# クルマエビの卵巣発達に対する飼料性ビタミンA,EおよびCの効果


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# Effects of Dietary Vitamins A, E, and C on the Ovarian Development of *Penaeus japonicus*

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A feeding experiment was conducted to evaluate the effect of non-supplementation of individual vitamins A, E, and C on the ovarian development of *P. japonicus* (mean weight=20.21±0.87 g). Vitamin A palmitate, DL-α-tocopherol, and L-ascorbyl-2-phosphate magnesium (APM) were used as the vitamin A, E, and C sources, respectively. A control diet was supplemented with these three vitamins. Bilateral eyestalk ablation was performed within 30–37 days after feeding with the experimental diets, and weight gain and gonadosomatic index (GSI) were examined 20 days after each prawn's ablation.

The weight gain of prawns fed with the control diet was significantly higher ($P<0.05$) than of those fed with diets not supplemented with vitamins A or E; however, this was no different ($P>0.05$) from that of prawns fed with the APM-unsupplemented diet. The highest GSI ($P<0.05$) was observed in prawns fed with the control diet. The control and initial prawn tissues contained higher vitamins A, E, and C than those of prawns fed with diets unsupplemented with vitamins A, E, or APM respectively. The present study showed the essentiality of supplemental vitamins A, E, and C in the diets of broodstock prawns for enhanced ovarian development.

Several studies on vitamin nutrition have demonstrated the importance of vitamins A, E, and C on growth, differentiation, and several reproductive processes of animals. However, information on the essentiality of these vitamins specific to gonadal maturation in penaeid prawns is scant and fragmentary. The development of effective prawn maturation diets requires an understanding of nutritional requirements for the species. As part of an investigation to determine nutritional requirements for the ovarian development of *P. japonicus*, the present study evaluated the effect of suppiementation or non-supplementation of vitamins A, E, and C on growth, survival, hepatosomatic index, and gonadosomatic index of prawns. Tissue lipid compositions and vitamin A, E, and C contents were also determined.

## Materials and Methods

*Diets, Prawns, and Experimental Conditions*

The experimental treatments consisted of a control diet, and diets not supplemented with vitamin A, E, or C (Table 1). The control diet was enriched with 15 mg vitamin A plamitate, 50 mg DL-α-tocopherol and 50 mg L-ascorbyl-2-phosphate magnesium (APM, as source of vitamin C) per 100 g semi-purified diet. APM was used as a vitamin C source because it has been demonstrated to have good vitamin C activity for *P. japonicus*.*2 The experimental diets were prepared every 15 days as described previously.[2]

Pond-reared *P. japonicus* were obtained from Mitsui Norin Kaiyosangyo Co., Kagoshima, Japan. These were acclimated to laboratory conditions and fed on a diet containing no lipids, vitamins A, E, and C for two weeks before the experiment. Thirty-two female prawns at the intermolt stage[3] were selected (mean weight= 20.21±0.87 g) and randomly distributed into the 8 rectangular 54 liter tanks. To identify each prawn, part of its exopod or endopod was cut. Four experimental groups were set up in duplicate, 4 prawns per tank. Eight prawns were dissected

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*2 Y. Takahashi, T. Itami, and Y. Aoki: Effect of phosphitan C (Mg-L-ascorbyl-2-phosphate) on the growth and body defense mechanism of kuruma shrimp. *Jap. Soc. Fish. Pathology Meeting Report*, March 1989 (1989).

**Table 1.** Percent composition of experimental diets

| Diet | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Vit. A palmitate*1 | 0.015 | — | 0.015 | 0.015 |
| DL-α-tocopherol*2 | 0.050 | 0.050 | — | 0.050 |
| Vitamin C (APM)*3 | 0.050 | 0.050 | 0.050 | — |
| Basal ingredients*4 | 97.800 | 97.800 | 97.800 | 97.000 |
| α-cellulose | 2.085 | 2.100 | 2.135 | 2.135 |

*1 Vitamin A palmitate (mol. wt. 524.87; Nakalai Tesque Inc., Japan). After feed processing, mean content of Diets 1, 3, and 4 was 18 mg/kg. Diet 2 contained 4 mg/kg.

