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Comparing the effects of dietary selenium and mannan oligosaccharide supplementation on the growth, immune function, and antioxidant enzyme activity in the cultured marron *Cherax cainii* (Austin, 2002)

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Abstract This study was conducted to compare the effects of dietary supplementation of selenium (Se, both inorganic and organic) and mannan oligosaccharide (MOS) on the growth, immune function, and antioxidant enzyme activities in the haemolymph of the marron *Cherax cainii* (Austin, 2002); 0.4 mg kg^{-1} of sodium selenate, 0.2 g kg^{-1} Sel-Plex®, or 0.4 % Bio-MOS® was added to the basal diet as sources of inorganic selenium (IS), organic selenium (OS), and MOS, respectively. After 90 days, marron fed all 3 supplemented test diets had significantly higher final weight, specific growth rate (SGR), survival, total haemocyte count (THC), percentage of hyaline cells, glutathione-*S*-transferase (GST), and glutathione peroxidase (GPx) activities than marron fed without any supplements. The marron fed OS showed significantly higher THC and percentage of hyaline cells than those fed IS and MOS. Marron fed MOS showed the highest final weight, SGR, and percentage of granular cells; however, there was no significant difference in the neutral red time retention between marron fed IS and OS, whereas marron fed OS resulted in the highest GPx activity and total Se levels in the haemolymph but the lowest lipid peroxidase activity; therefore, it is suggested that dietary supplementation with any source of Se and MOS is beneficial to improving growth, survival, and antioxidant activity.

Keywords Antioxidant activity · Growth and health performances · Marron · MOS · Selenium

Abbreviations

ANOVA	Analysis of variants
CARL	Curtin aquatic research laboratory
CDNB	1-Chloro-2,4 dinitrobenzene
DHC	Differential haemocyte count
DTNB	5,5′-Dithiobis-[2-nitrobenzoic acid]

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GPx	Glutathione peroxidase
GSH	Reduced glutathione
GST	Glutathione-*S*-transferase
IS	Inorganic selenium
LPO	Lipid peroxidase
MDA	Malondialdehyde
MOS	Mannan oligosaccharide
NRRT	Neutral red retention time
OS	Organic selenium
PVC	Polyvinyl carbonate
SGR	Specific growth rate
Se	Selenium
TCA	Trichloroacetic acid
THC	Total haemocyte count

Introduction

Many diets enriched with various supplements have been used in aquaculture to improve the growth and health of cultured animals (Dörr et al. 2008; Chiu et al. 2010; Sang and Fotedar 2010). Selenium (Se) and mannan oligosaccharide (MOS) are common dietary supplements that have been used in cultured prawns (*Macrobrachium rosenbergii*) (Chiu et al. 2010), marron (*Cherax tenuimanus*) (Sang et al. 2011), and rainbow trout (*Oncorhynchus mykiss*) (Kucukbay et al. 2009). In nature, Se is present in both inorganic and organic forms. In the inorganic form, Se is present as selenate or selenite, whereas in the organic form, it is present as selenocysteine and selenomethionine (Barceloux 1999). Both inorganic and organic forms of Se have a pivotal role in the normal metabolism that enhances health, immune function, and antioxidant enzyme activity (Abdel-Tawwab et al. 2007; Han et al. 2011). Another effective supplement, MOS, derived from the cell wall of the yeast, *Saccharomyces cerevisiae*, is used as an effective dietary immunostimulant for improving growth in yabbies (*C. destructor*) (Sang et al. 2011) and survival in rainbow trout (Dimitroglou et al. 2008). In addition, dietary MOS amplifies antioxidant enzyme activity in sea cucumbers (*Apostichopus japonicus)* (Gu et al. 2011).

Antioxidant enzymes, either glutathione-*S*-transferase (GST) or glutathione peroxidase (GPx), protect cellular tissues and membranes against oxidative damage caused by free radicals (Michiels et al. 1994; Felton 1995; Felton and Summers 1995). GST and GPx have also been identified as antioxidant agents to reduce lipid peroxidase (LPO) generated by oxidative metabolism (Monteiro et al. 2009; Lavarías et al. 2011); therefore, it is imperative to maintain the highest possible levels of GST and GPx activity in the haemolymph to counteract the damaging impacts of free radicals. Both GST and GPx convert hydrogen peroxide and fatty acid hydroperoxides into water and fatty acid alcohol through the reduced glutathione (GSH) pathway (Watanabe et al. 1997). The GST and GPx activities can be quantified as GSH, which is consumed per minute during the free radical deactivation process.

LPO is known as an indicator of oxidative stress in cells and tissues. Malondialdehyde (MDA) naturally occurs during the lipid peroxidation process; therefore, MDA levels can also be used to monitor LPO (Marnett 1999). Increasing levels of MDA are associated with

various conditions and pathological states of animals; therefore, MDA levels can be measured to quantify LPO and can be used as a tool to monitor the health of an animal. In addition to MDA, neutral red retention time (NRRT) has been used to evaluate lysosomal membrane stability and, in turn, membrane stress levels (Lowe and Pipe 1994; Dailianis et al. 2003). The stability of animal cell membranes decreases as the level of LPO increases (Ochoa et al. 2003; Catalá 2006). In addition, total and differential haemocyte counts (THC and DHC) are common tools that can be used to monitor the health of cultured animals such as the marron, *C. cainii* (Sang et al. 2009).

