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Fermentation by *Lactobacillus* spp. can Improve Apparent Digestibility and Hydrolyze the Protein of Soybean Meal for White Shrimp *Litopenaeus vannamei*

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ABSTRACT

The apparent digestibility coefficients (ADCs) of dry matter and protein of soybean meal and *Lactobacillus* fermented soybean meal in diets for white shrimp, *Litopenaeus vannamei*, were determined by using 0.5% Cr_2O_3 as inert indicator. Fish meal was used as comparison. The test diets were formed by a combination of 30% test ingredients and 70% reference diet. There was a total of 4 experimental diets (a reference and 3 test diets), and each was fed in triplicate to groups of shrimp (mean weight: 3.93 ± 0.12 g) in a seawater recirculating system. The ADCs of dry matter for the shrimp was higher ($p < 0.05$) in fish meal and fermented soybean than that in soybean meal. The ADC of protein was the highest in fish meal, followed by fermented soybean, and lowest in soybean meal. Molecular weight of soluble protein fraction in soybean meal and fermented soybean meal were mainly distributed at 30 – 70 kDa (66.7%) and ≤ 30 kDa (75.39%), respectively. The results showed that soybean meal fermented with *Lactobacillus* spp. can hydrolyze the protein and improve their ADCs of dry matter and protein for *L. vannamei*.

***Key words**: digestibility, white shrimp, soybean meal, fermented soybean meal.*

1. INTRODUCTION

White shrimp, *Litopenaeus vannamei* is the most important cultured shrimp species in the world. The annual aquaculture production of white shrimp has increased from 8,286 MT in 1980 to 3,178,721 MT in 2012 (FAO, 2014). Besides fast growth, white shrimp can well utilize more plant ingredients compared to other shrimp species, such as Kuruma shrimp or tiger shrimp. The major protein ingredients of shrimp aquaculture are largely dependent on fish meal. Whilst 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 shrimp feeds. Soybean meal is the most widely used plant ingredient for aquaculture industry. However, the use of soybean meal has some limitation, for example deficient in methionine and lysine, antinutritional factors such as trypsin inhibitors, hemagglitinin, phytic acid, saponin, phytoestrogen and anti-vitamin (Francis et al., 2001).

In Asia, fermentation was performed traditionally by microorganism like *Lactobacillus* spp., *Bacillus* spp. and *Aspergillus* spp, and this process can improve the quality of soybean meal. It has been demonstrated to decrease antinutritional factors and protein molecular weight of soybean meal processed by fermentation. Fermented soybean meal is widely applied in domestic animals. However, the information in aquatic animals

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is still limited (Zhou et al., 2011). Digestibility is 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 and compare with soybean meal and fish meal for white shrimp.

2. MATERIALS AND METHODS

2.1. Experimental diets

The formulation of reference and test diets for white shrimp was shown in Table 1. Test diet contained 70% of reference diet and 30% test ingredients, including brown fish meal (Pesquera Diamante, Peru), soybean meal (TTET Union, Taiwan) and *Lactobacillus* spp. fermented soybean meal (DaBomb Protein, Taiwan). The proximate compositions of test ingredients were analyzed according to AOAC (1995) and shown in Table 2. Both reference and test diet was supplemented with 0.5% chromium oxide (Cr2O3, Sigma Chemical, St. Louis, MO, USA) as inert indicator (Shiau and Chen, 1993). The ingredients of the experimental diet were mixed, cold water was added to the mixture and then the mixture was mixed further until stiff dough resulted. The dough was then passed through a mincer with die, and the resulting strands were dried using an electrical fan at 20°C. After drying, the strands were broken up, sieved into pellets (2.0 mm in diameter) and stored at -20°C until they were needed.

2.2. Experimental procedure

This 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 with approval (NPUST-IACUC-102-039).

Juveniles white shrimp *Litopenaeus vannamei* obtained from local hatchery (Pingtung, Taiwan) were used in the study. Upon arrival, the shrimp were acclimated to laboratory conditions for four weeks in a 3,500-L FRP tank and fed with a commercial diet (Uni-President Enterprise Corp., Tainan, Taiwan). At the beginning of the experiment, 25 shrimp (mean weight: 3.93 ± 0.12 g) were stocked in each of 12 FRP tanks (300 L). Each experimental diet was assayed in triplicate. The diets were randomly assigned to groups of shrimp. Each aquarium was part of a closed recirculating system with seawater at 30 to 32 ‰ salinity. The system consisted of a common filter, biofilter, protein skimmer and UV light to maintain the water

Table 1. Formulation of reference and test diets.

