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An evaluation of replacing fish meal with fermented soybean meal in the diet of *Macrobrachium nipponense*: Growth, nonspecific immunity, and resistance to *Aeromonas hydrophila*

Zhili Ding [a, 1], Yixiang Zhang [a, 1], Jinyun Ye [a, *], Zhenyu Du [b], Youqin Kong [a]

[a] *Zhejiang Provincial Key Laboratory of Aquatic Resources Conservation and Development, Key Laboratory of Aquatic Animal Genetic Breeding and Nutrition, CAFS, College of Life Science, Huzhou University, Huzhou, Zhejiang 313000, China*

[b] *School of Life Sciences, East China Normal University, Shanghai 200062, China*

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ABSTRACT

Partial or complete replacement of fish meal (FM) with fermented soybean meal (FSM) was examined in *Macrobrachium nipponense* over an 8-week growth trial. Growth and immune characteristics were evaluated. Fermented soybean meal replaced 0 (FM, control), 25% (R25), 50% (R50), 75% (R75), or 100% of the FM (R100) in five isocaloric and isonitrogenous diets. Each diet was fed to juvenile prawns (mean weight, 0.103 ± 0.0009 g) twice daily to apparent satiation in five replicates. Weight gain and specific growth rate of *M. nipponense* were significantly higher in prawns fed the R25 diet than that of prawns fed the FM diet. No significant differences were observed among the other treatments. Total hemocyte count and hemolymph phagocytic activity decreased as the proportion of FSM increased. Total antioxidant activity competence and malondialdehyde level in the hepatopancreas were highest in prawns fed the R100 diet. mRNA levels of the antioxidant genes Cu–Zn superoxide dismutase and catalase, heat shock cognate protein 70, and heat shock protein 90 were significantly differentially regulated in the prawn hepatopancreas. In addition, percent mortality increased after challenge with live *Aeromonas hydrophila*. Percent mortality of prawns fed the R100 diet was significantly higher than that of prawns fed the FM and R25 diets. These findings demonstrate that (1) *M. nipponense* growth performance was not affected by including a high proportion of FSM in the diet, and the best growth performance was obtained when 25% of the FM was replaced with FSM; (2) nonspecific immunity was impaired when all of the FM was replaced with FSM.

1. Introduction

Pursuing good-quality, low-cost protein sources has been a continuing interest of aquaculture. Fish meal (FM) is a highly nutritious and highly palatable protein source that is a major component of aquaculture feeds. However, FM production has declined because of decreases in wild fisheries populations, resulting in a supply shortage and escalating prices [1]. Thus, numerous plant-derived materials are being considered as alternate nutrient sources for aquaculture feeds [2]. As soybean meal has high protein content, a balanced amino acid profile, easy availability, and low cost, it has been one of the most common FM alternatives used in aquafeeds [2,3].

Nevertheless, the use of high levels of soybean protein can potentially alter aquatic animal growth, metabolism, and health status because of soy anti-nutritional factors (ANFs) in soybean meal, which limit including soybean as a protein source [4–6]. It has been suggested that soybean meal ANFs can be removed or partially inactivated by fermentation [7]. It has been demonstrated that using fermented soybean meal (FSM) as a protein source in formulated terrestrial animal diets enhances weight gain and immune status [8,9]. In addition, FSM has been used in place of FM in the diets of some fish species, such as orange-spotted grouper, *Epinephelus coioide* [10], Japanese flounder, *Paralichthys olivaceus* [11], hybrid tilapia, *Oreochromis mossambicus* × *Oreochromis niloticus* [12], ovate pompano, *Trachinotus ovatus* [13], black sea bream, *Acanthopagrus schlegelii* [14], and rainbow trout, *Oncorhynchus*

* Corresponding author. Tel./fax: +86 572 2322728.
E-mail address: yjy@hutc.zj.cn (J. Ye).

[1] These two authors contributed equally to this work.

