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The effect of vitamin E on growth, survival and hepatopancreas structure of the Argentine red shrimp *Pleoticus muelleri* Bate (Crustacea, Penaeidea)

Analia V Fernández Gimenez, Jorge L Fenucci & Ana M Petriella

Departamento de Ciencias Marinas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, CONICET, Funes 3350, Mar del Plata, Argentina

Correspondence: J L Fenucci, Departamento de Ciencias Marinas, Facultad de Ciencias Exactas y Naturales, UNMP, Funes 3350, B7602AYL, Mar del Plata, Argentina. E-mail: jfenucci@mdp.edu.ar

Abstract

Two trials were conducted to evaluate the growth, survival and hepatopancreas histology of the Argentine red shrimp *Pleoticus muelleri* (Bate, 1888) fed different levels of vitamin E and butylated hydroxytoluene (BHT) in a semipurified diet. The diets contained 0, 100, 600 or 1500 mg vitamin E kg^{-1} and 16 mg BHT kg^{-1} diet (trial 1) and 0, 1250, 1500, 1750 or 2000 mg vitamin E kg^{-1} diet, squid mantle and vitamin-free diet as a control (trial 2). After 30 days (trial 1), survival ranged between 43% and 64%, and the percentage weight gain of the shrimp varied from 22% to 31% with no significant differences among treatments ($P < 0.05$). After 40 days (trial 2), survival of shrimp fed the diet with no vitamin E and squid mantle was significantly lower (62%) than the other treatment (86–90%). Shrimp fed diets containing vitamin E from 1250 to 1750 mg kg^{-1} exhibited increased weight gain (34–65%); however, a significant difference was observed for shrimp fed the diet containing 2000 mg kg^{-1}. Histological results yielded differences among treatments. In shrimp fed 1750 mg kg^{-1} of vitamin E, the functional morphology of the organ was normal, with abundant secretion in the tubules. Signs of malnourishment such as cellular and nuclear retraction, desquamation of cells and hipertrofia, were evident in the hepatopancreas of shrimp fed the other diets. The results indicate that optimal vitamin E requirement for *P. muelleri* under the present experimental conditions appears to be approximately 1750 mg vitamin E kg^{-1} diet.

Keywords: crustacean, hepatopancreas, nutrition, vitamin E

Introduction

The Argentine red shrimp *Pleoticus muelleri* is an open thelycum species of high commercial value, occurring along Southwest Atlantic Ocean from Rio de Janeiro (Brazil) to Santa Cruz (Argentina) (Boschi 1986). Yearly and seasonal fluctuations in catches stress the importance of establishing commercial culture of this species.

One of the main problems of growing penaeid shrimps is related with inadequate knowledge of their dietary nutritional requirements. Numerous studies have revealed that fat-soluble vitamins A, D, E and K are essential in the diets of most animals for normal health and life functions, such as growth, development, maintenance and reproduction (He, Lawrence & Liu 1992). Early studies vitamin nutrition in crustaceans showed that dietary vitamin E was required by *Daphnia magna* (Viehoever & Cohen 1938) and *Moina macrocopa* (Conklin & Provasoli 1977). Several studies have been conducted to evaluate the dietary essentiality of vitamin E for penaeids (Cahu, Villete, Quazuguel & Guillaume 1991; He *et al.* 1992; Cahu, Cuzon & Quazuguel 1995; Hsu & Shiau 1999), although the vitamin E requirement of *P. muelleri*, has yet not been studied.

There is an increasing evidence that vitamins C and E have vital antioxidant roles in protecting lipid in tissues of aquatic animals (Conklin 1997).

Vitamin E may play a significant role in shrimp nutrition as an antioxidant, preventing polyunsaturated fatty acid oxidation in feeds as well as in shrimp tissues (Kanazawa 1985). Synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and ethoxyquin, are used in animal feeds to reduce oxidative rancidity (He & Lawrence 1993).

Kanazawa (1985) found that addition of vitamin E to diets resulted in improvement of survival of larval *Marsupenaeus japonicus*. He and colleagues (1992) reported that *Litopenaeus vannamei* showed significantly lower survival and weight gain when fed a vitamin E-free diet for 8 weeks. Alava, Kanazawa, Teshima and Koshio (1993) reported that vitamins A, E and C enhanced ovarian development in *M. japonicus*. Tocopherol is known to be one of the components affecting the quality of penaeid eggs, in terms of hatching rate and larval viability (Cahu *et al*. 1991; Fakhfakch, Villete & Cahu 1991). These studies also demonstrated that nutritional requirements for growth or reproduction are variable in penaeiod shrimp (Cahu *et al*. 1991).