Ingredients (%)	Reference diet	Test diet
Fish meal	55	38.5
Fish oil	1.7	1.2
Squid liver meal	5	3.5
Gelatinized-starch	26	18.2
Gluten	8	5.6
Vitamin mixture	1	0.7
Mineral mixture	2	1.4
Cholesterol	0.05	0.035
Choline chloride	0.1	0.07
Cr_2O_3	0.5	0.5
Alpha-cellulose	0.65	0.0295
Test ingredient	-	30

[1] Vitamin mixture supplied the following (mg/g mixture): pyridoxine HCl, 3.2963 mg; *para*-aminobenzoic acid, 10 mg; retinyl acetate, 0.6222 mg; riboflavin, 0.8333 mg; nicotinic acid, 0.2667 mg; thiamin-HCl, 0.5185 mg; menadione, 1.4815 mg; α-tocopheryl acetate, 10 mg; vitamin B12, 0.0074 mg; cholecaliferol (D3), 0.0037 mg; folic acid, 0.0777 mg; choline HCl, 200 mg; L-ascorbyl-2-monophosphate- Mg, 1.4815 mg; d-biotin, 0.0889 mg; Ca pantothenate, 5.1481 mg; *myo*-inositol, 125.9 mg. All ingredients are diluted with α-cellulose to 1 g.

[2] Mineral mixture supplied the following (mg/kg diet): K_2HPO_4, 100 mg; Na_2HPO_4, 215 mg; $Ca(H_2PO_4)_2 \cdot H_2O$, 265 mg; $CaCO_3$, 105 mg; Ca lactate, 165 mg; KCl, 28 mg; KI, 0.23 mg; $MgSO_4 \cdot 7H_2O$, 100 mg; ferric citrate, 10 mg; $CuCl_2 \cdot 2H_2O$, 0.15 mg; $AlCl_3 \cdot 6H_2O$, 0.24 mg; $MnSO_4 \cdot H_2O$, 1.07 mg; $ZnSO_4$, 1.5 mg; $CoCl_2 \cdot 6H_2O$, 1.4 mg; All ingredients are diluted with α-cellulose to 1 g.

Table 2. Proximate composition (%) of test ingredients.

	Fish meal	Soybean meal	Fermented soybean meal
Moisture	6.52	10.51	7.03
Crude protein	65.68	47.59	51.31
Ether extract	10.41	2.67	3.71
Ash	14.19	5.84	6.23
Crude fiber	ND	3.31	2.48
NFE1	3.2	30.08	29.24

NFE: nitrogen free extract = 100 – moisture – crude protein – ether extract – ash – crude fiber.
ND: not detectable.

quality. The water temperature of the rearing system was controlled at 28 ± 1°C.

Shrimp were fed 6% of their body weight per day. The daily ration was divided into two equal meals (09:00 and 17:00). The uneaten feed and fecal residues were removed at 10:00 and the feces were collected at 11:00, 12:00, 13:00, 14:00 and 15:00 by feces collection column.

2.3. Chemical analysis

Feed and feces were analyzed the proximate composition by the AOAC (1995) method and chromium (Cr) concentration by atomic absorption spectrophotometer (ZA-3000, Hitachi Co., Tokyo, Japan) after wet digestion (Furukawa and Tsukahara, 1996). Soluble protein fractions and distribution of soybean meal and fermented soybean meal were determined according to the method described by Chen et al. (2010) using high performance liquid chromatography (HPLC).

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

ADC of dry matter (%) = 100 × (%Cr in feed/%Cr in feces)
ADC of protein (%) = 100 × [1-(%Cr in feed/%Cr in feces) × (%CP in feces/%CP in feed)]
ADCi = ADCt + [(0.7 × Nr)/(0.3 × Nt)] × (ADCt – ADCr)
(test ingredient; t: test diet; r: reference diet; N: nutrient composition)

2.4. Statistical analysis

Each experimental diet was fed to three groups of fish in accordance with a completely randomized design. The results were analyzed by a one-way analysis of variance (ANOVA). When the ANOVA identified differences among the groups, multiple comparisons were made among the means using Student Newman-Keuls (SNK) test. Statistical significance was determined by setting the aggregate type I error at $p < 0.05$.

3. RESULTS

Shrimp fed different diets had similar growth (from 3.93 ± 0.12 g to 4.68 ± 0.24 g, $p > 0.05$ among all dietary groups, data not shown) during feces collection period. No shrimp died in all dietary treatments.

The results showed that ADC of dry matter was higher ($p < 0.05$) in fish meal and fermented soybean meal than that in soybean meal (Table 3). The ADC of protein was the highest in fish meal, followed by fermented soybean meal and lowest in soybean meal.

