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Organic selenium supplementation promotes shrimp growth and disease resistance to Taura syndrome virus

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ABSTRACT: To examine the effect of selenium (Se) supplementation on shrimp growth and disease resistance after Taura Syndrome virus (TSV) challenge, groups of Pacific white shrimp (*Penaeus vannamei*) from a strain known to be susceptible to TSV were fed 3 diets: (1) standard commercial shrimp feed (0.58 ppm Se content) without Se supplementation (standard diet group); (2) standard commercial feed plus 0.3 ppm inorganic Se (Inorg Se-group); and (3) standard commercial feed plus 0.3 ppm organic Se (Org Se-group) over a 5-week period. Samples (3 shrimp) were collected from all replicates before feeding (pre-feeding or week 0) and at the 1st, 2nd, and 3rd weeks post feeding to determine weight, length, and number of granular haemocytes. After the feeding trial, the remaining shrimp were used for a final TSV challenge test. Significant differences in weight between groups ($P < 0.05$) were found on the fifth week post feeding in which the average weight of shrimp fed the standard feed was 7.2 ± 1.2 g compared to 6.1 ± 1.2 g in the inorg Se-group and 9.0 ± 1.1 g in the org Se-group. Significant increase in the number of total haemocytes and granular haemocytes (GHs) were found only in the org Se-group on the third week post feeding. On day 6 post TSV challenge, the number of survivors in the org Se-group (66.7%) was also significantly higher than those of the inorg Se- (35.5%) and standard diet- (13.3%) group. Nested RT-PCR analysis showed that the number of shrimp with severe infection in the org Se-group (2/5) was less than the inorg Se- (4/5) and standard diet-group (4/4). The RT-PCR results were similar to those observed by histological examination. The number of total haemocytes and GHs isolated from survivors of the standard diet-group were significantly lower than those isolated from survivors of the inorg Se- or org Se-group. The present results indicated that organic Se supplementation in shrimp feed could improve shrimp growth and survival after an experimental challenge with TSV.

KEYWORDS: aquaculture, *Penaeus vannamei*, nutrient, trace element

INTRODUCTION

Selenium (Se), an essential dietary nutrient, plays an important role in normal functioning of the immune system, promoting a normal cellular immune response, and helping the body to resist viral infection[1]. The effect of Se on viral infection in humans has been studied extensively. As shown in HIV-positive patients, decline in Se concentration potentiates immunosuppression, an increase in viral replication, and acceleration in disease progression[2,3]. The severity of viral infection such as Coxsackievirus B3 and Influenza virus was increased in Se deficient-mice[4,5]. Selenium is incorporated into proteins to make selenoproteins such as glutathione peroxidase (GPx), an important antioxidant enzyme[6]. The antioxidant properties of selenoproteins help prevent cellular damage from free radicals. Low levels of it may increase oxidative stress on the immune system leading to an inadequate antioxidant system for protecting the host against viral infection. The significance of Se in fish nutrition is markedly growing along with the intensification of the industry. As with humans, Se also plays an important role in fish growth,

development, reproduction, flesh quality, maintenance of fish health, and in particular fish immunity. High dietary Se supplementation reduces oxidative stress and improves the immune response of fish[7]. In southern bluefin tuna fish, Se supplementation slows the myoglobin oxidation process thus resulting in a longer colour shelf-life, an important product quality feature of tuna meat[8]. Supplementation of organic Se to fry Nile tilapia enhances the growth and feed use, and reduces susceptibility to *Aeromonas hydrophila*[9].

A serious threat to the shrimp industry is from viral pathogens and particularly from white spot syndrome virus, yellow head virus, and Taura syndrome virus (TSV). Enhancing the immune system through vitamins and nutrients is important in improving host defence capabilities in shrimp. The study of the trace element Se on the immune system against viral infection in shrimp is very limited, although it has been shown by many researchers that feed supplemented with Se improves shrimp growth. Davis reported that the maximum growth of juvenile *P. vannamei* was achieved when fed with purified diets supplemented with 0.2–0.4 mg Se/kg diet (0.2–0.4 ppm)[10]. Wang et al proposed a dietary Se threshold of 0.44 µg/g (0.44 ppm) for *P. chinensis*[11]. Yuchuan and Fayi showed that *P. chinensis* fed with diet containing 20 ppm (20 mg/kg) Se had higher weight gain than those fed with 0.8 ppm Se[12].