The hepatopancreas is the major organ in decapod crustaceans and has many biological functions (Ceccaldi 1997). This gland occupies a large volume inside the cephalothorax. The histology of the *P. muelleri* hepatopancreas in intermolt is morphologically similar to that described for other Decapoda (Petriella, Díaz & Cuartas 1998) (Fig. 1).

The aim of this study was to evaluate the effect of various dietary levels of vitamin E and BHT on the shrimp *P. muelleri* to determine possible alterations in growth, survival and hepatopancreas structure as indicators of vitamin deficiency.

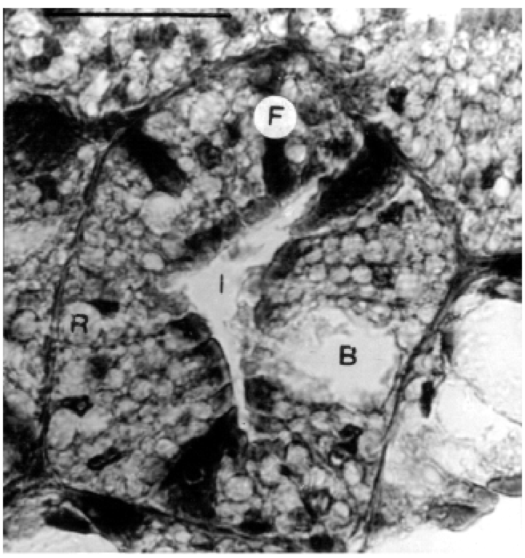

Figure 1 Hepatopancreas of wild *Pleoticus muelleri*: transverse section through a tubule showing the types of epithelial cells. Note the narrow lumen and the scarce intertubular space. B, cell B; F, cell F; R, cell R; l, lumen (bar = 25 μm).

Materials and methods

Several semipurified diets were designed containing different amounts of vitamin E (α-tocopherol acetate in form of Rovimix E 50®; Productos Roche S.A.Q., Buenos Aires, Argentina). A diet with 16 mg BHT kg^{-1} diet was also prepared. Dietary ingredients (Table 1) were mixed and diets were prepared by using a cold extrusion process, then oven-dried at 45 °C for 24 h (Fenucci & Zein-Eldin 1976). Dietary lipid used in the present study was fat-soluble stripped fish oil, prepared in the laboratory (Cuello & Carrizo 1986).

Two trials of 30 and 40 days, respectively, were conducted with *P. muelleri*. In the first trial, shrimp feeds having 0, 100, 600 or 1500 mg vitamin E kg^{-1} and a feed with 16 mg BHT kg^{-1} diet were tested. In the second trial, the effect of diet containing 0, 1250, 1500, 1750 or 2000 mg vitamin E kg^{-1} diet and fresh squid mantle (*Illex argentinus*) as standard feed, were tested. The shrimp were fed once a day in the morning

Table 1 Ingredient composition of standard semipurified diets

Ingredient	$g\,kg^{-1}$
Casein, vitamin-free	400
Manioc starch	220
Gelatin	120
Cellulose	100
Free fatty acids*	70
Sodium alginate	37
Cholesterol	20
Lecithin	10
Vitamin premix†	20
Mineral premix‡	3
Proximal composition	%
Moisture	7.1 ± 0.77
Total protein	44.5 ± 3.65
Total lipids	13.2 ± 2.63
Ash	4.16 ± 1.25

*Free fatty acids: 14:8 (4.5%); 15:0 (1%); 16:0 (18.9%); 16:1 (6.7%); 17:0 (1.3%); 16:2 (2%); 18:0 (2.7%); 18:1 (16.6%); 18:2 (2%); 18:3:3 (1.5%); 20:1 (6.7%); 20:2 (0.5%); 20:3:3+20:4:6 (1.3%); 20:5 (10.5%); 22:1 (4.2%); 22:3+22:4 (0.6%); 22:5 (0.6%); 22:6 (16.5%); 24:1 (1.9%).

