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Growth, Survival and Physiological Condition of Cultured Marron, *Cherax tenuimanus* (Smith, 1912) Fed Different Levels of Organic Selenium

Rudy Agung Nugroho and Ravi Fotedar

Sustainable Aquatic Resources and Biotechnology, Faculty of Science and Engineering, Curtin University, Bentley WA 6102, Australia

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Abstract: A 90-day feeding experiment was conducted to evaluate the effects of three levels of dietary supplementation of organic selenium (OS) on physiological and immunological responses of marron (*Cherax tenuimanus*). Three levels viz. 0.1, 0.2 and 0.3 g kg^{-1} of basal diet of Sel-Plex® as a source of OS. were prepared and tested against the basal diet, which was used as a control. Each diet was fed to four replicated groups of marron, (initial mean weight of 39.43 ± 0.55 g), at a rate of 3% body weight every second day. At the end of the feeding experiment, dietary inclusion of all Sel-Plex® levels significantly improved specific growth rate (SGR) and survival of marron. Inclusion of Sel-Plex® above 0.1 g kg^{-1} resulted in significantly higher hepatopancreatic moisture level (HM) and wet hepatosomatic index (Hiw), of marron than marron fed without Sel-Plex. Total Se level in the hepatopancreas was the highest (5.41 ± 0.28 μg g^{-1}) in the marron fed 0.2 g kg^{-1} of Sel-Plex, whereas muscle accumulated significantly higher level of Se when marron were fed 0.3 g kg^{-1} of dietary Sel-Plex. It is therefore suggested that the beneficial dietary supplementation levels of Sel-Plex in marron should range from 0.2 to 0.3 g kg^{-1}.

Key words: Organic selenium, physiological responses, marron.

1. Introduction

Selenium (Se) is an important trace element required for growth, survival and immune function in rainbow trout (*Oncorhynchus mykiss*) [1, 2], Atlantic salmon (*Salmo salar*) [3], juvenile grouper (*Epinephelus malabaricus*) [4], crucian carp (*Carassius auratus gibelio*) [5, 6], prawn (*Macrobrachium rosenbergii*) [7] and coho salmon (*Oncorhynchus kisutch*) [8]. Se can be categorized into two forms, inorganic and organic Se (OS) [9]. Inorganic Se is found as selenate while OS is present in Se containing amino acid such as selenomethionine [10, 11]. OS from yeast can be retained and absorbed at a higher rate, it is less toxic and has relatively higher bioavailability than inorganic Se [5, 12-15].

OS from yeast is permitted by US-FDA as a feed supplement [16] and as per the Directive 70/524/ECC, the maximum permissible Se level in aquaculture diets should be 0.5 mg kg^{-1}, whereas, the suggested threshold dietary Se in prawn (*Penaeus vannamei*) for enhanced immunity is 0.44 μg g^{-1} of diet [17, 18]. However, there is no information available on the effects and retention levels of dietary supplementation of Sel-Plex in marron (*Cherax tenuimanus*).

Marron is known as the third largest freshwater crayfish in the world and are indigenous species in the Southwest of Western Australia. As a result of the interest in marron farming, the need for knowledge in the area of marron nutrition and the use of supplementing microelement to improve productivity have also significantly increased. Previous research stated that organosomatic indices such as

Corresponding author: Rudy Agung Nugroho, HDR Student, research field: aquaculture. E-mail: rudysatriana@yahoo.com.

hepatosomatic indices are important tools to evaluate the healthstatuse of marron [19-21]. Further, the positive physiological performance can finally be manifested by high growth and survival rates of marron [5, 22].

There is no information on the use of Sel-Plex in the formulated diet of marron. Therefore, the aim of the experiment was to evaluate the effects of different levels of dietary Sel-Plex supplementations on the growth, survival, hepatosomatic indices, and total Se accumulation in the hepatopancreas and the tail muscle tissues of laboratory-reared marron.

