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Effect of Dietary Fish Meal Replacement by *Lactobacillus* spp. Fermented Soybean Meal on Growth Performance of Asian Sea Bass, *Lates calcarifer*

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Abstract

The present study was to evaluate the effects of dietary *Lactobacillus* spp. fermented soybean meal protein inclusion on growth performance of Asian sea bass, *Lates calcarifer*. The fermented soybean meal protein was substituted for fish meal protein at 0, 10, 15, 20, 25 and 30%. Total of 6 experimental diets were each fed to triplicate groups of Asian sea bass juveniles (initial weight: 9.2 ± 0.1 g) in 18 indoor concrete-ponds (4 × 3 × 1 m^3) static rearing system with brackish water (10‰) for 6 months. Growth performance was monitored each month. The final weight and feed conversion rate (FCR) of fish fed treatment diets were not significant differences. The results suggested that up to 30% fish meal protein can be replaced by *Lactobacillus* spp. fermented soybean meal without any negative effect on growth performance of Asian sea bass. It clearly demonstrated that fermented soybean meal is potentially used as a fish meal replacer for Asian sea bass.

***Key words**: Asian sea bass, Lactobacillus spp., fermented soybean meal, fish meal, growth.*

1. INTRODUCTION

In aquaculture industry, diet is the most expensive cost item, often ranging from 40% to 60% of the total variable expenses. Proteins are indispensable nutrients for the structure and function of all living organisms including fish (Shiau, 2001). The major protein ingredients for carnivorous species of aquaculture are largely dependent on fish meal. Total global fish meal production has remained relatively static over the past quarter century (FAO, 2015). Therefore, there is a need to identify and utilize less expensive and more sustainable alternative protein sources for fish feeds.

Soybean meal is considered one of the most promising ingredients and widely used as a protein source in aquafeed because of its acceptable amino acid profile, consistent composition, availability and reasonable price (Sookying and Davis, 2012). However, the use of soybean meal is limited because of its insufficient methionine and lysine and the presence of a wide variety of antinutritional compounds (Francis et al., 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 (Zhou et al., 2011). High protein digestibility of *Lactobacillus* spp. fermented soybean meal has been demonstrated in white shrimp (Zhuo et al. 2014) and grouper (Zhuo et al., 2016). Use of fermented soybean meal to replace fish meal for fish growth and immune responses is still unclear. The purpose of the present study was to evaluate the effects of dietary *Lactobacillus* spp. fermented soybean meal replacement of fish meal on growth of Asian sea bass, *Lates calcarifer*.

2. MATERIALS AND METHODS

2.1. Experimental diets

Formulation of experimental diets used in this study was shown in Table 1. Fish meal (Pesquera Diamante, Peru) and *Lactobacillus*

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Table 1. Formulation and proximate composition of experimental diets.

	Fermented soybean meal substituting to fish meal protein (%)					
	0	10	15	20	25	30
Ingredients (%)						
Fish meal (67.6% protein)	50	45	42.5	40	37.5	35
Fermented soybean meal (52% protein)	0	6.44	9.66	12.88	16.11	19.33
Fish oil	0	0.4	0.6	0.8	1.0	1.2
Soybean oil	5.15	5.10	5.07	5.05	5.02	5
Methionine	0	0.06	0.09	0.11	0.14	0.17
Lysine	0	0.05	0.08	0.11	0.14	0.17
Cellulose	5.85	3.95	3.00	2.05	1.10	0.14
Common ingredients1	39	39	39	39	39	39
Proximate composition (%)						
Moisture	8.6	7.9	8.2	8.3	8.4	8.4
Ash	11.0	11.5	11.8	11.6	11.2	11.3
Crude protein	45.0	44.9	46.0	45.6	45.5	44.8
Crude lipid	11.0	11.6	11.8	11.5	11.4	11.6

[1] Common ingredients contain vitamin premix, 2%; mineral premix 4%; gluten, 6%; alpha-starch, 22%; squid liver meal, 5%. Vitamin and mineral premix were referenced to Lin and Mui (2016).