Before we initiated this study, our survey of local shrimp feed producers revealed that no Se supplement was added to the premix used to produce shrimp feed. They considered that the Se present in natural feed ingredients (fish meal, soybean meal, etc.) and seawater (0.0009 ppm in 3.5% salinity) was sufficient to satisfy the shrimp nutritional requirement[13]. However, due to the beneficial effects of Se supplementation described above for other animal feeds, including fish feed, this study was undertaken to investigate the effect of Se supplementation in the shrimp diet on growth performance and disease resistance to TSV infection. In order to understand the functional mechanism of Se in shrimp, the effect of inorganic and organic forms of Se on the number of total and granular haemocytes, and severity of infection were determined. Although this is the first report of a positive effect of Se supplementation on shrimp growth and viral resistance, the mechanism for the effect is largely unknown.

MATERIALS AND METHODS

Experimental animals

Juveniles of Pacific white shrimp, *Penaeus vannamei*, Kona strain, with a mean fresh weight of 1–2 g were purchased from the Oceanic Institute in Hawaii. This strain of shrimp was selected as they have a high susceptibility to TSV[14]. They were reared in an aquarium until they reached an average size of 5 g in preparation for feeding trials. Shrimp were divided into three groups with 5 replicates of 25 shrimp in each group. Before experimental feeding (week 0), 3 shrimp from each replicate of all groups were sampled for determination of weight, length, number of granular haemocytes, and TSV infection. Total RNA extraction was performed using only one shrimp from each replicate to ensure that the shrimp were free of TSV during acclimatization in the non-biosecure wet lab by using a sensitive nested RT-PCR method (IQ2000 kit, Farming IntelliGene, Taiwan). The remaining shrimp (22 shrimp per replicate) were fed with three different formulated feeds: Group 1 (Standard diet) basal feed (Charoen Pokphand, Thailand); Group 2 (Inorg Se-group) basal feed supplemented with 0.3 ppm inorganic Se; Group 3 (Org Se-group) basal feed supplemented with 0.3 ppm organic Se.

Experimental fibreglass aquaria contained 200 l artificial seawater with a salinity of 20‰. Shrimp were fed a total of approximately 10% of body weight twice per day. Experimental shrimp were fed formulated diets for approximately one month. Each week (from week 1–3) during the experimental trial, 3 shrimp were removed from each replicate aquarium to determine weight, length, and number of GHs per ml of haemolymph. Samples were not collected on week 4 post feeding due to the low sample number after the 4 week feeding period. All sampled shrimp were discarded after examination. The survival rate was recorded every week. After 4 weeks on the test diets, the number of shrimp was adjusted to 9 shrimp per replicate for the TSV challenge test. During the challenge period, experimental shrimp were continuously fed with the same feed given during the feeding trial. The experiment was terminated on day 6 post-challenge since all shrimp in 2 replicates of the standard diet group had died. Survivors in each group were assessed for growth performance and number of GHs per ml of haemolymph. A total of 4–5 surviving shrimp from each group were checked for the presence of TSV by nested RT-PCR as described above.

Preparation of feed

Sel-Plex powder containing organic Se was kindly supplied by Alltech Biotechnology Co. Ltd. The basal feed (comprising of 32% protein, 5.5% fat, less than 4% fibre, and 0.5% moisture) was purchased from Charoen Pokphand Co. Ltd., Thailand. The amount of Se in basal feed pellet was 0.58 ppm as determined

by an inductively coupled plasma-mass spectrometry method. The basal feed pellets were crushed and Se products sufficient to yield 0.3 ppm Se in the final feed were mixed thoroughly with powdered gluten (4% gluten per kg feed) prior to blending with the crushed pellets. The reconstituted pellets were dried at 37 °C for 3 h before storage at −20 °C for the feeding trials.