†Vitamin premix without α-tocopherol acetate (mg kg^{-1}): cholecalciferol 35; thiamin 163; rivoflavin 156; pyridoxine 213; calcium pantothenate 250; biotin 250; niacin 500; folic acid 25; B_{12} HCL 20; ascorbic acid Rovimix STAY C 781; menadione 34; inositol 300; choline chloride 200; vitamin A acetate 140; wheat semolina csp; cellulose was replaced by appropriate amounts of α-tocopherol acetate (50%) in the premix to give different levels of vitamin E (Roche, Buenos Aires, Argentina).

‡Mineral premix: calcium 1000 mg; magnesium 500 mg; potassium 99 mg; zinc 30 mg; 10 mg; iron 10 mg; copper 2 mg; iodine 150 μg; selenium 200 μg; chromium 200 μg; molybdenum 500 μg (Twin Laboratories, Ronkonkoma, NY, USA).

at 5% of shrimp biomass initially, and then the ration was adjusted according to consumption. Exuviae and dead individuals were removed and recorded daily.

For trial 1, 105 shrimps were obtained from a commercial fisherman in the coastal waters of Mar del Plata, Argentina (38°S). For the second trial, 126 shrimps were reared from hatchery-raised postlarvae (wild broodstock from Mar del Plata, Argentina). All individuals were kept in 150-L glass aquaria with under-gravel filters as described by Boschi (1972) and fed squid mantle for 7 days prior to initiation of the feeding trial.

Triplicate groups of 7 animals (10 animals m^{-2}) ranging from 4.4 to 4.5 g and 1 g mean weight, were stocked in each aquaria for trials 1 and 2 respectively. Water temperature ranged from 17 to 23 °C, salinity was 34 g L^{-1} and pH 6.5. At the beginning and at the end of each trial, shrimps were weighed to the nearest 0.01 g.

At the end of trials, animals were anaesthetized on ice and the hepatopancreas of all individuals in intermolt (Díaz & Petriella 1990) were aseptically removed. In all cases, tissues were fixed in Davidson fluid (Bell & Lightner 1988) for 24 h, dehydrated in a progressive series of ethanol and embedded in butyl-paraffin and paraffin. Sections of 5 μm were stained with hematoxylin–eosin.

Diets were analysed for ash (550 °C) and dry matter (60 °C); total protein was estimated by the micro-Kjeldahl method (Barnes 1959) and total lipids were determined using Soxhlet method (IRAM 1985).

For the statistical analysis, the following tests were performed: Cochran or Barltlett, analysis of variance (ANOVA), Student's *t* and χ^2. For all analysis, statistical differences were considered significant at $P<0.05$ (Sokal & Rolhf 1979).

Results

In the first trial, after 30 days, survival ranged between 43% and 64%, and the percentage weight gain varied from 22% to 31% with no significant differences among treatments (Table 2).

In the second trial, after 40 days, shrimp fed squid mantle or vitamin-free diet both showed 62% survival; this value was significantly lower than those obtained with diets containing 1250 mg vitamin E kg^{-1} diet or more (86–90%). Shrimp fed diets containing vitamin E from 1250 to 1750 mg kg^{-1} exhibited an increase in mean weight; however, significant differences were only observed for shrimp fed the diet containing 2000 mg kg^{-1} (Table 3).

Table 2 Mean initial and final weight, percent weight gain and survival of shrimp fed different diets for 30 days (trial 1)

Vitamin E (mg kg^{-1} diet)	Mean weight (g ± s)* Initial	Final	ΔW (%)	Survival (%)
0	4.4 ± 0.73	5.7 ± 1.06	29 ± 6.53	50
100	4.5 ± 0.67	5.9 ± 0.69	31 ± 5.41	43
600	4.5 ± 0.82	5.5 ± 0.79	22 ± 2.66	57
1500	4.5 ± 0.75	5.6 ± 0.93	27 ± 6.02	64
BHT	4.4 ± 0.83	5.4 ± 1.17	23 ± 3.09	50

*Values are means ± SD of triplicates.
Δ*W*, weight gain; BHT, butylated hydroxytoluene.