spp. fermented soybean meal (DaBomb Protein, Taiwan) were used as the main protein source. *Lactobacillus* spp. fermented soybean meal was substituting to dietary fish meal protein at 0 (as a control diet), 10, 15, 20, 25 and 30%. All diets were kept isonitrogenous. Diet preparation was according to Changchien et al. (2013). All diets were stored at -20°C until used. Proximate compositions (AOAC, 1995) of the experimental diets were: crude protein 45.3 ± 0.5%; crude lipid, 11.5 ± 0.3%; crude ash, 11.4 ± 0.4%.

2.2. Experimental procedure

This study, involving animal experiment, conformed to the principles for the use and care of laboratory animals, in agreement with the Institutional Animal Care and Use Committee (IACUC) in National Pingtung University of Science and Technology (NPUST).

The Asian sea bass (*Lates calcarifer*) juveniles (6-7 cm) were obtained from a local hatchery (Pingtung, Taiwan) and acclimated to experimental condition in concrete ponds containing brackish water (10‰). The fish were fed with the control diet twice a day for 2-wks acclimation period.

At the beginning of the experiment, 1,440 fish (mean weight: 9.2 ± 0.1 g) were stocked in 18 experimental concrete ponds (4 × 3 × 1 m^3) of indoor static rearing system with brackish water (10‰). The experimental diets were each fed to triplicate groups of fish (80 fish/pond). The fish were chosen for the experiment and the diets were assigned to groups of fish randomly. The water temperature, dissolved oxygen, nitrate and ammonia were monitored daily, ranging from 24-28°C, 6.5-6.8 mg/l, 0.005-0.02 mg/l and 0.021-0.035 mg/l, respectively. The fish were fed to apparent saturation twice per day at 0900 and 1700 h. The uneaten feed were collected by a siphon immediately, then dried and weighed for recording the feed intake during the feeding period. During the experimental period, the rearing water was exchanged twice a week. Fish were weighed once every month.

2.3. Growth performance

At the end of the feeding trial, fish were bulk weighed and growth performance including final weight and feed conversion rate (FCR) were calculated. The equation of FCR was shown as below:

FCR = feed intake/(final body weight – initial body weight)

2.4. Statistical analysis

One-way analysis of variance (ANOVA) with Tukey's multiple comparison test was conducted to compare the significant differences ($p < 0.05$) between control and treated groups by using SigmaStat software (Jandel Scientific Co. Ltd., USA).

3. RESULTS AND DISCUSSION

The Asian sea bass were fed with the feed substituted fish meal with *Lactobacillus* spp. fermented soybean meal at 0%, 10%, 15%, 20%, 25%, and 30% for six months, and the final weight and feed conversion rate (FCR) were showed in Figures 1 and 2. There was no significant difference both in final weight and feed conversion ratio. All fish survived during the 6-month feeding trial.

