

Vitamin A effects and requirements on the juvenile Kuruma Prawn *Marsupenaeus japonicus*

Efectos y requerimientos de vitamina A en los juveniles del camarón Kuruma *Marsupenaeus japonicus*

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ABSTRACT

A 40-day feeding experiment was conducted to assess the effect and requirements of dietary vitamin A (vitA) of juvenile Kuruma Prawn *Marsupenaenus japonicus*. Nine semi-purified diets were prepared with supplementations of 0, 3,000, 6,000, 9,000, 12,000, 15,000, 18,000, 21,000 and 24,000 international units of vitA per kg (IU vitA/kg) and fed once a day to triplicate groups of 15 juveniles per tank (initial weight of 0.14 ± 0.001 g, mean \pm standard error). Weight gain (WG), specific growth rate (SGR) and feeding efficiency ratio (FER) were significantly lower in the juveniles fed with the diets contained 0 and 3,000 IU vitA/kg than in the other groups. Similar values of total retinol (ROL) in whole body were detected only in the juveniles fed on the diets supplemented with 21,000 and 24,000 IU vitA/kg: 10.22 ± 2.74 and 10.70 ± 0.14 μ g ROL /g of wet weight, respectively (mean \pm standard error). It was not possible to detect retinol in the whole body samples in any of the experimental groups. According to the WG analyzed by the broken line regression, the dietary requirements of vitA should be of 7,500 IU vitA/kg.

Key words: *Marsupenaenus japonicus* , vitamin A , retinol, retinal, Kuruma Prawn.

RESUMEN

Un experimento de alimentación de 40 días se realizó para determinar el efecto y requerimientos de vitamina A (vitA) en la dieta de juveniles de camarón Kuruma *Marsupaenus japonicus*. Nueve dietas semipurificadas se prepararon con suplementaciones de 0, 3,000, 6,000, 9,000, 12,000, 15,000, 18,000, 21,000 y 24,000 unidades internacionales de vitA por kg (IU vitA/kg) y se utilizaron para alimentar una vez por día a grupos por triplicado de 15 juveniles por tanque (peso inicial de 0.14 ± 0.001 g, promedio \pm error estándar). La ganancia en peso (WG), la tasa de crecimiento específico (SGR) y la tasa de eficiencia del alimento (FER) fueron significativamente más bajas en los juveniles alimentados con las dietas que contenían 0 y 3,000 IU vitA/kg que en los otros grupos. Valores similares de retinol (ROL) total en el cuerpo fueron detectados exclusivamente en los juveniles alimentados con las dietas suplementadas con 21,000 y 24,000 IU vitA/kg: 10.22 ± 2.74 and 10.70 ± 0.14 μ g ROL/g de peso húmedo, respectivamente (promedio \pm error estándar). No fue posible

detectar retinal en las muestras de cuerpo en ninguno de los grupos experimentales. De acuerdo con el WG analizado con una regresión de línea de rompimiento, los requerimientos de vitA en la dieta deben de ser 7,500 IU vitA/kg

Palabras clave: *Marsupenaeus japonicus*, vitamina A, retinol, retinal, camarón Kuruma.

INTRODUCTION

Vitamin A (vitA) is the generic descriptor for compounds with the qualitative biological activity of all-*trans*-retinol, and can be found in the animals as retinol (ROL), retinal (RAL) and retinyl esters (RNE) (Bearer-Rogers *et al.*, 2001). VitA has been related to multiple physiological processes in vertebrates, including vision, growth, cell differentiation and reproduction (Combs, 1998). In crustaceans, vitA has been related to the vision process (Dall, 1995; Conklin, 1997; Goldsmith & Cronin, 1993; Srivastava & Goldsmith, 1997), but also it has been reported as a necessary compound for normal growth in species such as *Litopenaeus vannamei* Bonne, 1931 (He *et al.* 1992), *Penaeus monodon* Fabricius, 1798 (Reedy *et al.*, 1999), *Marsupenaeus japonicus* (Kanazawa, 1985) and *P. chinensis* Osbeck, 1765 (Chen & Li, 1994). As well, vitA has been related to the normal ovarian development of *M. japonicus* (Alava *et al.*, 1993), *L. vannamei* (Liñan-Cabello *et al.*, 2002; Liñan-Cabello *et al.*, 2003), *Cherax quadricarinatus* Martens, 1868 (Liñan-Cabello *et al.*, 2004) and *P. chinensis* (Mengqing *et al.*, 2004).