*2 DL-α-tocopherol (mol. wt. 430.72; Wako Pure Chem. Ind., Ltd., Japan); After feed processing, mean content of Diets 1, 2, and 4 was 482 mg/kg. Diet 2 contained 128 mg/kg.

*3 L-ascorbyl-2-phosphate magnesium (Phosphitan C, mol. wt. 379.61; Showa Denko Co., Japan). APM (mg/kg diet): After feed processing, mean content of Diets 1, 2 and 3 was 492 mg/kg; Diet 4, 0 kg/mg.

*4 Casein, 55.0; squid liver oil, 4.00; soybean lecithin, 3.00; arginine HCl, 3.00; sucrose, 9.0; α-starch, 3.00; vitamin*5, 1.80; mineral*6, 8.00; cholesterol, 1.00; glucosamine HCl, 1.00; sodium citrate, 0.50; sodium succinate, 0.50; and agar, 8.00.

*5 Vitamin composition (mg/100 g diet): ρ-amino benzoic acid, 15.80; biotin, 0.63; inositol, 632.00; niacin, 63.20; Ca-pantothenate, 94.80; pyridoxine-HCl, 18.96; riboflavin, 12.64; thiamine-HCl, 6.32; folic acid, 1.26; cyanocobalamine, 0.13; choline chloride, 948.00; menadione, 6.34; and calciferol, 1.88.

*6 Mineral composition (g/100 g diet): $K_2HPO_4$, 1.86; $Ca_3(PO_4)_2$, 2.55; $MgSO_4 \cdot 7H_2O$, 2.84; and $NaH_2PO_4 \cdot 2H_2O$, 0.75.

for gonadosomatic index (GSI, gonad weight/body weight×100) and hepatosomatic index (HSI, hepatopancreas weight/body weight×100) as an initial group. The prawns were reared in aerated, flow-through sea water (about 1 liter/min) at a temperature of 23±1°C and a salinity of 34. Filtration was set-up through a sand-bed system that also functioned as a shelter for the burrowing prawns. A photoperiod of 100% darkness was maintained using an opaque hard plastic cover; the tanks were exposed to ceiling fluorescent light only during siphoning out of excess feeds and feces in the morning, at feeding time, ablation, and maturation stage check-up.

Prawns were fed with the experimental diets (moisture content=54.3±0.9%) slightly in excess, once daily at 16:30. Every morning, uneaten feed was collected from each tank, pooled, and frozen. After the feeding trial, the dry weight of the accumulated uneaten feed was obtained. Feed conversion efficiency (FCE in %, weight gain of prawn/feed consumed×100) was therefater calculated.

Prawns were bilaterally ablated by tying with surgical silk thread (Nescosuture, 2-0) at the base of the eyestalks. These were ablated during intermolt stages, within 30–37 days after feeding with the experimental diets. All animals were sacrificed, and GSI and HSI were measured 20 days after each prawn's ablation.

Weight gain, HSI, and GSI values were compared using analysis of variance and Duncan's multiple range test. All references to statistical significance were at the 5% level.

*Lipid Analyses*

The ovaries, hepatopancreas, and muscle at each stage of gonadal development were pooled and analyzed separately for lipid composition. Ovarian development was grouped into Stage I (GSI <2.0, immature ovaries) and stage II (GSI 2.0–4.9, slightly mature ovaries).[4] Total lipids (TL) were extracted with chloroform-methanol-water,[5] and separated into neutral (NL) and polar (PL) fractions in Sep-pak Silica Cartridges (Waters, S.A., U.S.A.)[6] using chloroform-methanol (98:2) for NL and methanol for PL. NL and PL were quantified photometrically[7] and lipid class compositions were analyzed by a thin-layer chromatography-flame ionization detector using the Iatroscan TH-10 in combination with a computing integrator, Iatrocorder TC-11 (Iatron Laboratories Inc., Japan). The developing systems were as described previously.[8] Fatty acid compositions (%) were determined by gas-liquid chromatography (Shimadzu GC-3BF) on 5% Shinchrom E-71.[8] Individual fatty acids were identified using pollack liver oil as a secondary standard and references to previous analyses.