Table 3 Mean initial and final weight, percent weight gain and survival of shrimp fed different diets for 40 days (trial 2)

Vitamin E (mg kg^{-1} diet)	Mean weight (g ± s)* Initial	Final	ΔW (%)	Survival (%)
Squid mantle	1.1 ± 0.30	1.7 ± 0.51	59 ± 6.36	62
Vitamin-free	1.0 ± 0.27	1.4 ± 0.35	45 ± 9.19	62
1250	1.1 ± 0.36	1.6 ± 0.51	50 ± 2.79	90
1500	1.1 ± 0.38	1.7 ± 0.69	57 ± 1.02	86
1750	1.0 ± 0.34	1.6 ± 0.62	65 ± 1.79	90
2000	1.1 ± 0.34	1.4 ± 0.63	34 ± 5.53	86

*Values are means ± SD of triplicates.
Δ*W*, weight gain.

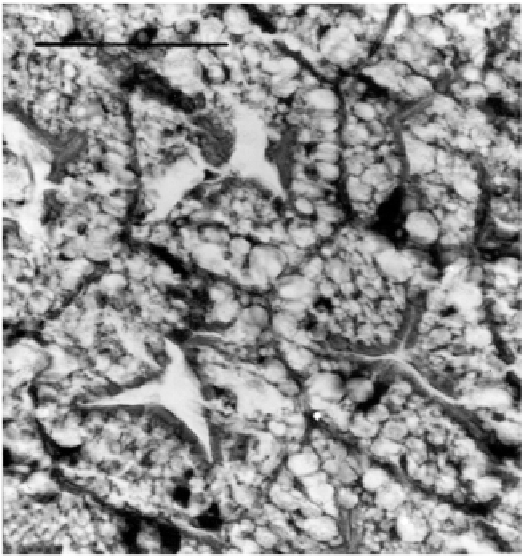

Figure 2 Hepatopancreas of *Pleoticus muelleri* fed a diet deficient in vitamin E, transverse section through tubules showing severe morphological alterations. Note that the tubules are surrounded by abundant connective tissue (bar = 50 μm).

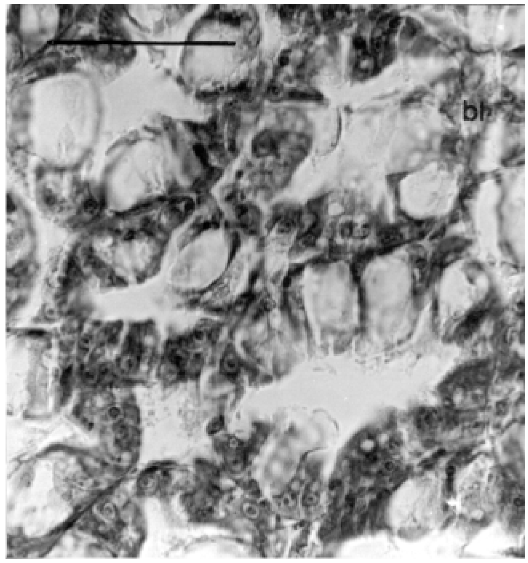

Figure 3 Hepatopancreas of *Pleoticus muelleri* fed a diet with 1250 mg vitamin E kg $^{-1}$, transverse section of tubules, showing damage of the basal lamina and cellular retraction. bl, basal lamina (bar = 50 μm).

Histological examination of the hepatopancreas showed conspicuous changes depending on dietary treatment. In the hepatopancreas of individuals fed diets without vitamin E or containing up to 600 mg kg $^{-1}$, cellular death was evidenced by cellular and nuclear retraction and the lack of nuclei in several cells. Tubules were surrounded by abundant connective tissue. In all individuals part of the organ maintained its normal structure (Fig. 2).

The hepatopancreas of shrimps fed diet containing 1250, 1500 or 2000 mg vitamin E kg $^{-1}$ diet showed apical desquamation of cells, damaged basal lamina and, in some cases, cellular retraction and hypertrophy (Fig. 3).

In shrimps fed with 1750 mg kg $^{-1}$ of vitamin E, the morphology of the organ was normal, with abundant secretion in the tubules. Different cell types were well recognized and possessed a conspicuous brush border (Fig. 4). Animals fed the diet containing BHT exhibited cellular hypertrophy and tissue disorganization (Fig. 5).

Discussion

In the first trial, the results indicate that there was no relationship between per cent weight gain or survival and the level of vitamin E in diets. However in the second trial, there was a positive relationship between the amount of vitamin E in diets and growth. The per cent weight gain obtained in these experiments were similar to normal values determined for this species under experimental conditions (Harán & Fenucci 1997; Díaz, Fernández Gimenez & Fenucci 1999).