2. Materials and Methods

2.1 Test Diets Preparation

A basal diet mixture was formulated (Table 1) using Feed LIVE® software package version 1.52 from Live Informatics Company Limited, Thailand. All ingredients of basal diet were purchased from Specialty Feed Pty Ltd., Western Australia except for the OS which was provided through a commercial product, Sel-Plex®, Alltech Inc. USA [23]. All ingredients were thoroughly mixed with 500 mL of distilled water per kg of basal diet. To this mixture, 0.1, 0.2 or 0.3 g kg^{-1} of Sel-Plex® was added to obtain the test diets. Each test diet and the basal diet (without the addition of Sel-Plex®) were passed through a mincer to obtain wet strands. The resulting strands were then dried in direct sunlight for 6 h and allowed to cool at room temperature. The dried strands were broken into pellets of 0.5 mm diameter and 3 mm length and stored in a dark room. Moisture, ash and dry matter of the test diets were determined by the methods of the Association of Official Analytical Chemists [24]. Actual Se levels in the diet were measured based on the spectrophotometry methods [25].

2.2 Experimental Design

The experiment was conducted in 16 blue plastic cylindrical tanks (800 mm diameter, 500 mm high, 250 L capacity, 70 L of freshwater in each tank) at Curtin Aquatic Research Laboratory (CARL), Curtin University, Western Australia. Mechanical filtration (fluval 205 filters, Hagen, USA), filtering at a rate of approximately 2 L min^{-1}, was used in each tank. Seven PVC pipes (55 mm diameter, 150 mm length) were also placed in each tank to provide shelter for each marron. Marron, mean initial body weight of 39.43 ± 0.55 g, (n = 112) which were supplied by Aquatic Resource Management Pty Ltd., Western Australia were used for the 90 days of the experiment. After transportation and placing in the blue cylindrical tanks, marron was acclimated to the culture conditions for a week.

During the acclimation period, the marron were fed with the basal diet at a rate of 3% of body weight every second day. The marron were then randomly distributed to 16 culture tanks at a density of seven marron per tank. Randomized blocks of four tanks, each block fed one of the test diets so that each diet was represented by four replicates, were used as an

Table 1 Ingredient of basal diet (g kg^{-1}) and proximate composition of the diets used in this experiment.

Ingredient	Content (g kg^{-1})
Fish oil[1]	32
Wheat bran	545.59
Soybean meal	101.5
Fish meal[2]	257.14
Calcium carbonate	0.2
Ascorbic acid	0.5
Betaine[3]	12
Premix[4]	1.5
Cholesterol	2.5
Wheat starch	47.07
	Proximate composition
Crude protein	27.05
Crude fat	8.02
Crude Fiber	6.39
Moisture content (%)	9.01
Ash (%)	6.56
Dry matter (%)	90.98
Energy (Cal/g)	1,833.249

All ingredients supplied by Specialty Feeds Pty Ltd, WA, Australia, [1]cod liver oil, [2]peruvian fishmeal, 56% CP. [3]betaine anhydrous 97%, [4]commercial vitamin and mineral premix for trout.

experimental design. The marron from each tank was fed the test diet at a rate of 3% of body every second day. This feeding rate was determined by previous experiments [21]. Uneaten food and feces were siphoned out before feeding and sufficient freshwater was added to maintain 70 L of water in each tank. Temperature was maintained at 20 °C by using automatic heaters (Sonpar®, Model: HA-100, China).

2.3 Data Collection

Temperature, pH and dissolve oxygen of water were recorded weekly using Cyberscan pH 300, Eutech Instruments, Singapore. Nitrate, nitrite and ammonium were monitored and recorded weekly using chemical test kits (Aquarium Pharmaceuticals™, Inc., USA). Nitrate and nitrite level were monitored to not exceed than 0.1 mg L^{-1}, whereas total ammonium was maintained lower than 0.2 mg L^{-1} to provide optimum water quality for cultured marron [26]. The marron was counted everyday to calculate the survival rate [27]:

$$\text{Survival rate } (S) = 100 \times (n_t/n_0)$$

where: S is the survival rate; n_t is the number of marron at time t and n_o is the initial number of marron at the beginning of the experiment.

The weight of each marron from each tank was measured at the day 0 and 90 of the feeding trial using electronic balance (GX-4000, A&D Company, Ltd., Japan) for weight. Specific growth rate (SGR % g day^{-1}) were calculated using the following equation [28]:

$$SGR = 100\ (\ln_{wt} - \ln_{wo})/d$$

where Wt and Wo are the weight of the marron at current time (t) and at the commencement of the experiment (0), d = culture period (day), respectively.

