine at higher level, and may caused gizzard erosion in chickens (Jacob, 2013). In case of soybean meal, the limitation use for poultry is associated with the imbalance essential amino acids content and high in trypsin inhibitor level. (Zollitsch *et al.*, 1993; Zhu *et al.*, 1996;). Processing can markedly influence the digestibility of amino acids in soybean meal due to reduced trypsin inhibitor. Soybean meal is known to be rich source of lysine, tryptophan, threonine, isoleucine, and valine, but containing low sulfuric amino acids, such as methionine and cystine (Banaszkiewicz, 2011).

Producing protein microparticle obtained from fish meal and soybean meal by transducer ultrasound treatment is the way to fulfill the purpose of improving protein and/or amino acids utilization. However, in relation to the degree of particle size, the birds may encountered difficulties in consuming very course or very fine particles (Pacheco *et al.*, 2013), thus pelleting would be a solution can be applied when feeding microparticle protein. It was previously reported that the better performance of broilers fed pelleted diets relative to those fed mash diets is well known in poultry production (Amerah *et al.*, 2007b; Dozier *et al.*, 2010). The result is associated with the higher dietary density and better digestibility due to changes in feed physical form resulting from processing, and reduced feed waste and increased energy efficiency. Zang *et al.* (2009) indicated that better performance of broilers receiving pelleted feeds is associated with the higher AME availability, better protein digestibility, as well as improved intestinal function. Performance improvement due to pelleting is more evident in fast growing poultry. For example, pellet length between 2.5 and 3.0 mm could be categorized as appropriate size in relation to growth and feed conversion in broiler

starters (Quentin *et al.*, 2004; Cerrate *et al.*, 2009; Abdollahi and Ravindran, 2013). However, pellet size with either 5 or 7 mm in length was reported to be suitable for broiler finishers (Abdollahi and Ravindran, 2013). Pelleting compensates the effects of dietary small particle sizes, because of particle size distribution need to be uniform, and reduced energy expenditure during digestion. Therefore, the present study was conducted to evaluate microparticle protein derived from fish meal and soybean meal in the forms of pellet and mash compared to intact sources.

MATERIALS AND METHODS

Microparticle Protein Preparation

Fish meal and soybean meal, as common protein sources for poultry, with their chemical composition and amino acid contents (Table 1) were ground to obtain reduced particle size. A slightly modified method of Jambrak *et al.* (2014) was adopted to obtain microparticle protein. The intact particle was then dissolved in distilled water with the ratio of 1 : 4 (w/v), and it was further added with 2 mL virgin coconut oil for every 500 mL solution prior to ultrasound transducer or ultrasonic bath treatment (Power Sonic 405, Hwashin Technoly Co., Korea). The ultrasonic bath treatment with the system of sound wave from the transducer radiate to the liquid at 40Hz was run for 60 min to obtain protein particle dispersion. In order to avoid protein damage in particular, solid component was firstly oven-dried at 40°C, and continued to sun-dried. Particle size determination was performed using particle size analyzer equipment (Nano Particle Analyzer SZ-100, Horiba Ltd., Japan), and particle size detected in average was 1.213 and 0.426 microns for fish meal and soybean meal, respectively.

Experimental Animal and Design

A total of 190 birds of one-day old sexed broiler of Lohman strain, with equal amount of male and female, were kept at a brooder cage prior to feeding microprotein test ingredient. When the birds were 21-day old they were allocated into individual cage (battery) by deviding into 2 criterions, namely 180 birds were used for forced feeding and 10 other birds were plotted and fasted for endogenous correction. Forced feeding procedure was performed when the birds were one and a half month old (45-day old) with body weight of 2475.5 ± 302.1 g. A completely randomized design with 3 treatments

(intact, mash, and pellet) and 6 replications (10 birds each, 5 males and 5 females) was arranged in the present study for the respective protein source, namely fish meal and soybean meal. Microparticle protein of fish meal and soybean meal were tested separately in either mash or pelleted forms and compared to their intact protein.

