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Effects of dietary vitamins C and E and their interactions on reproductive performance, larval quality and tissue vitamin contents in kuruma shrimp, *Marsupenaeus japonicus* Bate

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ABSTRACT

A 3 × 3 factorial feeding trial was conducted to investigate effects and interactions of vitamins C (VC) and E (VE) on reproductive performance, larval quality and body vitamin concentrations in kuruma shrimp. Pond-reared females (50 g mean body weight) were fed 9 experimental diets containing 3 grade levels of VC (0, 500 and 1000 mg ascorbic acid equivalent kg^{-1} diet) and VE (0, 300 and 600 mg α-tocopherol kg^{-1} diet) for 2 months. When ovaries reached stage IV, females were unilaterally ablated to induce spawning in a separated 120 l spawning tank under flow-through water system. The average temperature of water during the course of the experiment was 23.3 ± 1.9 °C, dissolved oxygen 8.7 ± 0.9 mg l^{-1}, pH 8.2 ± 0.2, and salinity 31.6 ± 0.7 g l^{-1}, respectively.

Neither dietary VC nor VE was a significant factor on fecundity, but the hatching rates and metamorphosis rates of nauplii into zoea I significantly increased with increased either dietary VC or VE. On the other hand, there were significant interactions between two vitamins on those parameters. VE concentrations in hepatopancreas, ovaries, muscles and eggs significantly increased with increased dietary VE while those also increased with dietary VC concentrations, particularly in 1000 VC fed groups, the values were significantly higher than those of others in most cases regardless of dietary VE concentrations. VC concentrations in hepatopancreas, ovaries, muscles and eggs significantly increased with increased dietary VC, but effects of dietary VE on VC concentrations in those organs were varied. In other words, the regeneration effect of VC on VE concentrations and the sparing effect of VE on VC concentrations in the organs of female shrimp were demonstrated in this study. Supplementations of VC and VE in maturation feeds for female shrimp are recommended to obtain higher egg hatchability and better quality larvae.

1. Introduction

The importance of vitamin C and vitamin E has been demonstrated for development and reproductive processes of aquatic animals. The major benefits of these vitamins are due to their capacity of scavenging reactive oxygen species in biological fluid and membranes. The action of reducing tocopheroxyl radicals and generating α-tocopherol (α-Toc) *in vivo* has been hypothesized by Tappel (1968). Some studies have been conducted to confirm the hypothesis (Mukai et al., 1991; Packer et al., 1979).

It has been suggested that vitamins C and E are essential nutrients for reproductive physiology of fish and crustacean species. The roles of these two vitamins in fish reproduction have been studied (Emata et al., 2000; Lee and Dabrowski, 2004; Sandnes et al., 1984; Waagbø et al., 1989; Watanabe and Takashima, 1977; Watanabe et al., 1985). In penaeid shrimp, even though the direct mechanisms have not been well established, supplementation of vitamin C into maturation diets enhanced ovarian development in *Marsupenaeus japonicus* (Alava et al., 1993a, 1993b), and egg hatchability in *Penaeus indicus* (Cahu et al., 1995) and *Litopenaeus vannamei* (Du et al., 2004; Sangha et al., 2000). Vitamin E was reported to improve reproductive performance of crustacean species, such as ovarian growth in *M. japonicus* (Alava et al., 1993b), higher egg hatchability in *P. indicus* (Cahu et al., 1995), better spawning performance, fertilization and egg hatchability in *L. vannamei* (Du et al., 2006). In addition, dietary vitamin C and E requirements for growth of early development stages in penaeid shrimp species including *M. japonicus* were well established as reviewed by Koshio (2010).

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On the other hand, the interactive effects of dietary vitamins C and E have long been investigated in animals since the finding of Packer et al. (1979) that vitamin C has capacity to regenerate tocopherol from tocopheroxyl radicals. As the results, interactive effects of these two vitamins on growth performance have been reported for several fish species such as Atlantic salmon (Hamre et al., 1997), lake sturgeon (Moreau et al., 1999), and hybrid striped bass (Sealey and Gatlin, 2002). However, the interaction of dietary vitamins C and E on aquatic animal reproduction has not been well documented. Particularly, the study on the interaction of these two vitamins for kuruma shrimp reproduction has not been reported.

The aim of this study is to investigate the effects and interactions of dietary vitamins C and E on reproductive performance including spawning performance, fecundity, egg hatchability, and larval quality. Vitamin concentrations in hepatopancreas, ovaries, muscles and eggs were also measured to evaluate the effects.

2. Materials and methods

2.1. Experimental design and diets

3 × 3 factorial design experiment was conducted with 9 diets containing 3 grade levels of vitamin C derivative (0, 500 and 1000 mg ascorbic acid (AsA) equivalent kg^{-1} diet) and vitamin E (0, 300 and 600 mg α-Toc kg^{-1} diet). DL-α-tocopherol (Wako Pure Chemicals Ind. Ltd., Japan) as a vitamin E source and ROVIMIX Stay C-35 (DSM Nutrition K.K., Tokyo, Japan) as a vitamin C source were supplemented to a basal diet (Table 1), respectively. The basal diet contained almost 0 mg AsA and about 39.6 mg α-Toc kg^{-1} diet.