TSV detection

Shrimp gills were collected in Trizol (GibCo BRL) for total RNA extraction following the manufacturer's instructions. The resulting shrimp RNA extract was quantified spectrophotometrically so that 100 ng could be used as the template for nested RT-PCR tests (IQ2000 TSV detection kit, Farming IntelliGene, Taiwan). The PCR products were subjected to electrophoresis on a 1.5% agarose gel before being visualized by a gel documentation machine (Gene Cam Flexi, Syngene). This detection system can grade the severity of TSV infections. For example, a PCR result showing only one band at 284 bp indicates a light infection while two bands (284 bp and 476 bp) indicate a severe infection. Negative samples show only one internal control band at 680 bp.

Histological examination

Survivors ($n = 4$) from each group were prepared for histological study by injecting Davidson's fixative (0.5 ml) directly into the hepatopancreas and adjacent areas of the cephalothorax of each shrimp[15]. The cephalothorax was then excised at the junction with the first abdominal segment, the carapace slit just lateral to the dorsal midline, and the intact cephalothorax immersed in 10 times its volume of the same fixative. After 48 h in Davidson's fixative, the cephalothorax was transferred to 70% ethanol and processed for routine histological examination using standard procedures for paraffin embedding and haematoxylin and eosin (H&E) staining[15]. Stained sections were examined by light microscopy for the presence of typical TSV lesions in the cuticular epithelium of the body surface, appendages, and gills.

Haemocyte counts

During the feeding trial, haemolymph was collected individually and prepared for total haemocyte (THC) and granular haemocyte (GHC) counts. The GHC included both large-granular and small-granular haemocytes. Haemolymph (0.1 ml) was withdrawn from the ventral sinus of the first abdominal segment into a syringe containing 0.1 ml fixative (10% formalin in 0.45 M NaCl) and transferred to a microtube. After 10 min, 20 µl portions of the fixed haemocyte suspension were mixed with 20 µl Rose Bengal solution (1.2% Rose Bengal in 50% ethanol) and incubated at room temperature for 20 min before being used to determine THC by haemacytometer or to prepare smears on microscope slides to measure %GH. Haemocyte counts were made for 5/25 squares (volume of 1 square $= 0.2 \times 0.1$ mm^3) to calculate the THC in 1 ml of haemolymph[16]. For GHC, completely dried smears were counterstained with haematoxylin solution for 7–10 min. The slides were then rinsed with tap water for 10 min followed by dehydration with 95% and 100% ethanol. After dehydration, the slides were submerged in xylene before mounting with permount and covering with a coverglass. The proportion of GHs in 200 total haemocytes was recorded and used to calculate the total number of GHs per ml of haemolymph from the THC obtained above.

Statistical analysis

Statistical analysis of the results (raw data format) was carried out using one way ANOVA with SIGMASTAT (Jandel Scientific Software). When normality tests failed, a one way ANOVA on ranks was carried out instead. Individual differences were determined using the Student-Newman-Keul's method and a t-test. Differences were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

The emergence of new viral diseases or the increase in disease outbreaks from known viruses in shrimp aquaculture is often attributed to defects in farm management practices such as overly dense culture or poor biosecurity. Among the strategies to reduce the impact of shrimp viral disease has been the development of specific pathogen free and specific pathogen resistant stocks following the discovery of significant differences in susceptibility among different families of shrimp[14,17,18]. However, the effect of host nutritional factors has been less frequently considered.

The study of Se requirement in shrimp is limited. The work done in *P. chinensis* demonstrated a good linear correlation between concentration of Se supplemented (20 ppm) in basal diet and weight gain[11]. In this study, Se was added to the basal diet (containing 0.58 ppm Se) to provide an additional 0.3 ppm of Se supplementation in both inorganic (Na selenite) and organic (Se yeast) form. At the beginning of the trial, shrimp mean fresh weight was approximately 6.1 ± 1.4 g (Fig. 1). Significant differences in weight between groups were first observed at the fifth week of the feeding trial (Fig. 1). Shrimp fed