For crustaceans, only limited information on the vitA quantitative requirement is available: Akiyama *et al.* (1992) reported that commercial species of penaeids might require a supplementation of 10,000 International Units of vitA per kg diet (IU vitA/kg, 1 IU = 0.3 µg of all-*trans*-ROL), while Conklin (1997) reported an inclusion of 5,000 IU vitA/kg for general diets of penaeid species. Recently, Shiau & Chen (2000) reported that juvenile *P. monodon* requires a minimum inclusion of 8,200 IU vitA/kg for optimal growth.

As for the Kuruma Prawn *M. japonicus*, also little information on dietary vitA requirement has been reported. Kanazawa (1985) reported that the shortage of vitA in the diets of the larval stage caused the cessation or retardation of metamorphosis, growth and high mortality. As well, Alava *et al.* (1993) reported that a supplementation of 15,000 IU vitA/kg, enhanced the ovarian development in the brookstock of Kuruma Prawn. The quantitative requirements of dietary vitA have been not established for this species and for thus, the aim of the present study was to assess the dietary requirement of vitA for growth in the juvenile Kuruma Prawn, one of the most important species of aquaculture in Japan.

MATERIAL AND METHODS

Test Diets. The table 1 was formulated according to the recent of nutrient requirement information for Kuruma Prawn (Teshima *et al.*, 2001). Defatted krill meal and squid meal (Nippon Suisan Kaisha Ltd., Tokyo, Japan), and vitamin-free casein (Wako Pure

Chemical Ind., Osaka, Japan) were used as protein sources. Squid liver oil (Riken Vitamin, Tokyo Japan), soybean lecithin (Wako Pure Chemical Ind., Osaka, Japan), cholesterol (Nacalai Tesque Inc., Kyoto, Japan) and *n*-3 highly unsaturated fatty acids (*n*-3 HUFA, DHAc, Oriental Co., Chiba, Japan) were used as lipid sources; while α -starch, dextrin (Wako Pure Chemical Ind., Tokyo, Japan) and sucrose (Wako Pure Chemical Ind., Tokyo, Japan) were used as carbohydrate sources. VitA palmitate (1,000 IU vitA/mg, Nacalai Tesque Inc. Kyoto, Japan) was added to the test diets to provide concentrations of 0, 3,000, 6,000, 9,000, 12,000, 15,000, 18,000, 21,000 and 24,000 IU vitA/kg of diet (table 2). Diets were

Table 1. Basal composition of the test diets fed to Kuruma Prawn juvenile for 40 days. The proximate composition in dry basis (%) of the basal diet was (mean \pm standard error): protein, 59.2 \pm 1.5; lipid, 8.9 \pm 2.1; ash 7.26 \pm 1.2; moisture, 11.13 \pm 2.1.

Ingredient	g/kg diet
Vitamin-free casein	350
Squid meal ¹	100
Krill meal ¹	100
Squid liver oil	30
Soybean lecithin	30
<i>n</i> -3 HUFA	25
Cholesterol	5
α -Starch	50
Sucrose	50
Dextrin	50
Mineral mix ²	60
Vitamin mix ³	32
Attractants ⁴	10
Activated gluten	80
Cellulose + vitA palmitate	28

¹Defatted material

²Mineral mix (g kg⁻¹): MgSO₄·7H₂O, 21.4; NaH₂PO₄·2H₂O, 5.6; KH₂PO₄, 14.0; Ca₃(PO₄)₂, 19.0.

³Vitamin mix (g kg⁻¹): *p*-aminobenzoic acid, 0.23; biotin, 0.01; myo-inositol, 9.1; nicotinic acid, 0.91; Ca-pantothenate, 1.3; pyridoxine-HCl, 0.27; riboflavin, 0.18; thiamine-HCl, 0.09; menadione, 0.08; α -tocopherol, 0.4; cyanocobalamine, 0.001; calciferol, 0.02, L-ascorbyl-2-phosphate-Mg, 7.1; folic acid, 0.001 and choline chloride, 12.12.