2.4 Moisture Contents and Hepatosomatic Indices

To measure moisture levels and hepatosomatic indices, four marron from each treatment group were dissected at the end of the feeding trial. The following equations [29] were used to measure these indices as previously described by Jussila et al. [28]:

$$HM\% = (WH_{wet} - WH_{dry}) \times 100/WH_{wet}$$

$$Hiw = WH_{wet} \times 100/W_t$$

$$Hid = WH_{dry} \times 100/W_t$$

Where, HM% = the percentage of hepatopancreas moisture, WH_{wet} = weight of wet hepatopancreas (g), WH_{dry} = weight of dry hepatopancreas (g), Wt = weight of total marron (g).

2.5 Determination of Selenium

At the commencement of the experiment, day 45 and day 90 of the culture period, four marron from each treatment group, one from each tank, were sacrificed to determine total Se concentrations in hepatopancreas and in tail muscle tissues. Total Se was determined by spectrophotometric method according to the procedure described by Revanasiddappa and Dayananda [25]. Four hepatopancreas and muscles of marron from each diet group were placed separately in a 60 mL volumetric flask and then 4 mL of concentrated HNO_3 was added. These samples were then heated at 80 ^oC for 1 h and another 3 mL of HNO_3 was added to the solution. Heating was continued for an additional 3 h until the samples were completely mineralized. The solution was then cooled and diluted to 10 mL with distilled water. To reduce Se^{6+} to Se^{4+}, 1 mL of the resulting solution was transferred into a test tube and 1 mL of concentrated HCl was added. This solution was then heated at 100 ^oC for 10 min in a thermostat bath and diluted to 10 mL with a 2% of HCl solution after cooling down to room temperature. The final solution was used to analyze the total Se concentration.

To measure total Se, 1 mL of final solution was transferred into a 10 mL flask. Volumes of 1 mL of 1% potassium iodide and 0.5 mL of 1 M hydrochloric acid were then added and shaken. Leuco malachite green (LMG 0.05%, 0.5 mL) was then added and followed by gentle shaking. After 2 min, 3 mL of

acetate buffer (pH 4.5) was added and the reaction mixture was kept in a water bath at 40 ^{o}C for 3 min. The solution was then cooled to room temperature and diluted to 10 mL with water. The final 10 mL solution was thoroughly mixed and allowed to stand for 20 min. The absorbance was measured at 615 nm against the reagent blank. The concentration of total Se was established by reference to the calibration graph.

2.6 Statistical Analysis

Statistical analysis was performed using SPSS software version 17. All data were expressed as mean ± SE (standard error). The data of growth, survival, hepatosomatic indices, total Se in the diet and marron tissues (hepatopancreas and muscle) were subjected to one way ANOVA followed by Tukey's *post hoc* to evaluate significant differences among the group of organic Se supplementation. All significant tests were at $P < 0.05$ levels.

3. Results

3.1 Water Quality Parameters

During the feeding trial, the temperature, pH and dissolved oxygen were found within accepted optimum ranges of 20-23 ^{o}C, 7.16-7.48, and 5.55-5.81 mg L^{-1}, over the experimental period [30-32].

3.2 Selenium Levels in the Diet

The actual levels of Se in the diet showed significantly different ($F = 0.37$, $P < 0.05$) among any levels of Sel-Plex® supplementation in the diet. It also showed slightly higher level than the levels of Sel-Plex® that had been added to the diet (Fig. 1).

3.3 Growth and Survival

After 90 days of feeding trial, the inclusion Sel-Plex® in the diet higher than 0.1 g kg^{-1} improved SGR of marron. The highest SGR was achieved when marron were fed 0.3 g kg^{-1} Sel-Plex in the diet ($F = 11.33$, $P < 0.05$; Fig. 2). Meanwhile, survival of marron fed any levels of Sel-Plex supplementation were significantly higher ($F = 6.77$, $P < 0.05$) than the control and the inclusion of Sel-Plex® higher than 0.1 g kg^{-1} improved survival of marron (Fig. 3).