*Vitamin A, E, and C Analyses*

Dietary and tissue vitamin A, E, and C contents were determined by high performance liquid chromatography (HPLC, Shimadzu LC-9A) using a UV-VIS detector (model SPD-6AV, Shimadzu). For vitamin A and E analyses, about 0.2 g of a freeze-dried sample was homogenized with hexane, ethanol, and water (4:2:1, v/v) for 2 min. in an Ultra-Turrax blender. After centrifugation at 2000 rpm for 5 min., the supernatant was rotary-

evaporated to dryness at 35–40°C and flashed with a stream of nitrogen. The residue was dissolved in ethanol, 50% KOH, and 1% pyrogallol in ethanol (6: 1: 0.4, v/v), and was refluxed at 90°C for 40 min. After cooling, hexane and water were added and the hexane layer was removed, filtered through a 0.45 $\mu$m cellulose acetate membrane filter (Advantec, Toyo Roshi Co., Japan), and evaporated. The residue was dissolved in 0.5 m*l* isopropanol and 20 $\mu l$ was introduced into the HPLC injection port. The column used was Shim-pack CLC-SIL, 0.15 m/6.0 $\phi$ (Shimadzu Corp., Japan). For vitamin A determination, the analytical conditions of HPLC were as follows: detection at UV 325 nm; column temperature, 35°C; eluent, 95% *n*-hexane +5.0% isopropanol; flow speed, 1.0 m*l*/min; pressure, 21 kg/cm$^2$. For vitamin E, the analytical conditions of HPLC were as follows: detection at UV 295 nm; column temperature, 35°C; eluent, 98.5% *n*-hexane +1.0% dioxane +0.5% isopropanol; flow speed, 1.0 m*l*/min; pressure, 21 kg/cm$^2$. In the analysis of APM contents, sample preparation and HPLC condidions were as described previously.[9] Vitamin A palmitate (Nakalai Tesque Inc., Japan), DL-$\alpha$-tocopherol (Wako Pure Chem. Ind. Ltd., Japan), L-ascorbic acid (Kanto Chemical Co., Japan), and L-ascorbyl-2-phosphate magnesium (Showa Denko Co., Japan) were used as standards.

## Results

The significantly highest GSI was observed in prawns fed with the control diet supplemented with vitamins A, E, and APM (Table 2). The prawn weight gains were reduced significantly by feeding with vitamin A or E-unsupplemented diets, but no significant difference in weight gain was obtained between prawns in APM-unsupplemented treatment and prawns in the control treatment. The FCE of prawns fed with the control diet was also higher compared to the diets unsupplemented with either vitamin A, E, or APM. Moreover, the HSI of prawns fed with the control diet was significantly higher compared to the vitamin A, E, or APM-unsupplemented groups. No mortality occurred during the feeding period.

The control and initial prawn tissues contained higher vitamin A, E, and C than those of prawns fed with diets unsupplemented with vitamin A, E, or APM, respectively (Table 3).

The lipid components in the ovaries, hepato-

**Table 2.** Mean weight gain, feed conversion efficiency (FCE), survival, hepatosomatic index (HSI), and gonadosomatic index (GSI) of prawns

| Treatment | Weight gain (g) | FCE (%) | Survival (%) | HSI (%) | GSI (%) |
|---|---|---|---|---|---|
| Control | 3.6±1.2$^a$ | 78.9 | 100 | 3.8±0.3$^a$ | 3.0±0.8$^a$ |
| No vit. A added | 2.8±0.7$^b$ | 61.4 | 100 | 3.1±0.7$^b$ | 1.7±0.7$^b$ |
| No vit. E added | 2.2±0.6$^b$ | 59.6 | 100 | 2.9±0.6$^b$ | 1.7±0.8$^b$ |
| No APM added | 3.1±0.9$^{ab}$ | 72.5 | 100 | 3.0±0.5$^b$ | 1.9±1.1$^b$ |

Initial: weight=20.2±0.9 g (n=32); HSI=2.8±0.3 (n=8); GSI=0.6±0.2 (n=8). Treatment means with the same superscripts are not significantly different at 5% level. Mean±standard deviation.