Parameter and Statistical Analysis

Parameters measured were digestibilities of protein and selected essential amino acids (8 amino acids), and calcium (Ca) retention. Digestibilities of protein and amino acids, and Ca retention were measured based on wet forced feeding method adopted from the simply modified procedure of Lessire (1990). Each bird was force fed with approximately 50 g air dry tested ingredient that was moistened first with distilled water with the ratio of 1 : 2 (w/v). Birds were fasted for 24 h to completely emptying the digestive tracts prior to forced feeding was performed. Fecal digestibilities of protein and amino acids, and Ca retention as well were calculated from the totally collected excreta. Digestibilities of protein (Pr) and amino acid (AA) were corrected with respective endogenous value obtained from group of fasting birds with the following formula (McNab, 1994) :

$$\text{Pr or AA digestibility (\%)} = \frac{[\text{PrC or AAC} - (\text{PrE or AAE} - \text{Epr or EAA})]}{[\text{PrC or AAC}]} \times 100 \dots (1)$$

Where:

- PrC = protein consumed
- AAC = amino acid consumed
- PrE = protein in excreta
- AAE = amino acid in excreta
- EPr = endogenous protein
- EAA = endogenous amino acid

Calcium (Ca) retention was calculated according to the formula for mineral retention in general (Thomas and Ravindran, 2010) with slight modification as follows:

$$\text{Ca retention (\%)} = \frac{[(\text{Ca consumed} - \text{Ca excreta}) / \text{Ca consumed}] \times 100}{\dots} \dots (2)$$

Data were subjected to statistical procedure of analysis of variance to evaluate treatments effect. When the treatment showed significant effect, it was continued to Duncan test (Steel and Torrie, 1981) to determine the difference between

Table 1. Chemical Composition and Amino Acid Contents of Fish Meal and Soybean Meal

Nutritional parameters	Ingredients	
	Fish Meal	Soybean Meal
Chemical compositions*		
Crude protein	42.14	40.77
Ether extract	12.47	19.54
Crude fiber	1.85	3.02
Ash	24.11	5.08
Calcium (Ca)	4.20	0.35
Phosphorus (P)	2.92	0.57
Amino acid contents*		
Arginine	3.65	2.01
Isoleucine	2.36	1.15
Leucine	3.66	2.03
Lysine	2.58	1.83
Methionine	1.33	0.54
Phenylalanine	3.11	1.51
Threonine	1.37	1.06
Valine	2.73	1.62

*Laboratory analysis value (2017)

mean values.

RESULTS AND DISCUSSION

Protein and amino acid digestibilities, and calcium (Ca) retention in either pelleted microparticle protein of fish meal (Table 2) or soybean meal (Table 3) significantly increased. In contrast, some amino acid digestibilities such as arginine, isoleucine, phenylalanine, and threonine of mash microparticle protein of fish meal were significantly ($P < 0.05$) lower than those of intact and pelleted forms (Table 2). Similar case was also found in mash microparticle protein of soybean meal for isoleucine, lysine, phenylalanine, and valine digestibilities (Table 3). In general, protein and amino acid digestibilities, and also Ca retention of pelleted microparticle protein of fish meal and soybean meal indicated the highest values as compared to other forms.

Protein digestibility of mash form of both

ingredients did not also better than that of intact protein. There was no improvement in digestibilities of protein as well as some amino acids of mash formed ingredient is possibly due to the unbound fine particle which caused very fast away from the gastrointestinal tract. However, pelleted diets are assumed to have longer spent time in the intestine. It was reported previously that the better performance of broilers fed pelleted diets compared to those fed mash diets is well known in poultry production (Amerah *et al.*, 2007b; Dozier *et al.*, 2010). In other side, it has been reported elsewhere that the smaller particle size of either feed ingredients in general (Gabriel *et al.*, 2003; Amerah *et al.*, 2007a; 2008; Chewing *et al.*, 2012) or protein in particular (Jambrak *et al.*, 2014; Huang and Stein, 2016) allowing the greater access to digestive enzymes which then increased nutrients digestibility. Reduce particle size of protein with pelleted form would be the best choice as applied for poultry by considering that birds may encounter difficulties in consuming very coarse or very fine particles of feed. Decreasing particle size could improve the DE and ME of wheat in finishing pigs (Fan *et al.*, 2017), and pelleted corn particle size increased growth performance in growing broilers (Parsons *et al.*, 2006). Zang *et al.* (2009) indicated that better performance of broilers receiving pelleted feeds is associated with the higher AME availability, better protein digestibility, as well as better intestinal function. Pelleting compensates the effects of dietary small particle sizes, because the frequency of particle size distribution is uniform and energy expenditure is reduced during digestion. The more efficiency of pelleted diet has been proved by Suthama and Wibawa (2017), that the increased protease activity could be associated with higher protein digestibility in pelleted-diet fed broiler.