The molecular weight of soluble protein fraction in soybean meal and fermented soybean meal were shown in Table 4, and mainly distributed at 30 – 70 kDa (66.7%) and ≤ 30 kDa (75.39%), respectively.

Table 3. Apparent dry matter and protein digestibility (%) of tested feed ingredients of white shrimp.

	Dry matter	Protein
Reference diet	71.87 ± 1.09b	85.56 ± 1.20 c
Ingredients		
Fish meal	72.26 ± 1.12b	86.78 ± 0.19c
Soybean meal	64.79 ± 1.09a	80.44 ± 1.51a
Fermented soybean meal	73.05 ± 1.33b	84.23 ± 0.19b

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

Table 4. The protein fraction and distribution of soybean meal and fermented soybean meal.

	Soybean meal	Fermented soybean meal
Molecular weight (kDa)		
<10	3.5%	16.4%
<30	22.6%	75.0%
30-70	66.7%	23.2%
>70	10.7%	1.8%

The values in the table were analyzed by high performance liquid chromatography and presented as percentage of total peak area in each fraction.

Moreover, we also found the small peptides (< 10 kDa) is about 16.35% of total peptides in fermented soybean meal.

4. DISCUSSION

Our results clearly indicated that fermented process by *Lactobacillus* spp. can improve dry matter and crude protein digestibility of soybean meal in diets for white shrimp. During fermentation, microorganism can excrete protease to partially hydrolyze the protein in soybean meal and then degrade molecular weight of protein (Kim, 2004).

Yan et al. (2012) reported that nitrogen digestibility of *Lactobacillus* spp. fermented soybean meal was better than that of *Aspergillus oryzae* fermented soybean meal in diets for pigs. The authors explained that this is because the hydrolysis ability is greater in *Lactobacillus* spp. than that in *A. oryzae*. Fermented soybean meal also showed higher protein digestibility (94.0%) than soybean meal (83.2%) in diets for Chinese sucker (Yuan et al., 2010). This finding is similar with the present study. However, the apparent digestibility of soybean meal and fermented soybean meal were similar for tilapia (Dong et al., 2010) and white shrimp (Yang et al., 2009). This might be due to the different fermented soybean meal sources or species variation.

The molecular weight of soybean meal protein was really hydrolyzed by the *Lactobacillus* spp. Fermentation (Table 4). Moreover, we found that the small peptides (< 10 kDa) accounted about 16.35% of total peptides in fermented soybean meal. Hence, higher digestibility of fermented soybean meal than soybean meal for white shrimp seems to be expectable.

In addition, the organic acid (lactic acid) concentration determined in fermented soybean meal used in the present study was about 6% (unpublished data). It has been reported that dietary citric acid and lactic acid supplementation enhanced the utilization of iron and phosphorus by rainbow trout (Vielma et al., 1999; Pandey and Satoh, 2008). Potassium diformate supplementation in diets have been demonstrated to enhance intestinal microflora in hybrid tilapia (Zhou et al., 2009), and growth and nutrient utilization in Nile tilapia (Ng et al., 2009). Atlantic salmon fed diets with lactic acid increased intestinal protease activity (Ringo et al., 1994). Based on these researches, it suggests that organic acid supplementation in diet can influence positively digestive function for aquatic animals. In our study, higher digestibility of fermented soybean meal in diet than that of soybean meal for white shrimp may be associated with the lactic acid which 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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豆粉以乳酸菌發酵可提升白蝦對其之表觀消化率並降解豆粉蛋白

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本研究利用0.5%三氧化二鉻作為指示劑測定白蝦對豆粉與乳酸菌發酵豆粉之表觀乾物與蛋白消化率，並以魚粉作為對照。實驗飼料為添加30%測試原料於參考飼料中，實驗及參考飼料均餵予石斑魚，再利用養殖系統附加之糞便收集管收及糞便。實驗分為四組，包括一個參考飼料及三個測試飼料，分別餵予初重3.93 ± 0.12 g之白蝦，飼養於純海水循環系統中，每組三重複。結果顯示各原料之乾物消化率以魚粉與發酵豆粉顯著高於$(P < 0.05)$豆粉；而蛋白消化率以魚粉最高，其次為發酵豆粉，以豆粉最低。當測定豆粉與發酵豆粉可溶性蛋白分子量分佈，顯示豆粉與發酵豆粉主要蛋白分子量分別為30–70 kDa (66.7%) and ≤ 30 kDa (75.39%)。本研究結果顯示豆粉以乳酸菌發酵後，可有效水解其蛋白質，並提升白蝦對其之消化率。

關鍵詞：消化率，白蝦，豆粉，發酵豆粉。

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