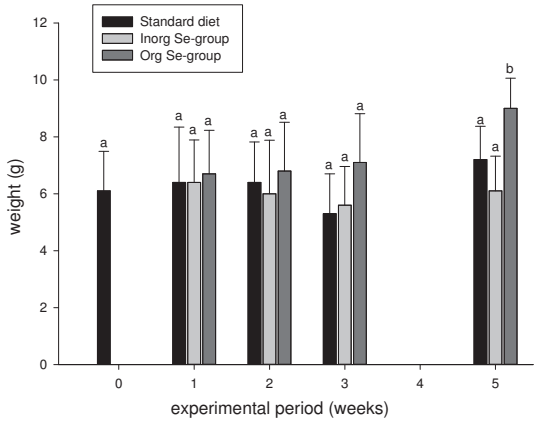

Fig. 1 Weekly shrimp weight recorded during the experimental period. Each bar indicates the mean $\pm$ SD (n = 15). Letter 'b' indicates a significant difference from the standard diet 'a'. Samples were not collected at week 4 post feeding. Different superscripts indicate significant differences between values at indicated times and the value at pre-feeding time.

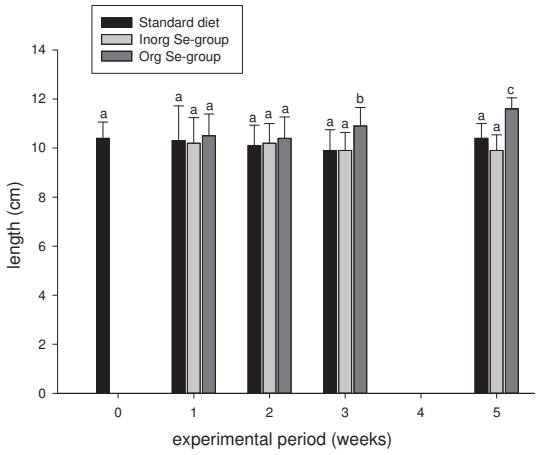

Fig. 2 Weekly shrimp length recorded during the experimental period. Each bar indicates the mean $\pm$ SD (n = 15). Numbers with different letters are significantly different from the pre-feeding group. Different superscripts indicate significant differences between values at indicated times and the value at pre-feeding time.

with the org Se-diet had a significantly higher mean weight (9.0 ± 1.1 g) than those fed with inorg Se-diet (6.1 ± 1.2) or the standard diet (7.2 ± 1.2). There was no significant difference between the latter two groups. Similar results were observed with shrimp length (Fig. 2). By the third week, the mean length of shrimp fed the org Se-diet (10.9 ± 0.7 cm) was significantly greater than that for the inorg Se-diet (9.9 ± 0.7) or standard diet (9.9 ± 0.8) groups. This trend continued until the end of the challenge period when the mean length of the org Se-group (11.6 ± 0.4) was significantly greater than that of the inorg Se- (9.9 ± 0.6) and standard diet- (10.4 ± 0.6) groups. The mechanism for improved growth performance as a result of organic Se supplementation is unclear and has to be further elucidated.

Shrimp circulating haemocytes play an important role in innate immunity. Many humoural defence molecules are contained in haemocyte granules and the release of which is stimulated by pathogen invasion[19]. The numbers of haemocytes, especially the granular cells, have been used as a measure for shrimp health status. THCs and GHCs were very variable amongst individual shrimp. In our study, the THCs in 15 shrimp sampled prior to the experimental trials varied from 4.2 to 25.8×10^6 cells/ml with a mean of $12.7 \pm 7.7 \times 10^6$ cells/ml (coefficient of variation = 61%). THC on the first and second weeks showed no significant differences (data not shown). Nor were there any significant differences in the number of GHs during the same period. On the third week of experimental feeding, the numbers of total haemocytes and GHs were significantly higher in the org Se-group, but not in the inorg Se-group, when compared to the standard diet group (Fig. 3). After the challenge with TSV, the THC decreased dramatically in all groups with the lowest GHC in the standard diet group. There was a significant difference in GHC between survivors receiving the standard diet and those receiving the inorg Se- and org Se-group. In contrast, there was no significant difference in GHC for survivors of the inorg Se- and the org Se-group.