3.4 Moisture and Hepatosomatic Indices

After 90 days of feeding, *HM*% of marron fed 0.2 g kg^{-1} Sel-Plex was significantly lower ($F = 3.55$, $P < 0.05$) than marron fed the control diet. The inclusion of 0.3 g kg^{-1} Sel-Plex® in the diet resulted in significantly higher *Hiw* ($F = 2.76$, $P < 0.05$) while the *Hid* of marron fed more than 0.1 g kg^{-1} of Sel-Plex was significantly higher ($F = 1.95$, $P < 0.05$) than the

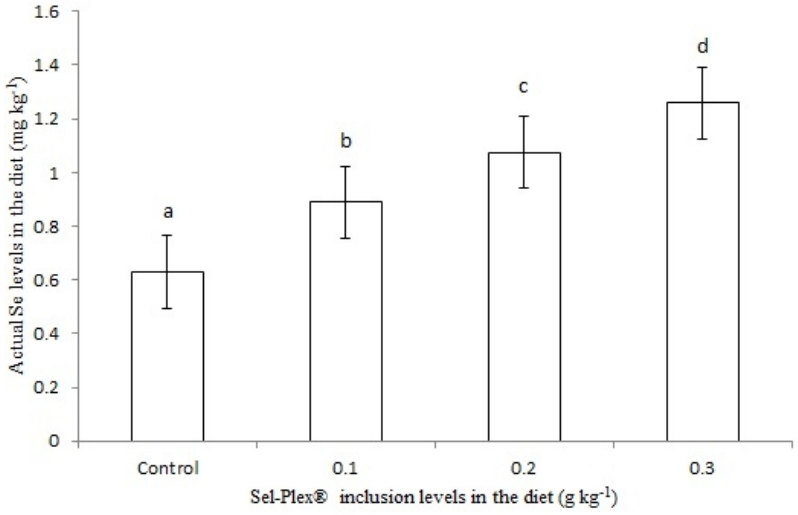

Fig. 1 Actual Se levels in mg kg^{-1} (mean ± SE) in diet after Sel-Plex® inclusion. Different letters indicate significantly different ($P < 0.05$) concentration.

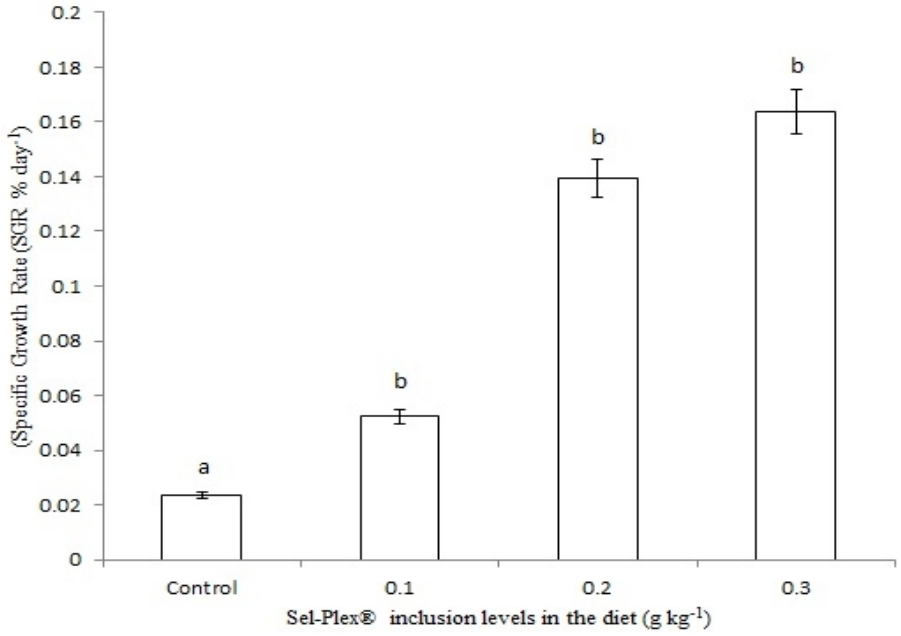

Fig. 2 Specific growth rate (% day^{-1}) of marron fed different levels of Sel-Plex® for 90 days, different letters indicate significantly different ($P < 0.05$).