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mykiss [15,16]. FSM has been used in place of at least 60% of FM without any negative consequences on growth of rainbow trout [15], and FM has been completely eliminated from the rainbow trout diet and replaced with FSM [16]. Only a few FSM studies have been conducted on economically important crustaceans, such as Pacific white shrimp, *Litopenaeus vannamei* [17], and tiger prawn, *Penaeus monodon* [18].

The freshwater prawn, *Macrobrachium nipponense*, is an important aquaculture species, and several studies have examined its protein requirements [19]. Although the feasibility of replacing FM with poultry by-product meal or meat and bone meal has been assessed in *M. nipponense* [20], only limited data are available on the nutritional value and physiological consequences of using plant proteins in this species. In this study, we evaluated prawn growth performance and health by examining nonspecific immunity and resistance to *Aeromonas hydrophila* associated with including high FSM content in the *M. nipponense* diet. It is anticipated that our results will provide beneficial information to the prawn industry.

2. Materials and methods

2.1. Experimental diets

The FSM in this study was supplied by Huzhou Huannong Microbiology Research Institute (Zhejiang, China; 9.35% small-size peptides on the protein level basis; 47.70% crude protein and 0.42% total lipid on a dry matter basis). Soybean meal was utilized as the substrate for fermentation after being milled through 0.45-mm screen. Fermented and enzymolysis agent (0.1% w/w) was added to soybean meal, which was fortified with a mixture of microorganism (*Pediococcus acidilactic*, *Enterococcus faecalis*, *Saccharomyces cerevisiae*, *candida utilis*, *Bacillus subtilis*, *Bacillus licheniformis*, *Rhodopseudomonas palustris*; 10, 15, 10, 10, 10, 10 and 15% w/w, respectively) and enzymes (protease, celluase and xylanase; 1.5, 2 and 1% w/w, respectively). The high quality FM was supplied by Huzhou Jinbao Group Co. Ltd. (Zhejiang, China; 66.69% crude protein and 9.10% total lipid on a dry matter basis). Five isonitrogenous (approximately 38% crude protein) diets with isocaloric value (15 kJ g^{-1}) were formulated to replace 0 (control), 25%, 50%, 75%, or 100% of FM protein by a corresponding amount of protein with FSM to form the experimental diets (FM, R25, R50, R75 and R100, respectively).

All ingredients of each diet were homogenized and further thoroughly mixed with distilled water. This mixture was extruded through a 1.5 mm die with a pelleting machine and dried at 40 °C to about 10% moisture before being stored at −20 °C. The approximate compositions of the experimental diets and essential amino acid (EAA) content were shown in Table 1 and Table 2, respectively.

2.2. Feeding trials and challenge test

Juvenile *M. nipponense* were obtained from a local farm (Huzhou, Zhejiang, China) and acclimated to laboratory condition by feeding on the commercial diet for 1 week. Five replicates were used per diet. Prawns (0.103 ± 0.0009 g) were randomly stocked into 25 indoor aquariums (length × width × depth = 1 m × 0.5 m × 0.5 m) with 50 prawns per aquarium. Some nylon fishing nets were placed in each aquarium as an artificial shelter to minimize disturbance. The temperature of the water ranged from 25 to 29 °C with dissolved oxygen more than 6.5 mg L^{-1} and ammonia and nitrate remained below 0.1 mg L^{-1}. Prawns were fed the experimental diets at 4–5% of body weight, divided into two equal feedings at 07:30 and 17:00 h daily, for 8 weeks. Uneaten feed on tank bottoms was siphoned out 2 h after feeding, dried, and weighed to estimate feed consumption.

Table 1
Ingredient composition and nutrient content of the test diets.

