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SHORT COMMUNICATION

Apparent digestibility of soybean meal and *Lactobacillus* spp. fermented soybean meal in diets of grouper, *Epinephelus coioides*

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Grouper are popular and high value marine fish in many parts of the world. They are also good candidates for intensive aquaculture because of their desirable taste, hardiness and rapid growth. Global grouper aquaculture production increased dramatically in recent years, recorded as 5480, 9467, 60,524 and 94,832 metric tonnes in 1990, 2000, 2005 and 2011 respectively (FAO 2014).

Aquafeed is the most expensive cost item, often ranging from 50% to 60% of the total variable expenses. The major protein ingredients are largely dependent on fish meal. While there is no doubt of the high-nutritional value of fish meal, the best quality meals are expensive, and according to some projections, their availability is expected to decline and the price will dramatically increase. Therefore, there is a need to identify and utilize less expensive and more sustainable protein sources within aquafeeds. Soybean meal, are the most widely used plant ingredient for aquaculture industry. However, the use of soybean meal is limited because of its unbalanced amino acid composition and the presence of a wide variety of antinutritional compounds (Francis, Makkar & Becker 2001).

Traditionally in Asia, the quality of soybean meal can be improved by fermentation of microorganisms, such as *Lactobacillus* spp., *Bacillus* spp. and *Aspergillus* spp. Both antinutritional factors and protein molecular weight of soybean meal have been demonstrated to decrease by the fermented processing (Kim 2004). Fermented soybean meal is widely applied in domestic animals. However, the information in aquatic animals is still limited (Zhou, Song, Shao, Peng, Xiao, Hua & Owari 2011). Digestibility data are very important to evaluate a new feed ingredient for culture species. Hence, the present study was aimed to determine dry matter and protein digestibility of fermented soybean meal in diets and compare with soybean meal and fish meal, for grouper.

The formulation of reference and test diets for grouper was shown in Table 1. Test diet contained 700 g kg^{-1} of reference diet and 300 g kg^{-1} test ingredients, including brown fish meal (Pesquera Diamante, Peru), soybean meal (TTET Union, Taiwan) and *Lactobacillus* spp. fermented soybean meal (DaBomb Protein, Taiwan). Both reference and test diet was supplemented with 5 g kg^{-1} chromium oxide (Cr_2O_3, Sigma Chemical, St. Louis, MO, USA) as inert indicator. The ingredients of the experimental diet were mixed, cold water was added to the mixture, and then the mixture was mixed further until a stiff dough resulted. The dough was then passed through a mincer with dye, and the resulting strands were dried using an electrical fan at 20°C. After drying, the strands were broken up, sieved into pellets (1.2 mm in diameter) and stored at −20°C until they were needed.

Our study, involving animal experimentation, conformed to the principles for the use and care of laboratory animals, in agreement with the Institutional Animal Care and Use Committee (IACUC) in NPUST.

Table 1 Formulation of reference and test diets

Ingredients (g kg^{-1})	Reference diet	Test diet
Fish meal	615	431
Corn oil	30	21
Squid liver meal	50	35
Scallop meal	50	35
Gelatinized starch	150	105
Wheat gluten	30	21
Vitamin premix*	10	7
Mineral premix†	20	14
Choline chloride	1	0.7
Chromic oxide	5	5
Alpha-cellulose	39	25.3
Test ingredient	–	300

*Vitamin mixture (mg g^{-1} mixture): thiamin hydrochloride, 2.5; riboflavin, 10; calcium pantothenate, 0.5; nicotinic acid, 37.5; pyridoxine hydrochloride, 2; folic acid, 0.05; inositol, 20; L-ascorbyl-2-monophosphate Mg, 5; choline chloride, 250; menadione, 2; alpha-tocopheryl acetate, 5; retinyl acetate, 1; cholecalciferol, 0.0025; biotin, 0.25; vitamin B_{12}, 0.05. All ingredients were diluted with alpha-cellulose to 1 g.

†Mineral mixture (mg g^{-1} mixture): calcium lactate, 327; K_2PO_4, 239.8; $CaHPO_4{\cdot}2H_2O$, 135.8; $MgSO_4{\cdot}7H_2O$, 132; $Na_2HPO_4{\cdot}2H_2O$, 87.2; NaCl, 43.5; ferric citrate, 29.7; $ZnSO_4{\cdot}7H_2O$, 3; $CoCl_2{\cdot}6H_2O$, 1; $MnSO_4{\cdot}H_2O$, 0.8; KI, 0.15; $AlCl_3{\cdot}6H_2O$, 0.15; $CuCl_2$, 0.1.