Diets were prepared by mixing dry ingredients with lipid mixture containing fat soluble vitamins and water, 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 analyzer (MA35, Sartorius AG, Germany). Finally, the diets were steamed for 1 min in an autoclave (MAX-S220, Ikiken Corp., Tokyo, Japan), cut into small pellets for a desired size, and stored at −30 °C until use.

Table 1
Composition of the basal diet.

Ingredient	g kg^{-1} dry diet
Squid meal	390
Fish meal	50
Krill meal	200
Soybean meal	52
Squid liver oil	40
Soybean lecithin	60
Cholesterol	9
Activated gluten	50
α-Starch	50
Vitamin mix* 1	39
Choline chloride	8
Mineral mix* 2	30
Attractants* 3	6
α-Cellulose	16
Proximate composition (% of dry weight)	
Crude protein	54.9
Crude lipid	18.4
Crude ash	12.5
Ascorbic acid and α-tocopherol contents (mg kg^{-1} diet)	
Ascorbic acid	Not detected
α-Tocopherol	39.6

* 1Vitamin mix (mg kg^{-1} diet): *p*-amino benzoic acid (470), D-biotin (200), inositol (20,710), niacin (4710), Ca-pantothenate (4710), pyridoxine-HCl (6700), riboflavin (810), thiamin-HCl (2020), folic acid (340), cyanocobalamine (0.67), menadione (318), vitamin A-palmitate (3973), and Calciferol (790).
* 2Mineral mix (g kg^{-1} diet): KH_2PO_4 (7.09), $Ca(H_2PO_4)_2 \cdot Ca(OH)_2$ (9.51), $MgSO_4 \cdot 7H_2O$ (10.63), and $NaH_2PO_4 \cdot 2H_2O$ (2.76).
* 3Attractants (g kg^{-1} diet): sodium citrate (2), sodium succinate (2), and glucosamine HCl (2).

2.2. Animals and feeding

Pond-reared *M. japonicus* broodstock of about 50 g mean body weight obtained from a local farm (Oita, Japan) were acclimated in laboratory conditions for 1 week and fed a commercial diet at the facilities of Momoshima Station, National Research Institute of Fisheries and Environment of Inland Sea (FEIS, Onomichi, Japan). 10 females of immature ovaries and 5 males were randomly distributed into 9 net cages of 2 × 2 × 2 m with about 15 cm sand bed and continuous aeration set up in a 10,000 m^2 pond. The water level was maintained at 1.0 m at low tide and 1.5 m at high tide. Water quality parameters were measured inside the net cages at about 80 cm water depth once a day at 5:00 pm by a water quality meter (U51 Multiparameter water quality meter, Horiba Ltd., Kyoto, Japan). The average temperature of water during the course of the experiment was 23.3 ± 1.9 °C, dissolved oxygen 8.7 ± 0.9 mg l^{-1}, pH = 8.2 ± 0.2, and salinity 31.6 ± 0.7 g l^{-1}. Each female was individually identified by cutting the telsons. Experimental animals were fed slightly excess of pellet diets once a day at 5:00 pm for a 2-month period. Excess feeds, feces and exuviae were removed in the following day morning.

After one month of the feeding trial, females were checked regularly for ovarian maturation by external examination at the first pleonite. When ovaries were determined at stage IV as described by Teshima and Kanazawa (1983), females were unilaterally ablated to induce spawning in a separated 120 l spawning tank. Females after spawning were returned to the respective net cages.

2.3. Reproductive performance and larval quality evaluation

Fecundity (egg number per spawn) was determined by counting three subsamples collected from spawning tanks. Triplicate samples of 100 eggs were randomly collected from the first spawning of each spawner and incubated at 28 °C for determining the hatching rate (%, 100 × number of nauplii/total number of incubated eggs). Triplicate samples of 100 hatched nauplii from each spawner were maintained in 1 l beakers at 28 °C with slight aeration and observed under light microscope to identify the number of survived nauplii which metamorphosed into zoea I according to the morphological descriptions of Hudinaga (1941). Metamorphosis rate of nauplii into zoea I (%, 100 × number of zoea I/total number of assessed nauplii) was calculated to evaluate the larval quality. Remaining eggs were rinsed with fresh water, pooled and stored at −80 °C for further biochemical analysis.

At the end of the experiment, hepatopancreas, ovaries and tail muscles dissected from 5 females of stage II ovaries were weighed, pooled and stored at −80 °C for calculating hepato-somatic index (%, HSI = 100 × hepatopancreas weight/body weight) and vitamin analyses.

2.4. Biochemical analysis

Crude lipid and crude protein of the basal diet were respectively determined by Soxhlet and Micro-Kjeldahl methods (Tecator Kjeltec System, Tecator Digestion Systems, 2100 Distilling unit and Titration unit, Foss, Denmark). Ash and moisture contents were analyzed according to the methods of the American Organization of Analytical Chemistry (AOAC, 1995). Wet shrimp tissues were dried in a freeze-dryer (Eyela FDU-110, Tokyo Rikakikai Co. Ltd., Japan) to calculate tissue dry matter (DM (%) = 100 × tissue dry weight/tissue wet weight).

AsA concentrations in the basal diet, hepatopancreas, ovaries, tail muscles and eggs were determined according to the method of Moe et al. (2004). Dietary concentrations of Stay-C were analyzed by