Ingredients (%)	Test diets				
	FM	R25	R50	R75	R100
Fish meal	58.00	43.50	29.00	14.50	0.00
Fermented soybean meal	0.00	20.00	40.00	60.00	80.00
Corn starch	21.00	16.00	10.00	6.00	0.00
Fish/soybean oil (2/1)	1.50	2.00	3.00	3.00	4.00
Soybean lecithin	0.50	0.50	0.50	0.50	0.50
Cholesterol	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.50	0.50	0.50	0.50	0.50
[a]Mineral mixture	3.00	3.00	3.00	3.00	3.00
[b]Vitamin mixture	2.00	2.00	2.00	2.00	2.00
Cellulose	10.90	9.90	9.40	7.90	7.40
Carboxymethyl cellulose	2.00	2.00	2.00	2.00	2.00
Proximate composition (% air dry matter)					
Moisture	10.63	10.51	10.65	10.72	10.71
Protein	38.37	38.25	38.28	38.32	38.38
Lipid	7.78	7.30	7.33	6.35	6.38
[c]Gross energy (kJ g^{-1})	15.47	15.45	15.49	15.52	15.50

[a] Vitamin mixture (100 g^{-1} mixture): vitamin A, 420,000 IU; vitamin C, 6000 mg; α-tocopherol acetate, 2000 mg; vitamin D3, 120,000 IU; vitamin K, 1000 mg; vitamin B1, 1000 mg; vitamin B2, 1000 mg; vitamin B6, 1600 mg; vitamin B12, 2 mg; niacin, 5000 mg; folic acid, 400 mg; inositol, 6000 mg; biotin, 10 mg; calcium pantothenic, 3500 mg.

[b] Mineral mixture (mg g^{-1} mixture): KCL, 28; $MgSO_4 \cdot 7H_2O$, 100; NaH_2PO_4, 215; KH_2PO_4 100; $Ca(H_2PO_4)_2 \cdot H_2O$, 265; $CaCO_3$, 105; $C_6H_{10}CaO_6 \cdot 5H_2O$, 165; $FeC_6H_5O_7 \cdot 5H_2O$, 12; $ZnSO_4 \cdot 7H_2O$, 4.76; $MnSO_4 \cdot H_2O$, 1.07; $AlCl_3 \cdot 6H_2O$, 0.15; $CuCl_2 \cdot 2H_2O$, 0.24; $CoCl_2 \cdot 6H_2O$, 1.4; KI,0.23; α-cellulose, 2.15.

[c] Gross energy was calculated based on protein = 16.7 kJ g^{-1}; lipid = 37.6 kJ g^{-1}; NFE = 16.7 kJ g^{-1}.

About 30% of the water was exchanged every day to maintain water quality.

At the end of the growth trial, 30 prawns were randomly sampled from each treatment group and were randomly assigned to three aquariums after final weighing. Prawns were challenged with 50 μL 3.5 × 10^7 live bacterial suspension of *A. hydrophila* from the Zhejiang Institute of Freshwater Fisheries (Zhejiang, China). Mortalities were recorded up to 96 h post-injection.

2.3. Sample collection

At the end of the feeding trial, all prawns were counted and measured in weight to determine survival rate, weight gain,

Table 2
Amino acid content of the test diets.

Amino acids	Test diets				
	FM	R25	R50	R75	R100
Indispensable					
Arginine	2.15	2.30	2.44	2.37	2.65
Histidine	1.59	1.54	1.47	1.45	1.41
Isoleucine	1.57	1.63	1.65	1.64	1.67
Leucine	2.86	2.94	2.96	2.95	2.99
Lysine	3.10	2.97	2.87	2.70	2.55
Methionine	0.91	0.79	0.65	0.54	0.38
Phenylalanine	1.49	1.59	1.74	1.71	1.81
Threonine	1.64	1.65	1.62	1.61	1.58
Valine	1.78	1.80	1.78	1.76	1.74
Dispensable					
Alanine	2.20	2.06	1.91	1.87	1.68
Aspartic acid	3.74	3.96	4.11	4.12	4.41
Glutamic acid	5.40	5.88	6.33	6.37	7.14
Glycine	2.01	1.95	1.84	1.79	1.66
Proline	1.49	1.69	1.76	1.79	2.01
Serine	1.62	1.75	1.86	1.88	2.07
Tyrosine	1.02	1.06	1.17	1.11	1.10