The 5-day LD_{50} dose for the initial TSV stock with Kona shrimp was found to be a dilution of approximately 1:100. Shrimp sampled before and after the experimental feeding trial were found to be free of TSV using a commercial TSV detection kit (data not shown). Small numbers of shrimp in all groups died during the four-week feeding trial prior to the TSV challenge. The dead shrimp were usually those that had recently molted and had been partially eaten by other shrimp. After feeding for 4 weeks, 9 shrimp from each replicate were injected with TSV at the LD_{50} dose (1:100). By day 6 post-TSV injection, shrimp in the 2 replicates from the standard diet group were all dead and the test was terminated. The mean number and percentage of surviving shrimp in the standard diet group was 1.2 ± 1.1 ($13.3 \pm 12.2\%$ survival), 3.2 ± 0.8 ($35.5 \pm 9.3\%$ survival) in the inorg Se-group, and 6.0 ± 0.7 ($66.7 \pm 7.8\%$ survival) in the

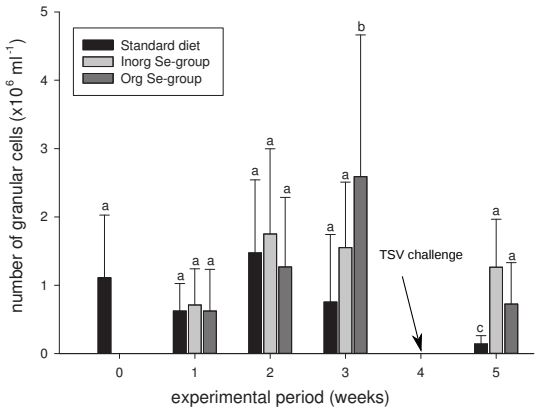

Fig. 3 Weekly shrimp total number of granular haemocytes (per ml of haemolymph) recorded during the experimental period. Each bar indicates the mean ± SD ($n = 15$). Different superscripts indicate significant differences between values at indicated times and the value at pre-feeding time.

Table 1 The percentage of survivors in each experimental group at 6 days post-TSV challenge and the number of survivors with severe infection (SI) determined by nested RT-PCR.

Experimental group	Number of survivors (±SD)	% Survivors (±SD)	SI (%)
Standard diet	1.2 ± 1.10	13.3 ± 12.2	100
Inorg Se-group	3.2 ± 0.84	35.5 ± 9.3	80
Org Se-group	6.0 ± 0.71	66.7 ± 7.8	40

org Se-group (Table 1). All differences were statistically significant.

Using equal amounts of total RNA template, 4 of the surviving shrimp from the standard diet group and 5 shrimp from each for the inorg Se- and org Se-group were checked for severity of TSV infection by RT-PCR. Most or all of the shrimp on the standard (4/4) and the inorg Se-diet (4/5) showed severe TSV infections compared to only 2/5 in the org Se-group (Table 1; Fig. 4). Histological examination of gills from survivors from the three groups on day 6 post-TSV challenge showed different levels of TSV infection. A high degree of pathological lesions typical of TSV, nuclear pyknosis/karyorrhexis, and numerous eosinophilic to basophilic cytoplasmic inclusions in the subcuticular tissue and cuticular epithelium within the appendages[20] were found in tissue collected from survivors of standard diet (Fig. 5B) and the Inorg Se group (Fig. 5C). The results were in contrast to the tissue collected from survivors of Org Se group as shown in Fig. 5D, in which TSV lesions of a lesser

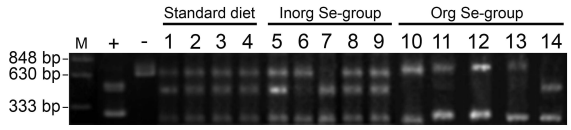

Fig. 4 RT-PCR results for TSV with experimental animals before the experiment and on day 6 of post TSV challenge. M1: molecular weight marker from the IQ2000 TSV detection kit.