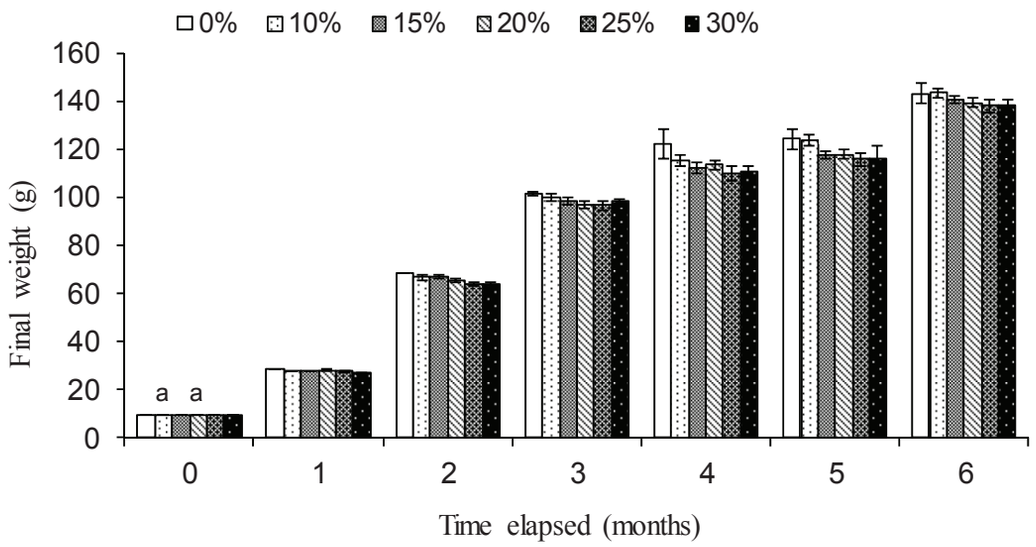

Fig. 1. The final weight of Asian sea bass fed different diets for 6 months. Each bar represents the mean value from three determinations with the standard error (SE).

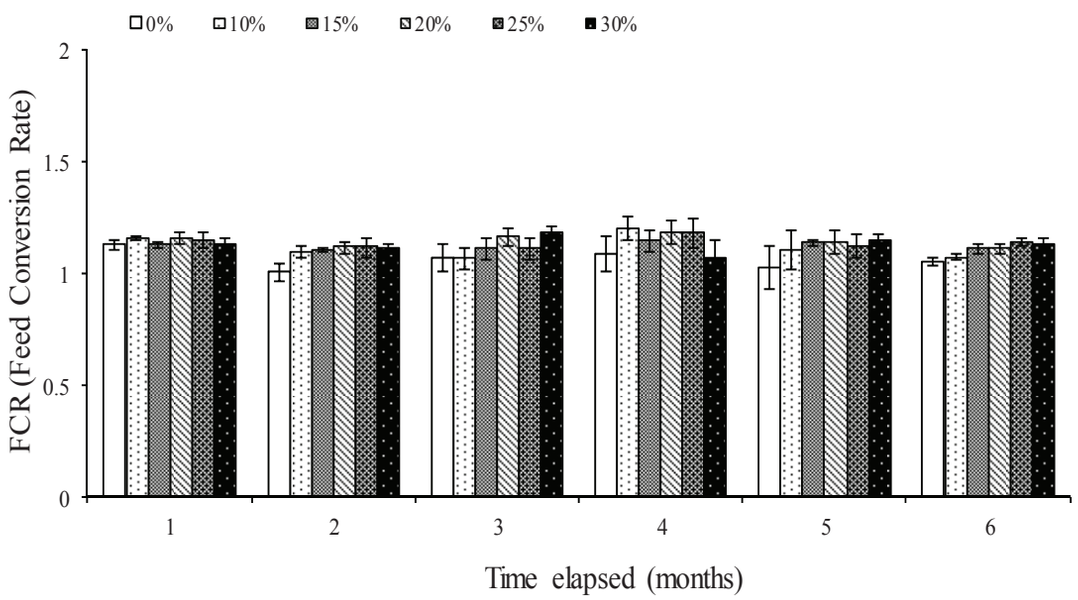

Fig. 2. The feed conversion rate (FCR) of Asian sea bass fed different diets for 6 months. Each bar represents the mean value from three determinations with the standard error (SE).

It clearly indicated that dietary fish meal protein can be replaced by fermented soybean meal up to 30% without any negative effects on growth and feed utilization of Asian sea bass. Francis et al. (2001) reported that there are several antinutritional factors (ANFs) in soybean meal, such as trypsin inhibitor, phytic acid, saponin…etc. These ANFs would disturb the absorption and utilization of nutrients in diet and suppress the growth for fish (Krogdahl et al., 2009). This will cause poor growth performance of the culture species when fed with high soybean meal diet. Microorganism fermentation is an ideal way to remove most of antinutritional factors. Glycinin and β-conglycinin derived from soybean meal have been demonstrated to be main antigenic components that caused allergic reaction in animals (Xu et al., 2010). These antinutrients has been demonstrated to depress growth and intestinal function in juvenile Jian carp (Jiang et al., 2015). Cheng and Lin (2016) indicated that the glycinin and β-conglycinin in soybean meal were completely degraded through the *Lacotobacillus* spp. fermentation. Similar results were reported by Yamamoto et al. (2010) that trout fed diets with fermented soybean meal for 60 days brought no effects on growth, intestinal morphology and hematological parameters compared to fish fed all fish meal diet.