⁴Attractants (g kg⁻¹ diet): sodium citrate, 3; sodium succinate, 3; glucosamine HCl, 4.

prepared by similar methods as described by Teshima *et al.* (2001). Briefly, all dry ingredients were mixed with the lipid mix (including vitA palmitate and the other fat-soluble vitamins), then, water (40 %) was added and mixed until a dough-like was obtained. The dough was passed through a pelletizer to obtain 1.6-mm pellets, which were dried at 60 °C in a constant temperature oven for 60 min. To improve the stability in water, dried pellets were steamed for 1 min at 100 °C. All diets were stored until used at -24 °C.

Feeding experiment. Oviparous Kuruma Prawns were obtained from Matsumoto Suisan, Miyazaki, Japan and transported to the Kamoike Marine Production Laboratory (Faculty of Fisheries, Kagoshima University, Japan) and were placed in a 500 L tank for spawning. Hatched larvae were reared by feeding live food (*Chaetoceros* sp. and *Artemia* nauplii) and a micro-diet (Shrimp Seed Production Micro-diet number 0-1, Himashigaru Co. Ltd., Kagoshima, Japan).

Ten days before starting the feeding trial, the juveniles were fed on the diet without vitA supplementation in order to get them adjusted to the test diets and deplete their vitA reserves, as reported by Shiao & Chen (2000). At the beginning of the feeding trial, juveniles were sorted to obtain equal size. Twenty-seven plastic tanks of 30 l capacity (filled with 20 l sea water) were used and 15 juveniles (initial weight of 0.14 ± 0.001 g, mean \pm standard error) were randomly stocked per tank and each test diet was fed to triplicate groups of juveniles. The juveniles were fed by hand the respective diets at 10 % of the mean body weight per day. The daily ration was offered once a day, during the evening. The juveniles were weighted every ten days and the ration size was adjusted accordingly. Fecal matter and uneaten diet were removed by siphoning from the tank. Uneaten diets were quantified for measurement of feed intake.

Each tank was equipped with an under-gravel filter covered with a sand bed and water was circulated through the action of an air-lift device. The water flow rate on each tank was 0.8 l min^{-1} . Through the feeding experiment water quality parameters (mean \pm SD) were: dissolved oxygen 5.8 ± 0.1 mg/l, pH 8.02 ± 0.1 , salinity 35 ± 0.3 g/l, and water temperature ranged from 28 to 30 °C. All tanks were maintained under a natural photoperiod of 12 h light, 12 hrs dark. The feeding experiment using the test diets was conducted for a period of 40 days.

At the end of the feeding experiment, the juveniles were starved for 24 h, then weighed and examined externally for symptoms of deficiency or excess of vitA. Five prawns for each dietary replicate tank were chosen randomly, pooled, dried in a freeze dryer and kept at -24 °C until proximate composition analysis. The rest of the prawns of each treatment were pooled and stored at -80 °C until vitA analysis.

Proximate analysis and vitA analysis. The diets and the whole body of Kuruma Prawn were analyzed according to

Table 2. Vitamin A (vitA) concentrations of the test diets used for rearing juvenile Kuruma Prawn for 40 days.

Treatment (IU vitA/kg)	Added vitA (mg/kg) ¹	Analyzed vitA (IU vitA/kg) ²
0	0	500 \pm 25
3,000	3	3,230 \pm 23
6,000	6	6,552 \pm 59
9,000	9	9,790 \pm 150
12,000	12	12,002 \pm 15
15,000	15	15,959 \pm 76
18,000	18	17,990 \pm 89
21,000	21	22,300 \pm 150
24,000	24	24,055 \pm 10

¹VitA added as retinol palmitate

²Mean \pm standard error

the Association of Official Analytical Chemists methods (1990) for moisture, crude protein and ash contents. Total lipids were determined by the method reported by Bligh & Dyer (1959).