**Table 3.** Vitamin A, DL-$\alpha$-tocopherol and ascorbic acid contents of prawns before and after the feeding trial ($\mu$g/g dry tissue)

| Treatment | Inititial | Control | No vit. A added | No vit. E added | No APM added |
|---|---|---|---|---|---|
| *Vitamin A* | | | | | |
| Ovaries | 1.50 | 0.97 | 0.14 | 0.59 | 0.92 |
| Hepatopancreas | 2.84 | 4.13 | 1.85 | 4.10 | 4.59 |
| Muscle | 0.31 | 0.23 | 0.04 | 0.15 | 0.30 |
| *Vitamin E* | | | | | |
| Ovaries | 110.0 | 258.1 | 276.5 | 70.5 | 238.9 |
| Hepatopancreas | 319.3 | 334.1 | 385.1 | 213.9 | 382.4 |
| Muscle | 130.6 | 121.9 | 119.4 | 74.7 | 107.3 |
| *Ascorbic acid* | | | | | |
| Muscle | 67.3 | 55.4 | 57.8 | 58.6 | 46.2 |

**Table 4.** Lipid class concentrations (mg/g dry tissue) of the ovaries, hepatopancreas, and muscles of prawns (Stage II)

| | Initial*1 | Control | No vit. A added | No vit. E added | No APM added |
|---|---|---|---|---|---|
| *Ovaries* | | | | | |
| TL*2 | 21.7 | 21.9 | 17.5 | 16.4 | 20.7 |
| NL | 13.7 | 9.6 | 7.3 | 7.4 | 10.0 |
| PL | 8.1 | 12.3 | 10.2 | 9.0 | 10.7 |
| SE | 0.6 | 0.3 | 0.2 | 3.7 | 2.6 |
| TG | 2.6 | 7.7 | 3.5 | 3.0 | 3.6 |
| FFA | 1.4 | 0.9 | 2.1 | 0.3 | 1.8 |
| CHO | 8.8 | 0.4 | 0.7 | 0.2 | 1.0 |
| DG | — | 0.1 | 0.3 | — | 0.3 |
| MG | 0.3 | 0.2 | 0.6 | 0.1 | 0.7 |
| PE | 0.8 | 0.2 | 0.1 | 0.1 | 0.9 |
| PI+PS | 0.3 | 0.6 | 0.6 | 0.5 | 0.5 |
| PC | 5.0 | 10.2 | 8.8 | 7.7 | 9.2 |
| SM | 1.9 | 1.2 | 0.6 | 0.7 | 0.1 |
| *Hepatopancreas* | | | | | |
| TL | 12.6 | 50.5 | 47.5 | 40.6 | 47.4 |
| NL | 9.7 | 43.4 | 41.7 | 35.0 | 42.6 |
| PL | 2.9 | 7.1 | 5.8 | 5.6 | 4.8 |
| SE | 0.1 | 0.3 | — | — | 1.2 |
| TG | 6.5 | 38.5 | 20.5 | 16.3 | 7.2 |
| FFA | 0.9 | 2.6 | 14.1 | 14.1 | 28.4 |
| CHO | 1.0 | 1.0 | 4.6 | 1.5 | 1.7 |
| DG | 0.5 | 0.4 | 0.8 | 1.0 | 1.4 |
| MG | 0.5 | 0.5 | 1.7 | 1.9 | 2.7 |
| PE | 0.1 | 5.0 | 0.1 | 0.9 | 0.4 |
| PI+PS | 0.1 | 1.7 | 0.4 | 0.2 | 0.5 |
| PC | 2.4 | 0.1 | 2.5 | 1.7 | 3.0 |
| SM | 0.2 | 0.2 | 2.3 | 2.1 | 0.8 |
| LPC | 0.1 | 0.1 | 0.5 | 0.7 | 0.1 |
| *Muscle* | | | | | |
| TL | 6.0 | 6.5 | 5.8 | 6.2 | 5.3 |
| NL | 1.0 | 1.4 | 1.3 | 1.3 | 1.7 |
| PL | 5.0 | 5.1 | 4.5 | 4.9 | 3.6 |
| FFA | tr | 0.1 | tr | 0.2 | 0.2 |
| CHO | 0.9 | 1.2 | 1.1 | 0.9 | 1.3 |
| PE | tr | 1.9 | 0.1 | 0.1 | 0.1 |
| PI+PS | 0.5 | 0.3 | 0.6 | 0.2 | 0.5 |
| PC | 3.9 | 2.5 | 3.4 | 2.3 | 2.7 |
| SM | 0.4 | 0.3 | 0.4 | 1.5 | 0.3 |
| LPC | 0.1 | 0.1 | tr | 0.8 | tr |

*1 Stage I.