Juveniles grouper, *Epinephelus coioides* obtained from local hatchery (Pingtung, Taiwan) were used in the study. Upon arrival, the fish were acclimated to laboratory conditions for 3 weeks in a 3500-L FRP tank. At the beginning of the experiment, 20 fish (mean initial weight ± SD: 22.01 ± 0.08 g) were stocked in each of 12 FRP tanks (300 L). Each tank was part of a closed recirculating system with seawater at 30–32 ppt salinity. The system was constructed with a common filter, biofilter, protein skimmer and UV light to maintain the water quality. Each experimental diet was assayed in triplicate [4 diets (reference diet and 3 test diets) × 3 tanks]. The diets were randomly assigned to groups of fish. The water temperature of the rearing system was controlled at 28 ± 1°C.

Fish were fed 40 g kg^{-1} of their body weight per day. The daily ration was divided into two equal meals (09:00 and 17:00 hours). The uneaten feed and faecal residues were removed at 10:00 hours and the faeces were collected at 11:00, 12:00, 13:00, 14:00 and 15:00 hours by faeces collection column. Feed and faeces were analysed the proximate composition by the Association of Official Analytical Chemists (A.O.A.C.) (1995) method and chromium (Cr) concentration by atomic absorption spectrophotometer (ZA-3000; Hitachi Co., Tokyo, Japan). Soluble protein fractions and distribution of soybean meal and hydrolysis soy protein were determined according to the method of Chen, Shih, Chiou and Yu (2010) by high performance liquid chromatography (HPLC).

Table 2 Apparent dry matter and protein digestibility (%) of reference diet and tested feed ingredients for grouper

	DM	CP
Reference diet	85.44 ± 1.02^c	95.24 ± 1.10^b
Ingredients		
Fish meal	86.78 ± 1.75^c	95.48 ± 0.81^b
Soybean meal	74.64 ± 0.70^a	90.56 ± 0.49^a
Fermented soybean meal	81.83 ± 0.64^b	93.62 ± 1.02^b

Different superscripts in the column indicate significant ($P \leq 0.05$) difference between different dietary treatments. Values are means ± SD from three groups of grouper fed on a same diet (n = 3 tanks).

The calculation of apparent digestibility coefficient (ADC) was shown as below:

$$\mathrm{ADC-DM}(\%) = 100 \times (\%\mathrm{Cr\ in\ feed}/\%\mathrm{Cr\ in\ feces})$$

$$\mathrm{ADC-CP}(\%) = 100 \times [1 - (\%\mathrm{Cr\ in\ feed}/\%\mathrm{Cr\ in\ feces}) \times (\%\mathrm{CP\ in\ feces}/\%\mathrm{CP\ in\ feed})]$$

$$\mathrm{ADCi} = \mathrm{ADCt} + [(0.7 \times \mathrm{Nr})/(0.3 \times \mathrm{Nt})] \times (\mathrm{ADCt} - \mathrm{ADCr})$$

DM, dry matter; CP, crude protein; i, test ingredient; t, test diet; r, reference diet; N, nutrient composition

The results showed that the ADC of dry matter was the highest in reference diet and fish meal, followed by fermented soybean meal and lowest in soybean meal for grouper (Table 2). The ADC of protein was the higher in reference diet, fish meal and fermented soybean meal than that in soybean meal. The results suggested that fermented process of microorganism (*Lactobacillus* spp.) can improve dry matter and crude protein digestibility of soybean meal for grouper. Microorganism can excrete protease during fermentation to partially hydrolyse the protein of soybean meal and then degrade molecular weight (Kim 2004). Yan, Wang and Kim (2012) found that nitrogen digestibility of

Lactobacillus spp. fermented soybean meal was higher than that of *Aspergillus oryzae* fermented soybean meal in pigs. The reason for this difference was likely to be the extensive hydrolysis of protein with *Lactobacillus* spp. fermentation in comparison to those with *A. oryzae* fermentation. Yuan, Gong, Yang, Lin, Yu and Luo (2010) also found the protein digestibility of soybean meal in diet (83.2%) for Chinese sucker can be improved to 94.0% through the processing of fermentation. This finding is in agreement with our present study. However, the apparent digestibility of soybean meal and fermented soybean meal were similar for tilapia (Dong, Guo, Ye, Song, Huang & Wang 2010) and white shrimp (Yang, Zhou, Zhou, Tan, Chi & Dong 2009). This might be due to the different fermented soybean meal sources or species variation.