VitA analysis in the diets and the whole body was performed by high performance liquid chromatography (HPLC). Total ROL was extracted and measured by the techniques reported by Estevez & Kanazawa (1995), while RAL was extracted and measured according with Hernandez *et al.* (2004). The HPLC system consisted of a pump LC-3A (Shimadzu Corp. Tokyo, Japan), a UV detector SPD-6A (Shimadzu Corp. Tokyo, Japan) set at 325 nm, a data processor Chromatopac C-R7Ae-plus (Shimadzu Corp. Tokyo, Japan), and a silica column Shim-pack CLC-SIL (4.6 x 150 mm, Shimadzu Corp. Tokyo, Japan). Hexane-isopropanol (95:5) and hexane-dichlorometane (2:1) were used as mobile phase for total ROL and RAL, respectively. A flow rate of 1 ml/min was used in both determinations.

Statistical analysis. Data on final body weight (BW), weight gain (WG), specific growth rate (SGR), feed efficiency ratio (FER), daily feed intake (DFI), survival rate, proximate analysis of whole body of each treatment were tested using one-way ANOVA (package super-ANOVA, Abacus Concepts, California, USA). Significant differences among the treatments were evaluated with Duncan's new multiple range test (Steel & Torrie, 1980). Statistical significance of differences was determined by setting the error at 5% ($p < 0.05$) for each set of comparisons. Dietary vitA requirement was estimated by the broken-line method (Robbins *et al.*, 1979). Regression analysis was performed using the software package Stat-View™ (Abacus Concepts, California, USA).

RESULTS

The means of BW, WG, SGR, FER, DFI and survival rate of the juvenile Kuruma Prawn are presented in table 3. Significant lower values of BW, WG, SGR and FER were observed in the juveniles fed with the diets containing 0 and 3,000 IU vitA/kg than the other treatments. The growth of the Kuruma Prawn in both groups started to decrease around the 30th day of the feeding trial. The highest values of BW, WG and SGR were observed in the juveniles fed with 18,000 IU vitA/kg, but no significant differences were observed with those fed the diets with 6,000, 9,000, 12,000, 15,000, 21,000 and 24,000 IU vitA/kg.

Significant lower survival rate was observed in the juveniles fed with the diets with 0, 12,000 and 24,000 IU vitA/kg than those fed with 18,000 IU vitA/kg. However, the mortality observed in those groups during the feeding trial was not related to the treatments with vitA. About 20 % of the juveniles fed the diet with 0 IU vitA/kg showed a lighter coloration than that observed in the other groups.

No significant differences were observed on the contents of moisture, ash and protein of the whole body among the groups (table 4). There was a tendency of low lipid content in the groups fed with no or low supplementation, but only lipid content was significantly lower in the groups fed with 3,000 and 6,000 IU vitA/kg than the group fed with 21,000 IU vitA/kg.

Total ROL was detected only in the juveniles fed on the diets supplemented with 21,000 and 24,000 IU vitA/kg. The contents of total ROL (mean of three samples \pm standard error) in these groups were: 10.22 ± 2.74 and 10.70 ± 0.14 μg ROL/g of wet

weight, respectively. No significant differences were observed among the means of both groups. As well, it was not possible to detect RAL in the whole body samples in any of the experimental groups.

Fig. 1 shows the dietary requirement of vitA for growth in terms of WG determined by the broken-line analysis. Based on mean WG, the regression equations were $y = 0.02x + 179$ ($r = 0.9$) and $y = 0.001x + 337$ ($r = 0.6$) and requirement was estimated to be 7,500 IU vitA/kg.

DISCUSSION

As vitA is mainly concentrated in the eyes of crustaceans, it is believed that its role is related to vision, and thus the requirements must to be lower than those reported for other species (Conklin, 1997). Dall (1995) reported that diets for penaeids might not need a vitA supplementation when fish oils are added to diets, while Conklin (1997) reported a maximum inclusion of 5,000 IU vitA/kg in the diets for commercial penaeids. The data of the present study indicated that dietary vitA was necessary for optimal growth of the juveniles of Kuruma Prawn *M. japonicus*, as also reported for *L. vannamei* (He et al., 1992), *P. monodon* (Reedy et al., 1999) and *P. chinensis* (Chen & Li, 1994). Based on the data of WG (table 3 and Fig. 1), the minimum dietary supplementation of vitA must be of 7,500 IU vitA/kg. This value is comparable to the value found for the juveniles of *P. monodon* (about 8,400 IU vitA/kg) (Shiau & Chen, 2000), the only species with quantitative requirements reported. A higher supplementation (15,000 IU vitA/kg) was recommended for the development of the ovaries of broodstock of Kuruma Prawn (Alava et al., 1993), but as in the