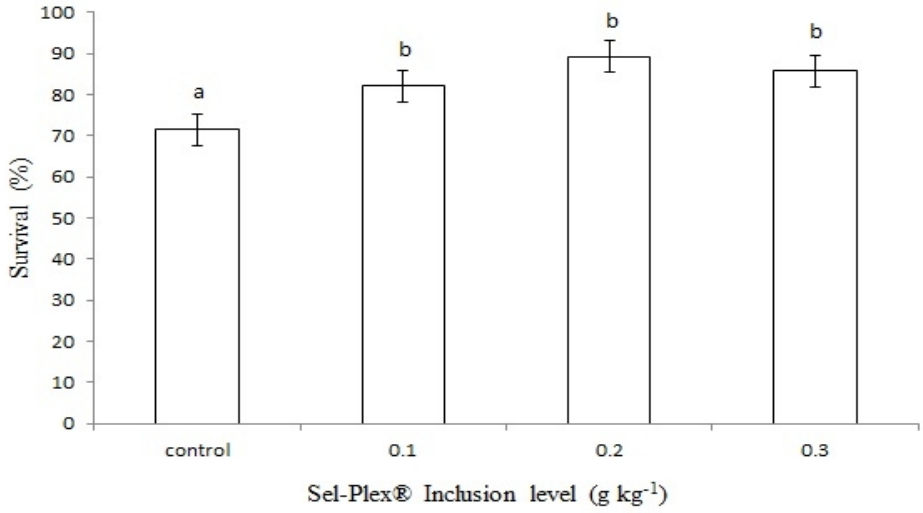

Fig. 3 Survival of marron fed different levels of Sel-Plex® for 90 days. Different letters indicate significantly different ($P < 0.05$).

control (Fig. 4).

3.5 Total Selenium Levels

After 90 days of feeding, marron fed the basal diet showed significantly lower total Se levels both in hepatopancreas ($F = 2.21$, $P < 0.05$) and in muscle ($F = 0.19$, $P < 0.05$) tissues than marron fed Sel-Plex supplementation (Fig. 5). The marron fed 0.3 g kg^{-1} Sel-Plex accumulated highest levels of the total Se in their muscle tissues while the highest level of total Se was in the hepatopancreas of marron fed 0.2 g kg^{-1} Sel-Plex.

4. Discussion

The levels of Sesupplementation in the diet may be different, depending on the levels that are permissible

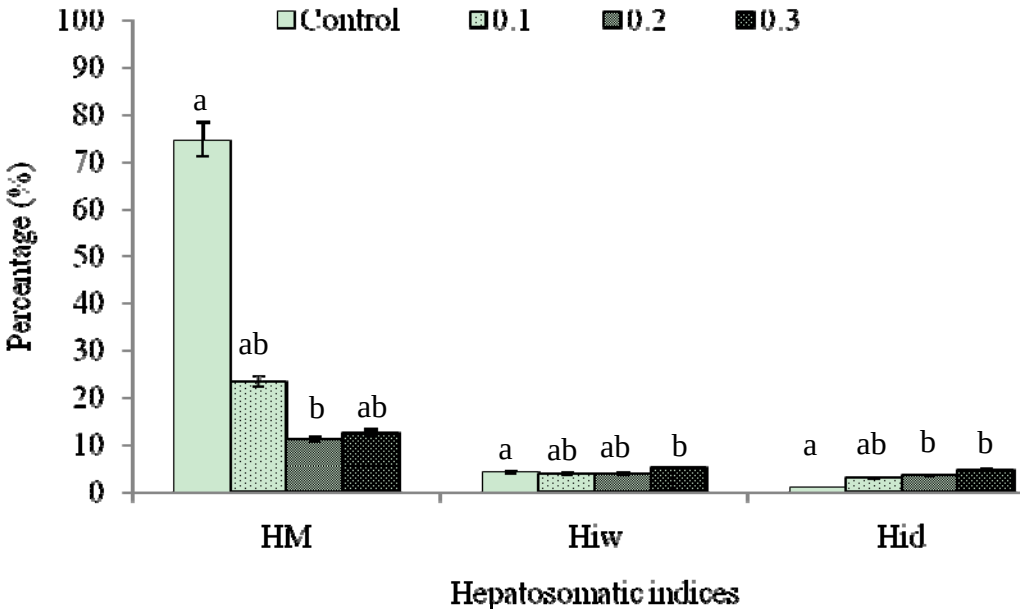

Fig. 4 The mean values of hepatosomatic indices of marron fed different levels of Sel-Plex® (g kg^{-1}) for 90 days. Different alphabet (a, b) above the bars at the same parameters denote significant differences at $P < 0.05$ in mean values. *HM* (%) = the percentage of hepatopancreas moisture, *Hiw* (%) = the percentage of wet hepatosomatic indices, *Hid* (%) = the percentage of dry hepatosomatic indices.