*2 TL, total lipid; NL, neutral lipid; PL, polar lipid; SE, steryl esters; TG, triglycerides; FFA, free fatty acids; CHO, cholesterol; DG, diglycerides; MG, monoglycerides, PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; SM, sphingomyelin; and LPC, lysophosphatidylcholine.

pancreas, and muscle of *P. japonicus* were markedly affected by non-supplementation in dietary vitamins A, E, and APM (Table 4). TL concentrations in these organs and tissues of prawns fed with diets unsupplemented in either vitamin A, E, or APM were lower than those of the control prawns. In ovaries and muscles, PL concentrations were higher than NL, regardless of dietary treatments. The hepatopancreas contained the highest TL, and NLs were dominant. Prawns fed with the control diet had higher triglycerides (TG) and phosphatidylcholine (PC) in the ovaries, higher phosphatidylethanolamine (PE), phosphatidylinositol+phosphatidylserine as well as TG in

**Table 5.** Fatty acid compositions (%) of neutral and polar lipids in the ovaries, hepatopancreas and muscle of prawn

| | Initial* | | Control | | No vit. A added | | No vit. E added | | No APM added | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NL | PL | NL | PL | NL | PL | NL | PL | NL | PL |
| *Ovaries*: | | | | | | | | | | |
| 14:0 | 3.2 | 1.ç | 2.5 | 1.1 | 1.9 | 0.9 | 1.4 | 0.8 | 3.3 | 1.5 |
| 16:0 | 21.2 | 22.3 | 23.4 | 18.5 | 22.9 | 17.9 | 21.6 | 18.2 | 26.8 | 20.1 |
| 16:1 | 6.0 | 6.6 | 8.2 | 6.1 | 12.9 | 10.8 | 11.8 | 10.6 | 14.2 | 12.9 |
| 18:0 | 7.4 | 8.2 | 2.5 | 8.9 | 2.7 | 7.8 | 2.6 | 7.6 | 3.0 | 6.1 |
| 18:1n-9 | 22.8 | 22.7 | 23.1 | 23.0 | 29.3 | 27.2 | 32.6 | 27.0 | 29.6 | 26.8 |
| 18:2n-6 | 8.7 | 8.6 | 15.4 | 13.9 | 12.3 | 11.9 | 12.2 | 10.9 | 10.3 | 10.1 |
| 20:1n-9 | 2.8 | 2.8 | 2.1 | 2.5 | 2.5 | 2.8 | 2.6 | 3.5 | 2.3 | 3.1 |
| 20:2n-6 | 0.7 | — | 0.7 | 1.0 | 0.8 | 1.3 | 0.9 | 1.2 | 0.6 | 1.3 |
| 20:3n-3+20:4n-6 | 4.0 | 4.9 | — | 1.8 | 1.0 | 1.2 | 1.1 | 1.3 | 1.2 | 1.4 |
| 20:5n-3 | 11.9 | 12.0 | 6.9 | 10.3 | 3.5 | 7.9 | 3.3 | 7.1 | 2.3 | 6.8 |
| 22:6n-3 | 6.0 | 7.4 | 10.1 | 8.1 | 6.1 | 5.7 | 6.2 | 6.9 | 4.2 | 6.4 |
| *Hepatopancreas*: | | | | | | | | | | |
| 14:0 | 2.0 | 0.7 | 1.3 | 0.8 | 0.7 | 1.9 | 0.9 | 0.6 | 1.3 | 0.3 |
| 16:0 | 14.8 | 15.8 | 21.6 | 18.5 | 16.9 | 19.6 | 19.4 | 16.4 | 19.8 | 16.6 |
| 16:1 | 6.5 | 5.9 | 3.4 | 3.4 | 5.7 | 4.5 | 6.0 | 7.9 | 7.0 | 8.8 |