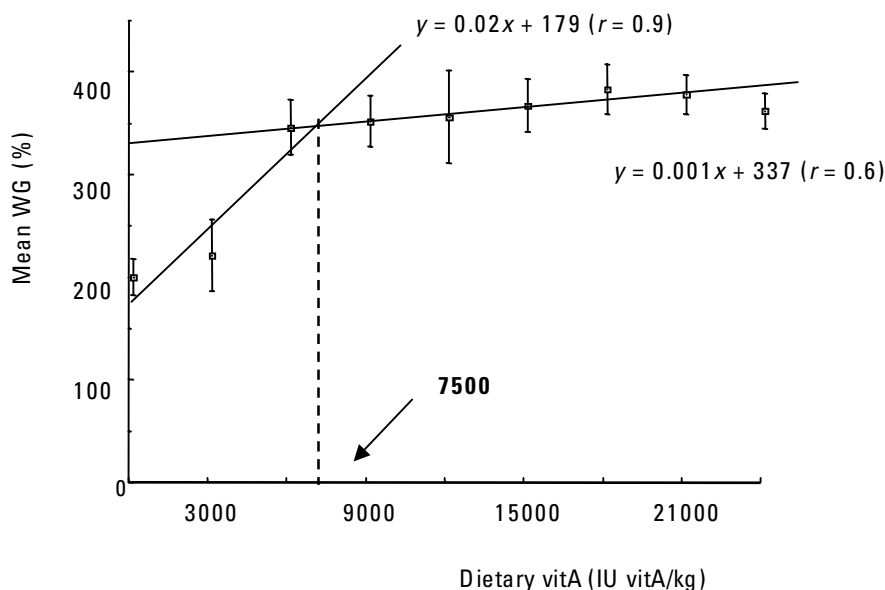


Figure 1. Relationship between the weight gain of juvenile kuruma prawn and the dietary vitamin A (vitA) level. Each point represents the mean of three groups of fish with 15 prawns per group. Requirement derived with the broken-line method for weight gain (WG) is 7,500 IU vitA/kg.

Table 3. Final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed efficiency ratio (FER), daily feed intake (DFI) and survival rate of juvenile Kuruma Prawn fed different levels of vitamin A (vitA) for 40 days. Values are the means of three replicate groups ± standard error. Means with different letters in the same column differ significantly ($p < 0.05$).

Treatment (IU vitA/kg)	FBW (g/prawn)	WG (%) ¹	SGR (%/day) ²	DFI (g/prawn/day)	FER ³	Survival (%)
0	0.41±0.02a	196 ± 18a	2.7±0.1a	0.04±0.01	0.02±0.01a	54±7a
3,000	0.44±0.04a	217 ± 36a	2.9±0.3a	0.04±0.01	0.02±0.01a	58±2ab
6,000	0.61±0.04b	342 ± 27b	3.7±0.2b	0.04±0.01	0.03±0.01b	63±2ab
9,000	0.62±0.02b	348 ± 15b	3.7±0.1b	0.04±0.01	0.04±0.01b	67±7ab
12,000	0.63±0.06b	353 ± 46b	3.7±0.3b	0.04±0.01	0.04±0.01b	50±5a
15,000	0.65±0.04b	363 ± 26b	3.8±0.1b	0.03±0.01	0.04±0.01b	67±1ab
18,000	0.67±0.04b	380 ± 24b	3.9±0.1b	0.03±0.01	0.03±0.01b	76±11b
21,000	0.66±0.02b	375 ± 19b	3.9±0.1b	0.04±0.01	0.03±0.01b	65±4ab
24,000	0.64±0.03b	359 ± 17b	3.8±0.1b	0.03±0.01	0.04±0.02b	50±4a

¹Weight gain = [(Final BW – Initial BW)/Initial BW] x 100

²Specific growth rate = [(ln Final BW – ln Initial BW)/40] x 100

³Feed efficiency ratio = weight gain (g) / total feed intake in dry basis (g)

case of other nutrients, vitA seems to be required in higher levels during the reproduction cycle (Conklin, 1997).