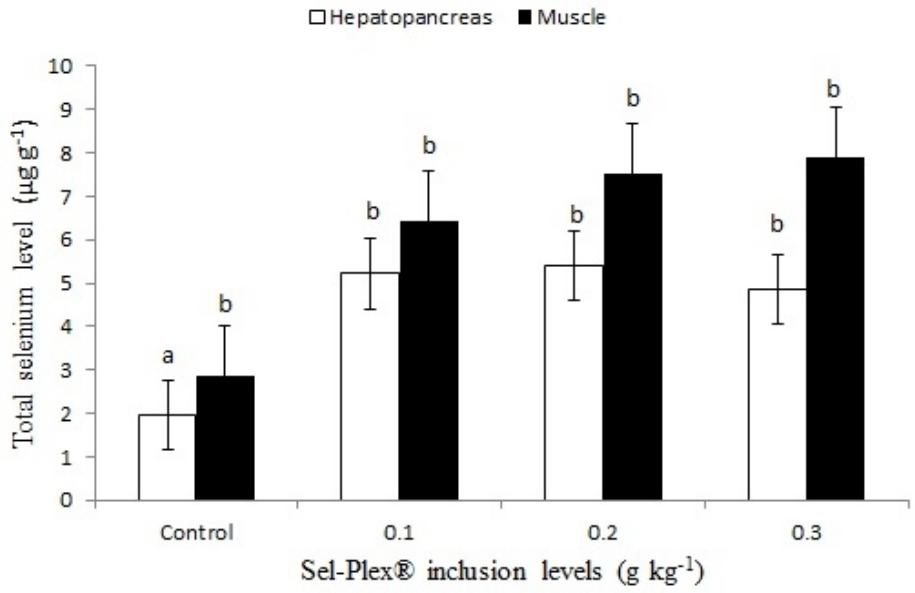

Fig. 5 The mean values of total Se level on the hepatopancreas and muscles tissue of marron fed different levels of Sel-Plex® for 90 days. Different letters at the same color bars denote significant differences ($P < 0.05$) in mean value.

on the certain animals [18, 33, 34]. Current research was the first attempt on the effects of Se supplementation in the diet of marron that used levels 0.1, 0.2 and 0.3 g kg^{-1} Sel-Plex supplementation in the diet as suggested by Wang et al. [17] and Dörr et al. [18] who stated that 0.44 g kg^{-1} Se supplementation was threshold level on prawn (*Penaeus vannamei*). However, the actual Se content in the diet might be

different from the Se supplementation level. Present study showed that the actual Se levels in the control diet and the test diet were greater than the levels of Sel-Plex supplementation. This greater actual level of Se in the diet might be from fish meal and soybean meal that contain Se [35]. Similar results were observed in previous research by Lin and Shiau [4], Wang and Xu [34] and Lorentzen et al. [33] who reported that the actual Se level in the diet was slightly different from the level of Se supplementation.

4.1 Growth and Survival

Crayfish growth can be influenced by age, seasons, stocking density, type of food and supplementation of dietary immunostimulants [30, 31, 36-38]. This study showed that all the Sel-Plex supplementation levels in the diet resulted in an improved growth performance and survival of marron. These results are similar to previous studies on the role of Sel-Plex in improving growth performances of juveniles sea cucumber (*Apostichopus japonicus*) [39] and allogynogenetic crucian carp (*Carassius auratus gibelio*) [5]. The Sel-Plex in the diet gets incorporated with protein structure in the marron tissues and then could interact with iodine to prevent abnormal hormonal metabolism [40, 41], resulting in higher growth performance. In addition, Sel-Plex can be deposited and retained as seleno protein in the muscle and liver tissues of marron for approximately three years where it can be extensively utilized and re-utilized [42]. Higher marron growth performance can also be mediated through the activation of antioxidant enzymes such as Glutathione peroxide (GPx), Superoxide dismutase (SOD) and catalase which are responsible for the improvement in the growth performance, survival and disease resistance [43-45].