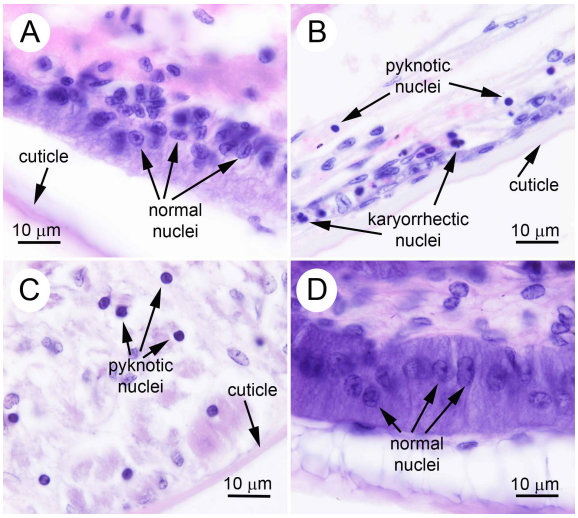

Fig. 5 Histological examination of epidermal tissue from surviving shrimp at day 6 post-TSV challenge. (A) normal shrimp (non-challenged), (B) shrimp from standard diet group, (C) shrimp from Inorg Se-group, (D) shrimp from Org Se-group.

degree were found.

Taken together, organic Se supplementation of the shrimp feed improved growth, haemocyte number, and survival after TSV challenge while inorganic Se had no effect on growth or number of haemocytes and showed a lesser degree of improvement in post-challenge survival. This might be explained by differences in bioavailability. The bioavailability of Se obtained from an organic source was higher than that obtained from an inorganic source for Atlantic salmon and channel catfish[21–23]. A number of supplementation studies have shown that organic Se from Se-yeast has greater bioavailability than inorganic Se[1,24,25]. The increase in absorption and storage of organic Se is probably due to its direct incorporation into proteins[26]. It has been previously reported in growing-finishing swine and rats that feeding diets with selenomethionine as a source of Se resulted in greater tissue accumulation of Se than other forms of Se[27–29].

The effect of organic Se in growth improvement has also been reported in channel catfish. Catfish fed Se from organic sources had better growth than those fed Se from inorganic sources at suboptimal levels but not at levels above the minimum dietary requirement[30].

Se deficiency is associated with decreased activities of Se-dependent antioxidant enzymes such as glutathione peroxidase (GPx) and thioredoxin reductase that have been linked to the occurrence, virulence, or disease progression of some viral infections[1,31]. Significant reductions in the activity of antioxidant enzymes such as superoxide dismutase, catalase, and GPx have been observed in white spot syndrome virus-infected shrimp when compared with uninfected shrimp, suggesting that viral infection in shrimp may lead to increased levels of oxidative stress[32]. Under low Se conditions, it has been suggested that increased oxidative stress and apoptosis are induced by viruses that then replicate at higher rates, escape from dying cells, and exhibit increased virulence[1]. In support of this suggestion, we found more severely-infected shrimp in the control group with no Se supplementation than in the groups with Se supplementation. The possible role of oxidative stress and apoptosis in this phenomenon needs to be examined in more detail. The tendency for less intense RT-PCR test results and histopathological studies in the org Se-group suggested that the ability of TSV to propagate had been reduced. This could be related to an improved shrimp defence response including numbers of haemocytes. The number of total haemocytes and GHs only increased significantly at the end of the third week of experimental feeding in the org Se-group. After TSV challenge, the number of haemocytes in circulation decreased, which might have been due to their migration to the site of viral infection[33]. However, we did not analyse specific cellular-mediated defence functions such as phagocytosis or encapsulation, and it is possible that these could have varied even though the total cell counts did not. It would be of interest to determine whether this apparent reduction in viral propagation also occurs with other shrimp viral pathogens such as white spot syndrome virus or yellow head virus since this might clarify if Se involvement is associated with a general, rather than a TSV-specific, anti-viral response.

The fact that the surviving shrimp were infected with TSV but were able to tolerate the infections due to some limitation of viral replication is similar to what is seen in shrimp that have been genetically selected for their ability to survive TSV challenge. These shrimp become infected, but the level of viral propagation is reduced when compared to unselected shrimp[34]. A similar result has been reported for *P. vannamei* survivors from a challenge with yellow head virus[35]. Again, the mechanisms underlying the low level of viral propagation are currently unknown, but it is possible that Se may serve as an important co-factor required by the animal to respond optimally to infection. Indeed, the extent to which supplementation with organic Se improved the post-challenge survival of a strain specifically selected for its susceptibility to TSV suggests that shrimp breeding programs should pay greater attention to nutritional status as a key component of their selection programs.