The most typical signs of nutrient deficiency reported for crustaceans are either the growth depression or mortality (Conklin, 1997; Chen & Li, 1994). The reduce growth observed in the juveniles of Kuruma Prawn fed with the diets of 0 and 3,000 IU IU vitA kg⁻¹ was considered as a vitA deficiency symptom. As well, the light coloration observed in the juveniles fed on those diets might be a sign of vitA deficiency, and similar results were

reported for juveniles of *P. monodon* when fed a vitA deficient diet (Shiau & Chen, 2000).

Shiau & Chen (2000) reported that juveniles of *P. monodon* showed increased content of total lipids in the hepatopancreas with increasing levels of dietary vitA. There was a similar tendency in the contents of total lipid in the whole body of juvenile Kuruma Prawn. These findings contrasts with those reported for other aquatic species, as high contents of dietary vitA decreased the total lipid content in whole body of fish, such as guppy *Poecilia reticulata* Peters, 1859 (Shim & Tan, 1990), greasy grouper *Epinephelus tauvina* Foskaskål, 1775 (Mohamed *et al.*, 2003) and Japanese flounder *Paralichthys olivaceus* (Hernandez *et al.*, 2005). However, the mechanisms of how might vitA affects the lipid deposition in crustaceans have been not studied yet and thus, require further research.

Table 4. Effects of different levels of vitamin A (vitA) on the body composition (% wet basis) of the juvenile Kuruma Prawn fed for 40 days. Values are the means of three replicate groups ± standard error. Means with different letters in the same column differ significantly ($p < 0.05$).

Treatment (IU vitA/kg)	Proximate composition			
	Moisture	Ash	Protein	Lipid
0	75.7 ± 1.1	5.1 ± 0.2	14.8 ± 0.2	4.1 ± 0.1ab
3,000	76.5 ± 0.8	4.4 ± 0.5	14.4 ± 0.1	3.8 ± 0.2a
6,000	77.0 ± 0.5	4.4 ± 0.1	14.6 ± 0.1	3.8 ± 0.1a
9,000	75.8 ± 0.8	4.6 ± 0.2	14.6 ± 0.2	4.3 ± 0.2ab
12,000	76.1 ± 1.1	4.6 ± 0.3	14.9 ± 0.1	4.2 ± 0.2ab
15,000	78.4 ± 1.3	4.7 ± 0.2	14.3 ± 0.5	4.1 ± 0.2ab
18,000	76.0 ± 0.6	4.6 ± 0.1	14.7 ± 0.1	4.5 ± 0.1ab
21,000	75.9 ± 1.2	4.5 ± 0.2	15.0 ± 0.1	4.5 ± 0.2b
24,000	76.4 ± 0.5	4.3 ± 0.2	14.8 ± 0.1	4.1± 0.2ab

The role of vitA in crustaceans was believed to be related to the vision process, and indeed RAL (the vitA metabolite that supports the synthesis of photopigments in the eyes; Combs, 1998) has been reported to be up to 85% of total vitA in the eye (Srivastava & Goldsmith, 1997) and an important compound for broodstock (Liñan-Cabello *et al.*, 2002, 2003) of some species of crustaceans. In contrast to those findings, it was not possible to detect RAL in the samples of whole body of Kuruma Prawn and total ROL was detected only in the samples of the juveniles fed on supplementations higher than 21,000 IU/kg. This might suggests that kuruma prawn storages vitA as ROL (either as free ROL or RNE) rather than RAL when fed high levels of vitA. Srivastava *et al.* (1996) reported that the crayfish *Procambarus* and the lobster *Homarus* sp. are able to storage ROL and RNE. During this study,

all vitA analysis were performed on whole body basis and it is difficult to establish where vitA is storage in the body of the Kuruma Prawn, but a similar trend of increased levels of total ROL were found in juveniles of *P. monodon*, when fed increasing levels of vitA (Shiau & Chen, 2000).

In conclusion, vitA was a necessary compound for the optimal growth of juveniles of Kuruma Prawn and the minimum supplementation for growth of vitA as ROL palmitate in this species must be of 7,500 IU vitA/kg. As well, it was possible to observed that juveniles of Kuruma Prawn are able to storage ROL when fed diets with inclusions more than 21,000 IU vitA/kg.

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