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Effects of polychaete extracts on reproductive performance of kuruma shrimp, *Marsupenaeus japonicus* Bate. – Part II. Ovarian maturation and tissue lipid compositions

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ABSTRACT

A 32-day feeding trial was conducted to examine effects of marine polychaete extracts on ovarian maturation and tissue lipid compositions of *Marsupenaeus japonicus*. Three fractions extracted from polychaetes such as trichloroacetic-soluble (TSF), neutral lipids (NLF) and polar lipids (PLF) fractions at 0.5% (dry matter basis) were supplemented to a basal diet (BD). Frozen polychaetes (FPC) and BD were used as positive and negative controls, respectively. Each treatment with triplicate groups of ten unilaterally eyestalk-ablated wild caught females at early stages of ovarian development were fed test diets above once a day at 5:00 pm with excess of pellet diets and 10% of biomass with FPC. The ovarian development was tracked every 4 days. The maximum ovary shadow ratios (OSR = ovary width × 100/body width) were not significantly different among treatments. Ovarian maturation time (OMT), defined as the time elapsed for ovaries to develop from initial to maximum OSR, of NLF and FPC groups were significantly shorter than those of other groups. Maturation response (MR = proportion (%) of females achieving ovarian maturity within each treatment) had some distinctive trends during the course of the trial. The study also indicated that NLF was the most effective on *M. japonicus* ovarian maturation and followed by TSF. Phospholipids were dominant in the ovarian lipid compositions while contents of neutral lipids were relatively high in hepatopancreas in all diet groups. Hepatopancreatic and ovarian fatty acid profiles showed significant differences in proportions of some important fatty acids. Furthermore, this study demonstrated that formulated feed being fed solely was able to promote the ovarian maturation in kuruma shrimp broodstock.

1. Introduction

In shrimp production industry, penaeid broodstocks are commonly caught from the seas and fed fresh marine organisms such as polychaetes, squids or bivalve mollusks under captive conditions. The use of such natural fresh foods is usually linked to unknown chemicals in marine organisms that stimulate the reproductive system development. Among marine organisms popularly used as fresh foods for penaeid shrimp, marine polychaetes have been widely considered superior for inducing ovarian maturation and spawning (Coman et al., 2007; Du et al., 2004; Lytle et al., 1990; Middleditch et al., 1980; Naessens et al., 1997; Nguyen et al., 2008; Wouters et al., 2002). In common practices, marine polychaetes were used to feed crustacean broodstock solely or more usually in combination with other natural foods and/or with pellet diets. Marine polychaetes are superior for their nutrient values particularly lipid components (Harrison, 1990; Laufer et al., 1998; Wouters et al., 2001a) which shrimp have very limited ability to synthesize *de novo* (Kanazawa et al., 1988; Teshima and Kanazawa, 1983; Teshima et al., 1992) and some unknown factors stimulating sexual maturation.

The sexual simulation effects of marine organisms used as foods for broodstock have been investigated in different penaeid shrimp species. Clam lipid extracts were effective in inducing ovarian maturation of *Penaeus japonicus* (Parazo, 1987). Hydro-alcohol soluble extract from squids had a positive effect on the secondary triggering of vitellogenesis in *Litopenaeus vannamei* (Mendoza et al., 1997). Steroids extracted from marine polychaetes *Perinereis* sp. stimulated *in vitro* oocyte development in *Penaeus monodon* (Meunpol et al., 2007; 2010). It was reported that marine polychaetes possess some hormonal substances that enhance reproductive performance in penaeid shrimp species (Laufer et al., 1997; Poltana, 2005) and other crustaceans (Laufer et al., 1998; Tsukimura and Kamemoto, 1991). However effects and nature of active

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compounds involving in stimulating reproductive system development in penaeid shrimp, particularly in *Marsupenaeus japonicus*, have not yet been clarified. Identification of these reproductive development stimulators can help developing broodstock diets by incorporating these elements in the diet taking into account that the optimal maturation feeds for *M. japonicus* have not been yet available.

Despite advantages as outstanding maturation foods for penaeid species, inconsistent supply and quality of fresh foods due to seasons and other factors may potentially limit provision of sufficient nutrients to animals (Bray and Lawrence, 1992). It was also reported that marine polychaetes were to be the vector transmission of white spot syndrome virus to penaeid shrimp species (Vijayan et al., 2005). Viral infections, recently, have been also reported in wild *M. japonicus* populations along the coastal areas of Japan (Maeda et al., 1998).

Therefore, the present research aimed to determine the functional elements of marine polychaetes on reproductive performance and tissue lipid compositions of kuruma shrimp. This paper forms the second part of the subject and presents the examining results of the influence associated with polychaete extracts on ovarian maturation of kuruma shrimp. Their effects on total lipids, lipid class and fatty acid compositions of shrimp tissues were also quantified and data are compared and discussed. The first part has been published (Nguyen et al., 2008) and the third paper of this series will report on the effects of combined polychaete extracts on reproductive performance and tissue lipid composition in different ovarian maturation stages of *M. japonicus* soon.