| 18.0 | 3.0 | 5.5 | 3.5 | 9.1 | 2.9 | 6.4 | 5.9 | 5.5 | 3.3 | 6.2 |
| 18:1n-9 | 20.6 | 19.0 | 22.9 | 20.9 | 25.6 | 25.2 | 28.3 | 26.9 | 29.7 | 26.2 |
| 18:2n-6 | 11.6 | 10.9 | 18.5 | 16.3 | 15.2 | 14.7 | 16.4 | 15,3 | 16.3 | 14.5 |
| 20:1n-9 | 10.0 | 3.5 | 6.9 | 3.2 | 6.5 | 2.7 | 7.9 | 3.3 | 7.7 | 3.0 |
| 20:2n-6 | 1.1 | 0.9 | 3.3 | 1.2 | 1.1 | 1.4 | 1.2 | 1.4 | 1.2 | 1.6 |
| 20:3n-3+20:4n-6 | 1.8 | 3.7 | 0.8 | 2.5 | 0.9 | 2.0 | 0.9 | 2.1 | 0.9 | 2.0 |
| 20:5n-3 | 7.4 | 14.9 | 3.5 | 10.4 | 2.2 | 8.3 | 1.5 | 6.0 | 1.9 | 6.7 |
| 22:6n-3 | 8.1 | 11.4 | 6.5 | 12.2 | 5.9 | 10.3 | 2.2 | 9.7 | 2.3 | 6.8 |
| *Muscle* | | | | | | | | | | |
| 14:0 | 1.2 | 0.8 | 2.9 | 1.7 | 2.0 | 0.3 | 0.5 | 0.9 | 0.8 | 0.6 |
| 16:0 | 21.4 | 20.1 | 27.9 | 23.8 | 24.8 | 23.6 | 26.5 | 24.0 | 24.9 | 24.8 |
| 16:1 | 3.8 | 4.3 | 4.1 | 4.3 | 5.8 | 4.5 | 4.1 | 4.6 | 4.1 | 4.7 |
| 18:0 | 6.9 | 8.6 | 7.6 | 9.3 | 10.4 | 9.3 | 11.5 | 10.1 | 10.8 | 10.6 |
| 18:1n-9 | 21.2 | 20.1 | 20.6 | 21.9 | 28.5 | 23.9 | 26.8 | 23.3 | 24.2 | 26.2 |
| 18:2n-6 | 18.3 | 10.6 | 12.8 | 14.9 | 10.8 | 14.5 | 11.0 | 15.1 | 11.9 | 14.1 |
| 20:1n-9 | 2.4 | 1.9 | 2.3 | 2.3 | 2.8 | 2.1 | 2.2 | 2.6 | 2.0 | 2.2 |
| 20:2n-6 | — | 0.5 | 0.6 | 0.6 | — | 0.8 | 0.6 | 0.8 | 0.6 | 0.8 |
| 20:3n-3+20:4n-6 | 3.0 | 2.7 | 1.7 | 1.8 | 1.6 | 1.1 | 1.6 | 1.2 | 1.7 | 1.4 |
| 20:5n-3 | 9.7 | 13.5 | 7.5 | 9.1 | 3.5 | 7.9 | 6.1 | 6.2 | 7.9 | 7.5 |
| 22:6n-3 | 9.1 | 12.3 | 8.9 | 8.0 | 7.4 | 6.8 | 6.9 | 6.6 | 7.1 | 6.0 |

* Stage I.

the hepatopancreas, and higher PE in the muscle than those fed vitamins A, E, or APM-unsupplemented diets. The relative percentages of selected fatty acids in the NL and PL of ovaries, hepatopancreas and muscles are shown in Table 5. The control diet resulted in higher 20: 5n-3 and 22: 6n-3 in the ovaries, hepatopancreas, and muscle of prawns than the vitamin A, E, or APM- unsupplemented diets.

## Discussion

The present suty showed the importance of supplemental vitamins A, E, and APM in diets of broodstock prawns for enhanced ovarian development. It appears that the fat-soluble vitamins A and E contained in the lipids in the basal ingredients were sufficient to maintain excellent survival; however for significantly better weight gain and gonadal development, supplementation of both vitamins A and E is necessary.