In conclusion, it is clear that Se supplementation and particularly supplementation with organic Se can have a dramatic effect on shrimp growth, haemocyte number, and survival after TSV challenge. Although the mechanisms for these potential benefits are still unknown, more detailed work on the effect of Se in shrimp is warranted. In addition, these results suggest that there would be potential benefits from similar studies to elucidate further relationships between the nutritional status of shrimp and their response to pathogens.

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REFERENCES

1. Rayman MP (2000) The importance of selenium to human health. *Lancet* **356**, 233–41.
2. Baum MK, et al (1997) HIV-1 infection in woman is associated with severe nutritional deficiencies. *J Acquir Immune Defic Syndr Hum Retrovirol* **16**, 272–8.
3. Baum MK, Shor-Posner G, Lai S, Zhang G, Lai H, Fletcher MA, Sauberlich H, Page JB (1997) High risk of HIV-related mortality is associates with selenium deficiency. *J Acquir Immune Defic Syndr Hum Retrovirol* **15**, 370–4.
4. Beck MA, Kolbeck PC, Shi Q, Rohr LH, Morris VC, Levander OA (1994) Increased virulence of human eterovirus (Coxackievirus B3) in selenium-deficient mice. *J Infect Dis* **170**, 351–7.
5. Nelson HK, Shi Q, Van Dael P, Schiffrin EJ, Blum S, Barclay D, Levander OA, Beck A (2001) Host nutritional selenium status as a driving force for influenza virus mutation. *FASEB J* **15**, 1846–8.
6. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Haefeman DG, Hoejstra WG (1973) Selenium: biochemical role as a component of glutathione peroxidase. *Science* **179**, 588–90.
7. Lin YH, Shiau SY (2007) The effects of dietary selenium on the oxidative stress of grouper, *Epinephelus malabaricus*, fed high copper. *Aquaculture* **267**, 38–43.
8. Thomas P, Surai P (2006) The increasing importance