Figure 1a showed the molecular weight of soluble protein fraction in soybean meal mainly distributed at 30–70 kDa (66.7%), whereas the molecular weight of fermented soybean meal mainly distributed at ≤30 kDa (75.39%; Fig. 1b). It clearly indicated that the molecular weight of soybean meal protein really degraded by the *Lactobacillus* spp. fermentation. Hence, higher digestibility of hydrolysis soy protein than soybean meal is expectable. Moreover, we also found the small

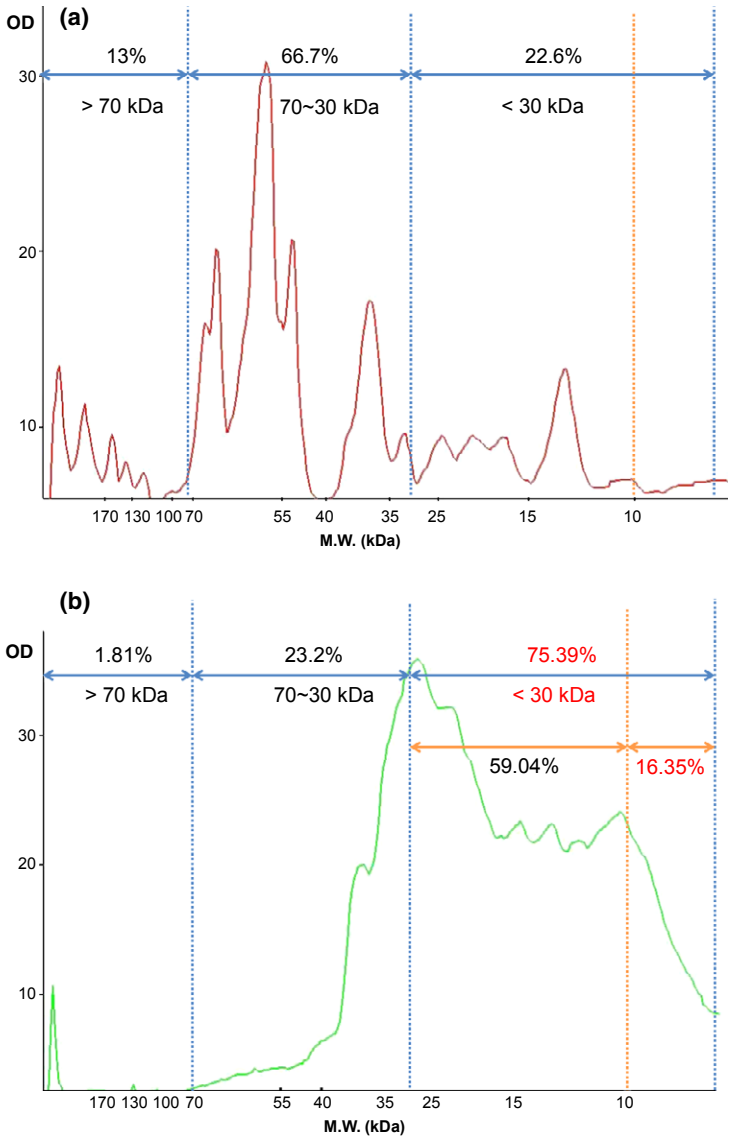

Figure 1 The protein fraction and distribution of (a) soybean meal; (b) fermented soybean meal.

peptides (M.W. <10 kDa) is about 16.35% of total peptides in fermented soybean meal.

In addition, the organic acid (lactic acid) concentration determined in fermented soybean meal was about 60 g kg^{-1} (unpublished data). It has been reported that dietary citric acid and lactic acid supplementation enhanced the utilization of iron and phosphorus by rainbow trout (Vielam, Ruohonen & Lall 1999; Pandey & Satoh 2008). Zhou, Liu, He, Shi, Gao, Yao and Ringo (2009) reported that potassium diformate supplementation in diets can improve intestinal microflora in hybrid tilapia. Ng, Koh, Sudesh and Siti-Zahrah (2009) indicated that Nile tilapia fed diets with potassium diformate showed high growth and nutrient utilization. Atlantic salmon fed diets with lactic acid increased intestinal protease activity (Ringo, Olsen & Castell 1994). In the present study, grouper fed diets with fermented soybean meal showing high dry matter and protein digestibility may be attributed to the lactic acid produced by the *Lactobacillus* spp. fermentation. The other function derived from organic acid, such as growth promotion, nutrient utilization and intestinal microflora, must be confirmed in the future study.

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