Imbalance amino acid profile is another problem of plant feed ingredients. Our present study is aimed to evaluate the maximum fish meal replacement level by fermented soybean meal without supplementation of limiting amino acid (e.g. methionine and lysine). The study demonstrated that at least 30% fermented soybean meal can be used to replace fish meal protein in diets without any supplemental limiting amino acids. The tolerance level of soybean meal substituted to fish meal was varied among fish species. For example, the fish meal replacement level by soybean meal over the adequate levels, e.g. 24% for Japanese flounder (Ye et al., 2011), 30% for tiger puffer (Lim et al., 2011), 90% for red seabream (Kader et al., 2012), 40% for cobia (Chou et al., 2004) and 60% for round batfish (Chiu et al., 2014) showed suppressed growth of the fish. Kaushik et al. (2004) reported that dietary fish meal can be reduced to 5% by soybean meal combined with wheat and corn gluten and canola meal without any adverse consequence in terms of growth or nitrogen utilization in European sea bass Hybrid striped bass study was found that fish fed the diet with 5% fish meal, 25.5% soybean meal and 38.1% fermented soybean meal showed similar weight gain with fish fed the diet with 30% fish meal and 26% soybean meal (Rombenso et al., 2013). Moreover, Yang et al. (2001) indicated that diets including FSB up to 24% in diet did not have an adverse effect on growth of silver perch. All the studies suggested sea bass is able to utilize plant protein sources well. However, there are only few reports regarding of alternative protein research in sea bass. Higher soybean product inclusion in diet for Asian sea bass is needed further research.

The organic acid (lactic acid) concentration determined in fermented soybean meal used in our study was about 6% (data not shown). It has been reported that dietary lactic acid supplementation enhanced the nutrient utilization by rainbow trout (Pandey and Satoh, 2008). This is often attributed to enhanced nutrient availability due to a reduction or alteration of gastrointestinal bacteria as well as the acidifying effects to the diet and gut, which can chelate or solubilize minerals, and/or to the stomach that may improve digestive enzyme activity (da Silva et al., 2013). Based on the above suggestion, good feed utilization of the fish fed with fermented soybean meal is expectable.

In conclusion, our results suggested that up to 30% replacement level of fish meal by *Lactobacillus* spp. fermented soybean meal did not suppress the growth performance of Asian sea bass. It clearly demonstrated that fermented soybean meal is potentially used as fish meal replacer for Asian sea bass.

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飼料中乳酸菌發酵豆粉取代魚粉對金目鱸成長表現之影響

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本研究為探討以乳酸菌發酵豆粉取代魚粉對金目鱸成長之影響。乳酸菌發酵豆粉分別取代飼料中魚粉蛋白0、10、15、20、25及30%，實驗飼料共分為六組，分別餵予初重9.2 ± 0.1 g金目鱸魚苗，每組三重複，飼養於18個4 × 3 × 1 m^3水泥池，使用半淡鹹水(10‰)養殖，實驗為期6個月，每個月監測金目鱸之成長表現。魚體末重與飼料轉換率(FCR)無論每個時間點及各組間均無顯著差異。本實驗結果顯示乳酸菌發酵豆粉取代魚粉蛋白至30%不會造成魚體成長之負面影響，顯示發酵豆粉具有取代金目鱸飼料中魚粉蛋白之潛力。

關鍵詞：金目鱸，乳酸菌，發酵豆粉，魚粉，成長。

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