Our observations are not in agreement with previous studies which mentioned a growth reduction in animals fed vitamin E-deficient diets (Kanazawa 1985; He *et al.* 1992; He & Lawrence 1993). Kanazawa (1985) found that addition of vitamin E to diets resulted in improved survival of *M. japonicus*. For *Fenneropenaeus indicus*, growth rate was lower when shrimp were fed vitamin E-deficient diets (Reddy, Ganapathi Naik & Annappaswa-My 1999).

In this study the best results for *P. muelleri* were obtained with a diet supplemented with 1750 mg vitamin kg $^{-1}$ diet. This level is significantly higher than those found in other penaeids. For example, an optimum requirement of 99 mg vitamin E kg $^{-1}$ diet was determined for *L. vannamei* (He & Lawrence 1993), this level is significantly lower than 300 mg kg $^{-1}$, previously recommended by Akiyama, Dominy and Lawrence (1992) to formulate commercial diets; these estimations were made following the changes in growth parameters. For *F. indicus* females maturation was determined a requirement of 300 mg vitamin E kg $^{-1}$ diet (Cahu & Fakhfakch 1990).

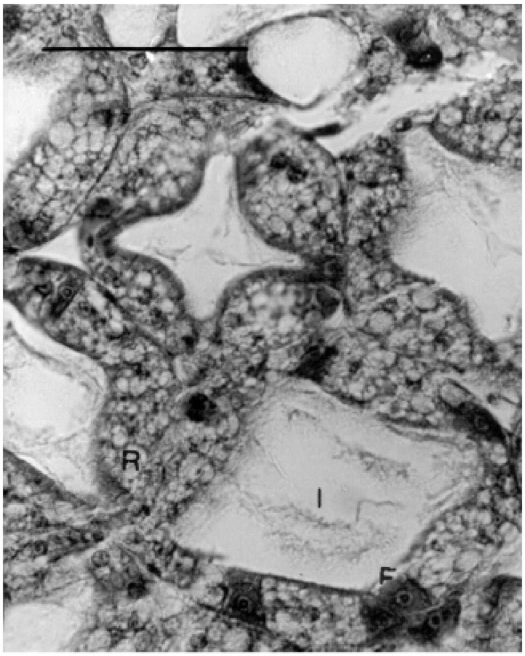

Figure 4 Hepatopancreas of *Pleoticus muelleri* fed a diet with 1750 mg vitamin E kg^{-1}, transverse section through tubules, showing the cellular types, similar to those of wild prawns. F, cell F; R, cell R; l, lumen (bar = 50 μm).

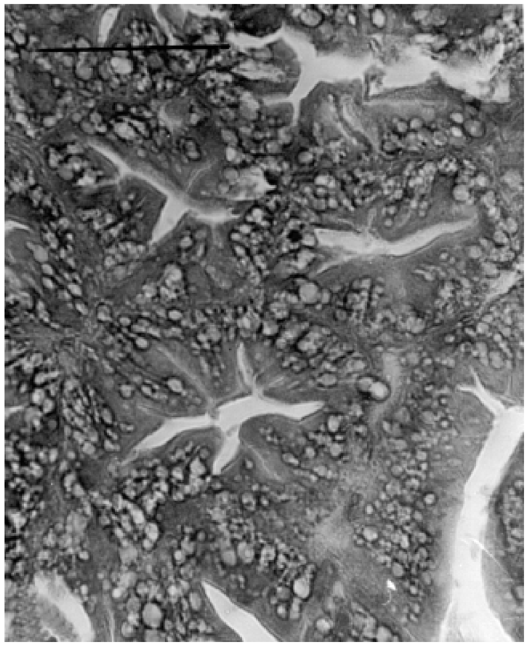

Figure 5 Hepatopancreas of *Pleoticus muelleri* fed a diet with 16 mg butylated hydroxytoluene kg^{-1}, transverse section through tubules revealed severe cytological alterations (bar = 50 μm).

Reddy and colleagues (1999) observed lack of appetite and a low food conversion rate in *Penaeus monodon* fed diets lacking α-tocoferol. Kanazawa (1985) and He and colleagues (1992), working with *F. indicus* and *L. vannamei*, respectively, noted a darkening of the shrimp hepatopancreas as a sign of vitamin E absence. This symptom of deficiency was not observed in Argentine penaeid species.