2. Materials and method

2.1. Polychaete extracts

Polychaete nutrient extraction procedure was mainly based on methods of Kanazawa et al. (1979) and Deshimaru (1981). In short, marine polychaetes obtained from local farm (Kochi, Japan) were lyophilized in a freeze-dryer (Eyela Freeze Dryer FD-1, Rikakikai, Japan) and ground into powder. Polychaete powder was fractionated into methanol–water soluble part and chloroform layer as the method of Bligh and Dyer (1959) adapted to large amount of tissues. The water soluble fraction was added with trichloroacetic acid (TCA) at 7% after being evaporated off to remove remaining methanol. The mixture was maintained overnight at 4 °C and centrifuged at 3000 *g* in 10 min at 4 °C (MX-160 high speed refrigerated micro-centrifuge, Tomy, Japan) to collect the supernatant. After remaining TCA was washed by diethyl ether, the residual part containing mostly free amino acids and peptides was assigned as TCA-soluble extract (TSF). The chloroform layer containing mostly lipids after solvents evaporated off was fractionated by chilled acetone treatment into acetone soluble and acetone insoluble fractions assigned as polychaete neutral lipid (NLF) and polar lipid (PLF) extracts, respectively. All polychaete extracts were stored at −80 °C until incorporated with other ingredients to prepare test diets.

2.2. Experiment design and feed preparation

Five dietary treatments with triplicates including basal diet (BD, Table 1), BD incorporated with TCA-soluble (TSF), neutral lipids (NLF), polar lipids (PLF) fractions at 0.5% of dry matter and fresh frozen polychaetes (FPC) were given to *M. japonicus* broodstock for a 32-day period.

When TSF or NLF/PLF was added to the basal diet (Table 1), the equivalent amount of soybean meal or squid liver oil was respectively reduced to maintain homogeneity of test diet proximate compositions. After adding NLF/PLF into the mix of squid liver oil, soybean lecithin, and cholesterol, fat-soluble vitamins were dissolved in the mixture, and then mixed well with other dry ingredients in a professional mixer (Kitchen Aid, USA). TSF was dissolved completely in water and added to the diet mixture. After water was added, those were mixed for 15 min, applied to the mincer for making spaghetti-like string, and dried using a convection oven (DK 400, Yamato Corp., Japan) with regular moisture content check for a desire level by a moisture detector (MM30, Yamato Scientific Corp., Tokyo, Japan). Finally, the diets were steamed for one minute in an autoclave (MAX-S220, Ikiken Corp., Tokyo, Japan), cut into small pellets for a desire size, and stored at −30 °C until use. Marine polychaetes were frozen and stored at −30 °C, and thawed in sea water right before feeding.

Table 1
Composition of basal diet.

Ingredient	g kg^{-1} dry diet
Squid meal	391
Fish meal	50
Krill meal	200
Soybean meal	30
Squid liver oil	45
Soybean lecithin	55
Cholesterols	9
Activated gluten	50
α-starch	70
Vitamin mix[a]	30
L-ascorbyl-2-monophosphate-Na/Ca[b]	20
Choline chloride	8
Mineral mix[c]	30
Attractants[d]	6
Carophyll Pink 10% CWS[e]	5
Lactoferrin[f]	1
Total	1000

[a] mg kg^{-1} diet: *p*-Amino benzoic acid, 350; d-Biotin, 150; Inositol, 15400; Niacin, 3500; Ca-pantothenate, 3500; Pyridoxine-HCl, 500; Riboflavin, 600; Thiamin-HCl, 1500; Folic acid, 250; Cyanocobalamine, 0.5; Menadione, 200; Vitamin A-palmitate, 2500; α-Tocopherol, 1500; Calciferol, 50.
[b] Stay-C 35: DSM Nutrition Japan K.K., Tokyo, Japan.
[c] g kg^{-1} diet: K_2HPO_4, 7.09; $Ca_3(PO_4)_2$, 9.51; $MgSO_4\,7H_2O$, 10.63; $NaH_2PO_4\,2H_2O$, 2.76.
[d] g kg^{-1}diet: sodium citrate, 2; sodium succinate, 2; glucosamine HCl, 2.
[e] Carophyll Pink 10% CWS by DSM Nutrition Japan K.K., Tokyo, Japan.
[f] Bovine lactoferrin: Morinaga Corp., Japan.

2.3. Animals and experiment conditions

Wild caught *M. japonicus* broodstock of about 68 g mean BW obtained from Matsumoto Suisan Co. Ltd., Miyazaki, Japan, in acclimation to experimental conditions, were fed a commercial diet (Vital-Prawn, Higashimaru Corp., Kagoshima, Japan) for 1 week. All females in inter-molt stage according to Robertson et al. (1987) were unilaterally ablated by tying the eye-stalks with surgical thread (Nescosuture, 2–0) and marked with eye-tags for individual identification on the commencement day of the feeding trials. Eye-stalks came off after 4 or 5 days after tying. Ablated females were randomly allocated at a density of 10 individuals per tank in maturation tanks assigned for each treatment. Females were fed slightly excess with formulated diets and 10% of body weight with fresh frozen polychaetes once a day at 5:00 pm. Excess feeds, feces and exuviae were removed in the following morning.

Broodstocks were housed in maturation composite tanks with sand bottom, continuous aeration, water flow-through of 300% day^{-1} filled by the filtered sea water with temperature of 26 ± 2 °C, salinity of 34 g l^{-1}, dissolved oxygen of 6.9 ± 0.4 mg l^{-1}, pH of 8.2 ± 0.3. Daily photoperiod of 14:10 hour light:dark was maintained. The experiment was conducted at Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University, Japan.

2.4. Maturation evaluation

The ovarian development was individually tracked every 4 days by measuring the body width and ovary shadow width at dorsal