Significantly reduced GSI in the vitamin A-unsupplemented treatment demonstrates that vitamin A is an essential nutrient for the gonadal development of *P. japonicus*. It has been reported that the mechanism of action of retinoic acid (the active derivative of vitamin A) is closely similar to that of steroid hormones and thyroxin, involving activation of the expression of specific genes, and thus placing retinoids in the category of hormones regulating growth, differentiation, and embryonic development.[10] It has been reported that crustaceans build up vitamin A reserves during maturation, which are then transferred to the oocytes.[11] To date, no study using using semi-purified diets to determine the effect of dietary vitamin A on the gonadal development of other penaeid prawns has been reported. In crustaceans, up to 90% of vitamin A is concentrated in the eyes.[12] It is an interesting line of research to see the mobilization of vitamin A when the broodstock prawn in eyestalk-ablated.

Since highly unsaturated fatty acids (HUFA) are essential in the diets of penaeids,[13] it is postulated that as a metabolic antioxidant, vitamin E will be also important.[14] Vitamin E may also be necessary in preventing the oxidation of HUFA in feed. Vitamin E is indispensable in broodstock diets for ayu, carp, rainbow trout, and red seabream.[15] Vitamin E plays a very significant role in reproduction, found to be required in *Penaeus indicus* broodstock diets affecting the quality of eggs in terms of hatching rate and larval survival.[16] No supplementation of vitamin E in the diet of *P. japonicus* broodstock yielded lower GSI compared to the control diet, indicating a requirement of this vitamin for gonadal development.

Vitamin C plays a role synergistically with vitamin E for the maintenance of intracellular antioxidants and free radical traps.[17] Moreover, vitamin C acts as an enzyme co-factor in the formation of collagen[18] and as a regulator of steroidogenesis in adrenal gland and gonads.[19] A positive effect of vitamin C in fish eggs on hatching performance has been demonstrated.[20-21] It has been suggested that vitamin C may have an important function in fish reproduction and the feeding of a vitamin C-free diet to broodstock could diminish reproductve performance.[22] Vitamin C is an essential nutrient for the juvenile penaeid shrimp.[23-26] However, this has been known to be unstable; thus, a relatively stable derivative of vitamin C, L-ascorbyl-2-phosphate magnesium magnesium (APM) was used in the present sudy. A supplement of 215–430 mg APM per kg diet is sufficient to prevent clinical signs of vitamin C deficiency in *P. japonicus* juveniles.[1] Normal growth was obtained in *P. japonicus* fed with diets containing 215 mg/kg vitamin C activity from APM, whereas diets with 43 mg/kg diet or less incurred mortality.* In the present study, no significant difference in weight gain was obtained between the control diet and the APM-unsupplemented diet. However, dietary non-supplementation of APM resulted in a significantly lower GSI than did the control diet. Possibly, large *P. japonicus* ($\geq$20 g) have increased storage capacity and endogenous reuse of vitamin C. This vitamin C store appeared to support good growth, but was inadequate for gonadal development. Results demonstrated the essentiality of APM as a vitamin C source in diets for gonadal development of *P. japonicus*.

Feed conversion efficiency appeared to be affected by the non-supplementation of the test micronutrients. The vitamin A or E-unsupplemented diets resulted in low FCE values. The APM-free diet was more efficient that the vitamin A or E-unsupplemented diets, but less efficient compared to the control. It is possible that because prawns consumed less of the diets unsupplemented in either vitamin A, E, or APM, the intake of nutrients (particularly n-3 HUFA) were also lower, resulting in lower deposits in the ovaries, hepatopancreas, and muscle.

In the present study, non-supplementation in either dietary vitamin A, E, or APM led to inferior GSI than the control diet. Although only incomplete ovarian maturation was obtained, results demonstrated that under the conditions of the present experiment, supplementation of 15 mg Vitamin A palmitate, 50 mg DL-$\alpha$-tocopherol acetate and 50 mg L-ascorbyl-2-phosphate magnesium per 100 g of diet significantly increased the GSI of ablated *P. japonicus*. The effects of graded dietary levels of vitamins A, E, and C on complete gonadogenesis, fecundity, spawning, egg, and larval quality need to be investigated further.

* Y. Takahashi, T. Itami, and Y. Aoki: Effect of phosphitan C (Mg-L-ascorbyl-2-phosphate) on the growth and body defense mechanism of kuruma shrimp. *Jap. Soc. Fish. Pathology Meeting Report*, March 1989 (1989).

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