of selenium in fish nutrition. *Aquaculture Asia Pacific Magazine*, pp 28–9.
9. Ahmad MH, El-Marakby HI, Seden MEA, Abdel-Twwab M, Abou-El-Atta ME (2006) The use of organic selenium (SelPlexO) in practical diets for Nile tilapia, *Oreochromis niloticus* (L.): effect on growth performance, feed utilization, whole-body composition and enteropathogenic *Aeromonas hydrophila*-challenge. In: *The 7th International Symposium on Tilapia in Aquaculture*, Veracruz, Mexico, pp 95–107.
10. Davis DA (1990) Dietary mineral requirements of *Penaeus vannamei*: Evaluation of the essentiality for thirteen minerals and the requirements for calcium, phosphorus, copper, iron, zinc and selenium. PhD thesis, Texas A&M Univ.
11. Wang AL, Wang WN, Liu CQ, Wang SA, Wag RD, Ma ZQ (1994) Effects of selenium concentrations in feed on the growth and selenium contents of *Penaeus chinensis*. *J Fish China* **18**, 245–80.
12. Yuchuan T, Fayi L (1993) Selenium requirement of shrimp *Penaeus chinensis*. *Chin J Oceanol Limnol* **11**, 249–53.
13. Turekian K (1968) *Oceans*, Prentice Hall, Englewood Cliffs.
14. Argue BJ, Arce SM, Lotz JM, Moss SM (2002) Selective breeding of Pacific White Shrimp (*Litopenaeus vannamei*) for growth and resistance to Taura Syndrome Virus. *Aquaculture* **204**, 447–60.
15. Bell TA, Lightner DV (1988) *A Handbook of Normal Penaeid Histology*, World Aquaculture Society, Baton Rouge, LA, pp 2–6.
16. Sritunyalucksana K, Gangnonngiw W, Archakunakorn S, Fegan D, Flegel TW (2004) Bacterial clearance rate and a new differential hemocyte staining method to assess immunostimulant activity in shrimp. *Dis Aquat Org* **63**, 89–94.
17. Xu Z, Yrzykowski J, Alcivar-Warren A, Argue BJ, Moss SM, Arce S, Traub M, Calderon FO, Lotz J, Breland V (2003) Genetic Analyses for TSV-Susceptible and TSV-Resistant Pacific White Shrimp, *Litopenaeus vannamei*, using M1 Microsatellite. *J World Aquacult Soc* **34**, 332–43.
18. Moss SM, Doyle RW, Lightner DV (2005) Breeding shrimp for disease resistance: challenges and opportunities for improvement. In: Walker PJ, Lester RG, Bondad-Reantaso MG (eds) *Diseases in Asian Aquaculture V*, Asian Fisheries Society, Manila, pp 379–93.
19. Johansson M, Keyser P, Sritunyalucksana K, Söderhäll K (2000) Crustacean haemocytes and haematopoiesis. *Aquaculture* **191**, 45–52.
20. Flegel TW (2006) Detection of major penaeid shrimp viruses in Asia, a historical perspective with emphasis on Thailand. *Aquaculture* **258**, 1–33.
21. Bell JG, Cowey CB (1989) Digestibility and bioavailability of dietary selenium from fishweal, seleite, selenomethionine and selenocystine in Atlantic salmon (*Salmo solar*). *Aquaculture* **81**, 61–8.
22. Lorentzen M, Maage A, Julshamn K (1994) Effects of dietary selenite or selenomethionine on tissue selenium level of Atlantic salmon (*Salmon solar*). *Aquaculture* **121**, 359–67.
23. Wang C, Lovell RT (1997) Organic selenium sources, selenomethionine and selenoyeast, have higher bioavailability than inorganic selenium source, sodium selenite, in diets for channel catfish (*Ictalurus punctatus*). *Aquaculture* **152**, 223–34.
24. Beilstein MA, Whanger PD (1986) Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins. *J Nutr* **116**, 1701–10.
25. Fairweather-Tait SJ (1997) Bioavailability of selenium. *Eur J Clin Nutr* **51**, S20–3.
26. Combs GF Jr, Combs SB (1986) *The Role of Selenium in Nutrition*, Academic Press, Orlando.
27. Mahan DC, Parrett NA (1996) Evaluating the efficacy of Se enriched yeast and inorganic selenite on tissue retention and serum glutathione peroxidase activity in grower finisher swine. *J Anim Sci* **74**, 2967–74.
28. Kim YY, Mahan DC (2001) Comparative effects of high dietary levels of organic and inorganic selenium on selenium toxicity of growing finishing pigs. *J Anim Sci* 79, 942-8.
29. Taylor JB, Finley JW, Caton JS (2005) Effect of the chemical form of supranutritional selenium on selenium load and selenoprotein activities in virgin, pregnant, and lactating rats. *J Anim Sci* **83**, 422–9.
30. Gatlin DM III, Wilson RP (1984) Dietary selenium requirement of fingerling channel catfish. *J Nutr* **114**, 627–33.
31. Taylor EW, Nadimpalli RG, Ramanathan CS (1997) Genomic structures of viral agents in relation to the biosynthesis of selenoproteins. *Biol Trace Elem Res* **56**, 63–91.
32. Mohankumar K, Ramasamy P (2006) White spot syndrome virus infection decreases the activity of antioxidant enzymes in *Fenneropenaeus indicus*. *Virus Res* **115**, 69–75.
33. Muñoz M, Vandenbulcke F, Saulnier D, Bachère E (2002) Expression and distribution of penaeidin antimicrobial peptides are regulated by haemocyte reactions in microbial challenged shrimp. *Eur J Biochem* **269**, 2678–89.
34. Srisuvan T, Noble BL, Schofield PJ, Lightner DV (2006) Comparison of four Taura syndrome virus (TSV) isolates in oral challenge studies with *Litopenaeus vannamei* unselected or selected for resistance to TSV. *Dis Aquat Org* **71**, 1–10.
35. Anantasomboon G, Poonkhum R, Sittidilokratna N, Flegel TW, Withyachumnarnkul B (2008) Low viral loads and lymphoid organ spheroids are associated with yellow head virus (YHV) tolerance in whiteleg shrimp *Penaeus vannamei*. *Dev Comp Immunol* **32**, 613–26.