The higher nutrients digestibility of pelleted fish meal can be associated with protein particle size was 3 to 4 fold larger than soybean meal (Suthama and Wibawa, 2016), and related to chemical properties. The differences in results between fish meal and soybean meal is assumably due to the presence of phytate component such as oligosaccharide and anti-trypsin in soybean meal. The inferior results found for soybean meal was seemingly attributable to the presence of phytate compound (Banaszkiewicz, 2011; Tahir *et al.*, 2012), non-protein soluble component such as oligosaccharide (Kocher *et al.*, 2002; Oliveira and Stein, 2016), and trypsin inhibitor

Table 2. Protein and Essential Amino Acids Digestibilities of Microparticle Protein Derived from Fish Meal in Broiler Chicken

Parameter	Intact protein	Microparticle protein	
		Mash	Pellet
Fish Meal			
Protein digestibility (%)	81.4 ^b	82.8 ^b	87.0 ^a
Amino acids digestibility (%)			
Arginine	78.0 ^b	75.6 ^c	84.9 ^a
Isoleucine	86.4 ^b	84.0 ^c	88.8 ^a
Leucine	87.2 ^a	85.5 ^b	88.1 ^a
Lysine	76.9 ^b	77.7 ^b	81.7 ^a
Methionine	77.3 ^b	75.0 ^b	88.4 ^a
Phenylalanine	65.5 ^a	63.7 ^b	66.3 ^a
Threonine	82.4 ^a	78.0 ^b	81.6 ^a
Valine	77.4 ^b	76.9 ^b	81.0 ^a
Ca retention (%)	62.9 ^b	63.3 ^b	69.1 ^a

^{a-c}Mean values within row followed by different superscripts are significantly different (P<0.05)

(Banaszkiewicz, 2011; Pacheco *et al.*, 2013). Phytate compound in soybean meal has been reported to be varied with the range of between 1 and 1.5% (Banaszkiewicz, 2012). Phytic acid may provide some advantages in binding affinity for calcium and protein which is beneficial in tofu-making, but this reactive mechanism has adverse effect on Ca and protein absorption in monogastric animals, especially poultry. This phenomenon might be proved by the data of Ca retention and protein digestibility of pelleted soybean meal (Table 3) were lower than those of similar form of fish meal (Table 2), although it has been treated with ultrasound transducer. Thus, longer ultrasound transducer treatment is needed for soybean meal to maximize nutrients utilization. Soluble carbohydrates and trypsin inhibitor can interfere with protein and/or amino acids utilization, therefore, protein and some amino acids digestibilities were still lower for soybean meal.

The present results can also be connected with the digestive enzyme activity which was specific for protein and a certain amino acid (Krehbiel and Matthews, 2003; Ren *et al.*, 2014).

It may be comparable with the report of Suthama *et al.* (2014) indicated that nitrogen (N) retention was improved without any change in intestinal protease activity although with different dietary protein content. This implied that the work of intestinal protease depended on the portion of ingredients-containing non protein soluble carbohydrate used in the diet. In some cases, digestibility of individual amino acid is found to be similar pattern to that of protein, and other phenomenon indicates that higher protein digestibility is not always followed by similar level of individual amino acid digestibility. Similar report by Ravindran (2013) that a given substrate or nutrient of a certain feedstuff was not completely the same as found in other ingredient. Therefore, similar nutrient of the different ingredient showed the different response to digestive enzyme. It was supported by the finding of Moughan *et al.* (2014) that the variation of individual amino acid digestibility was smaller among grains or its by products. A comparison of individual amino acid digestibility between animal protein sources, blood meal and fish meal, are the interesting example. Blood meal has

Table 3. Protein and Essential Amino Acids Digestibilities of Microparticle Protein Derived from Soybean Meal in Broiler Chicken

Parameter	Intact protein	Microparticle protein	
		Mash	Pellet
Soybean meal			
Protein digestibility (%)	80.6 ^b	82.0 ^b	86.1 ^a
Amino acids digestibility (%)			
Arginine	80.0 ^b	79.4 ^b	88.8 ^a
Isoleucine	74.8 ^a	69.4 ^b	73.8 ^a
Leucine	81.8 ^c	84.5 ^b	89.7 ^a
Lysine	81.0 ^a	75.0 ^b	79.6 ^a
Methionine	61.1 ^b	62.0 ^b	69.3 ^a
Phenylalanine	79.2 ^a	76.6 ^b	80.3 ^a
Threonine	73.8 ^c	78.6 ^b	86.3 ^a
Valine	71.8 ^b	67.4 ^c	77.9 ^a
Ca retention (%)	56.2 ^b	55.9 ^b	60.2 ^a

^{a-c}Mean values within row followed by different superscripts are significantly different (P<0.05)

higher amino acid digestibility with smaller variation than fish meal. This provides the important meaning that fish meal has large variation for individual amino acid digestibility.

CONCLUSION

It can be concluded that pelleted form of either fish meal or soybean meal improve protein and amino acids digestibilities, and Ca retention in broiler, although some individual amino acids digestibility of mash form decrease.

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