4.2 Moisture and Hepatosomatic Indices

Moisture level and hepatopancreas indices have been widely used as tools to evaluate the condition of marron [19, 20, 46, 47]. These indices are also affected by the nutrient profile and supplementation in the diet [19, 20]. The lower moisture and higher weight of hepatopancreas indicate higher total energy reserved reflecting improved health condition [48-50].

The present study showed that after 90 days of feeding trial, the marron fed 0.2 g kg^{-1} Sel-Plex had *HM*% lower than control groups. These condition were indicated that the marron fed Sel-Plex® supplementation were nutritionally healthier by having higher energy reserves in hepatopancreas. In addition, *Hiw* and *Hid* of marron fed Sel-Plex diet also indicated greater ability of marron to distribute and reserve minerals, organic substances such as lipid, carbohydrate and protein [51, 52]. Similarly, moisture content of hepatopancreas of tropical spiny lobsters juvenile (*Panulirus ornatus*, Fabricius 1798) fed mannan oligosaccharide (MOS) indicated improved physiological condition by showing lower *HM*%, and higher *Hiw* than control [53].

4.3 Total Selenium Deposition in Marron Tissues

Se bioaccumulation has been studied in several aquatic animals, including salmon [3], rainbow trout [2], juvenile grouper [4], medaka (*Oryzias latipes*) [54] and adult crayfish (*Procambarus clarkii*) [18]. The uptake of Se can be from water or diet and the uptake of water-soluble Sel-Plex by fish can be from gills, epidermis or gut [55]. In the current experiment, total Se accumulation in hepatopancreas and muscle of marron increased as Sel-Plex supplementation level in the diet increased. Similarly, Mahan and Parrett [12] and Wang et al. [5] reported that the Se supplemented diet resulted in Se accumulation in various tissues of channel catfish (*Ictalurus punctatus)* and can vary in their tissues and organs [56]. Particularly, hepatopancreas as a main digestive gland that are responsible for absorption of nutrients from the digestive track, a high level of Se might be occured in this organ. However, at higher level of Sel-Plex (0.3 g kg^{-1}) in the diet, Se can be deposited in the muscle tissue of marron. Study in hybrid striped bass (*Morone*

saxatilis × *M. chrysops*), channel catfish and Atlantic salmon revealed that high Se concentration occured in muscle tissues when Se was supplemented in their diets [3, 57, 58]. The ingested Sel-Plex has higher absorption, availability and retention than inorganic Sel-Plex [58] and is known to be incorporated into selenoprotein that could be accumulated in the body of animal [15, 59]. Se in organic form such as selenomethionine that binds to amino acid can efficiently be incorporated into animal tissue and has greater retention in muscles than inorganic form [3, 55, 60]. Though, the absorption, availability and tissue accumulation of Se in the body organs can be affected by dietary ingredients, hepatic metabolism and prevailing gut pH [61-63].

In this study, Sel-Plex® as asource of OS is also known as selenoyeast that contains selenoprotein. It is a dried product (non-viable) baker's yeast, derived from *Saccharomyces cereviceae* strain CNCM I-3060, cultivated in a Se-enriched fermentation medium to provide a high level of selenomethionine [23]. Selenomethionine may be incorporated into proteins in place of methionine or be metabolized to Selenocystein, which can be catabolized into hydrogen selenide (H_2Se) by a P-lyase enzyme [64-66]. In contrary to selenomethionine, inorganic Se such as selenate (Na_2SeO_4) and selenite (Na_2SeO_3) are metabolized to H_2Se through reduction and subsequently incorporated into selenoproteins including GSH-PX, iodothyronine 5'-deiodinases (TDI), thioredoxin reductases (TR), selenophosphate synthetase (SePsyn), plasma Selenoprotein P (Se-P), and muscle selenoprotein W (Se-W) [66]. Some of these enzymes are related to antioxidant activity such as GSH-PX [67, 68].

5. Conclusions

The present study suggested that dietary supplementation of Sel-Plex can be used to improve the physiological condition and health of the marron as indicated by growth, survival and acceptable levels of total Sel-Plex in the hepatopancreas and muscle of marron. It is also suggested that 0.2-0.3 g kg^{-1} Sel-Plex should be added in the diet of marron. Further research needs to be conducted to evaluate the direct effects of Sel-Plex supplementation on the digestive enzymes profile and antioxidant enzymes activity.

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