It is difficult to compare the requirements of the different penaeiod shrimp species due to the different experimental conditions. Kanazawa (1985) and He and colleagues (1992) carried out their investigations employing larval states of *F. Indicus* and *L. vannamei*, respectively, meanwhile juveniles or adults were used in the present work.

On the other hand, it is important to consider the lipid concentration in the diet. Several authors have shown in fish (Watanabe, Takeuchi, Wada & Ueharo 1981; Schwarz, Kirch Gessner, Steinhart & Runge 1988; Roem, Kohler & Stickney 1990) and even in crustaceans (Cahu *et al.* 1991) that vitamin E requirement increases when polyunsaturated fatty acid level increases in the diet. The vitamin E requirement also varies when other antioxidant factors are present in the diet (Hung, Cho & Slinger 1981). The incorporation of good quality oils to the diets protects the lipids during the manufacture and storing and apparently reduces the vitamin E requirement in the trout (Hung *et al.* 1981) and the channel catfish (Murai & Andrews 1974).

In the present work, refined fish oil (70 mg kg^{-1}), completely free of vitamins and antioxidants, was used to prepare the formulated diets. Besides, in these diets the only protection against lipid peroxidation was supplied by the vitamin supplement or synthetic antioxidant (BHT).

In contrast, He and Lawrence (1993) fed the animals with a diet containing 40 mg fatty acids kg^{-1} and 30 mg corn oil kg^{-1}. However, the corn oil employed in the diet only lacked of vitamin E, meanwhile the vitamin C, with great antioxidant power, was possibly present. Considering the previous investigations, it is possible that the differences in the estimated optimum levels of vitamin E are due to diet composition. The level and state of oxidation of polyunsaturated lipids in the diet, as well as the presence of other antioxidants and selenium, affect the dietary level of vitamin E required by fish (Roem *et al.* 1990). Variation in the vitamin E requirements among different species probably is associated with differences in experimental conditions, as well as quantity of polyunsaturated fatty acids in the diet, their degree of unsaturation or oxidative status.

The synthetic compound BHT is commonly used as an antioxidant in animal feeds (Rumsey 1980). The use of good quality oils and the incorporation of antioxidants into formulations of crustacean diets may decrease the need for dietary vitamin E as was found for trout (*Salmo gairdneri*) (Hung *et al.* 1981) and catfish (*Ictalurus punctatus*) (Murai & Andrews 1974). In the present study, shrimp fed diet supplemented with 16 mg BHT kg^{-1} diet, exhibited similar growth and survival than the other treatments, but showed several histopathological signs in the hepatopancreas cells. He and Lawrence (1993) determined that dietary BHT at 16 mg kg^{-1} diet prevented growth reduction of *L. vannamei*. This synthetic antioxidant was highly effective in inhibiting dietary lipid peroxidation, but showed less active in tissue protection and had no protective effect on membrane lipid peroxidation.

Histological analysis is important due to midgut gland cells of invertebrates reflecting the nutritional value of a diet (Vogt, Storch, Quinitio & Pascual 1985). Histological criteria constitute a practical means for the assessment of the nutritional value of diets in combination with statistical parameters such as growth and survival; short-term studies can be used for evaluation of feed formulation (Vogt, Quinitio & Pascual 1986; Rodriguez Souza, Sekine, Suzuki, Shima, Stüssmann & Takashima 1996). In the present study, histological alterations indicate that a vitamin E-supplemented diet is essential to maintain the normal structure of hepatopancreas of *P. muelleri*. The most notable changes reported in this work, such as cellular and nuclear retraction, desquamation or detachment of cells and damage of the basal lamina, have been reported for other crustacean species (Icely & Nott 1992; Rodriguez Souza *et al.* 1996; Reddy *et al.* 1999). Histological results of these experiments are supported by other studies carried out with penaeids. Reddy and colleagues (1999) observed in *P. monodon* that diets deficients in α-tocopherol resulted in detachment or destruction of the epithelial cells of hepatopancreas.

The results of this work indicate that vitamin E supplementation of prepared diets is necessary to maintain normal structure in the hepatopancreas of *P. muelleri*. Optimal vitamin E requirement for Argentine red shrimp under the present experimental conditions appears to be approximately 1750 mg vitamin E kg^{-1} diet.

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