As an important cultured species in Western Australia, marron, the third largest crayfish in the world, is recognized as a valuable aquaculture commodity (Nobes 2011). Increasing market demand of the marron has led to a significant boost in research to improve the health performance of the animal; however, the information regarding the effects of Se and MOS dietary supplements on the growth, survival, immunocompetence, and antioxidant enzyme activity of marron is limited. Thus, the current study was designed to evaluate growth, survival rate, THC and DHC, GST, GPx activity, LPO, NRRT, and total Se levels in the haemolymph of marron fed both inorganic and organic forms of Se and MOS.

Materials and methods

Experimental diets

Three different types of supplements were added to the basal diet of cultured marron. Either 0.4 mg kg^{-1} sodium selenate as a source of inorganic Se (IS) or 0.2 g kg^{-1} (approximately equates to 0.4 mg kg^{-1} OS) Sel-Plex® as a source of organic Se (OS) that contains at least 98 % OS, of which 62–68 % is selenomethionine (Burdock and Cousins 2010), and 0.4 % Bio-MOS® (MOS) was added to the basal diet. All levels of supplementation were selected as described in previous experiments (Sang and Fotedar 2010; Nugroho and Fotedar 2013). Dietary ingredients and the composition of the basal diet were formulated using Feed LIVE® version 1.52 (Live Informatics Company Limited, Nonthaburi, Thailand; Table 1). All chemicals and reagents were purchased from Sigma-Aldrich Chemical Company, St Louis, USA, while the ingredients for the basal diet were supplied from Specialty Feed, Pty. Ltd. (Glen Forrest, Western Australia), except for Sel-Plex® and Bio-MOS®, which were donated by Alltech, Nicholasville, USA. All ingredients were thoroughly mixed with sodium selenate, Sel-Plex®, or Bio-Mos® to prepare the test diets. Each test diet or basal diet was mechanically minced to obtain a uniform particle size (0.5 mm in diameter). 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 3 mm lengths and stored in a dark room.

Animals and experimental setup

Marron were purchased from Blue Ridge Marron Farm, Manjimup, Western Australia, and acclimated at Curtin Aquatic Research Laboratory (CARL), Curtin University, Western Australia, for 1 week. Marron were then randomly distributed into 4 triplicate groups ($n = 12$) consisting of 7 marron each. Each group was then placed in blue plastic cylindrical tanks (800 mm in diameter, 500-mm high, 250-L capacity, 70 L freshwater in each tank). Mechanical filtration (fluvial 205 filters; Hagen, Mansfield, Massachusetts, USA) by filtering at a rate of approximately 2 L min^{-1} was used in each tank. Seven PVC pipes

Table 1 Ingredients of basal diet (g kg^{-1}) and proximate composition of the diets

Ingredients	Content (g kg^{-1})
Fish oil[a]	32
Wheat bran	545.59
Soybean meal	101.5
Fish meal[b]	257.14
Calcium carbonate	0.2
Ascorbic acid	0.5
Betaine[c]	12
Premix[d]	1.5
Cholesterol	2.5
Wheat starch	47.07
Proximate composition	Percentage
Crude protein	27.05
Crude fat	8.02
Crude fibre	6.39
Moisture content	9.01
Ash	6.56
Energy (cal/g)	1,833.249

All ingredients supplied by Specialty Feeds Pty Ltd, WA, Australia. [a] Cod liver oil. [b] Peruvian fishmeal, 56 % CP. [c] Betaine Anhydrous 97 %. [d] Commercial vitamin and mineral premix for trout. Actual Se in the basal diet = 0.89 mg kg^{-1} Se

(55 mm in diameter, 150 mm long) were also placed in each tank to provide shelter for each marron. The water temperature was maintained at 24–25 °C by using automatic heaters (Sonpar®, Model: HA-100; Yong Cheng Aquarium Co., Ltd., China). Dissolved oxygen was monitored and maintained at 6 ppm every 2 days using a CyberScan pH 300 (Eutech Instruments, Singapore, China). NO_2–N, NO_3–N, NH_3/NH^{+4}, PO_4–P, pH, were monitored every 2 days using chemical test kits (Aquarium Pharmaceuticals™, Inc., McLean, Virginia, USA).The marron in each tank were fed either the test or basal diet at a proportion of 3 % of its body weight every other day. This feeding rate was determined by previous experiments (Sang and Fotedar 2010; Nugroho and Fotedar 2013). Uneaten food and faeces were syphoned out before the next feeding, and sufficient fresh water was added to maintain 70 L of water in each tank.

Sampling and analytical procedures

Surviving marron from each tank were counted every 15 days until the end of the feeding trial. The marron haemolymph was taken from the base of the fifth thoracic leg of each marron at day 90. Following collection of haemolymph samples, THC, DHC, GST, GPx, LPO, and NRRT were determined. Total soluble Se levels in the marron haemolymph was also analysed after 90 days of feeding.

The weight of each marron from each tank was measured at days 0 and 90 of the feeding trial to calculate initial weight, final weight, and specific growth rate (SGR) by using the following equation:

$$\mathrm{SGR} = 100 \times (\ln(\mathrm{Wt}) - \ln(\mathrm{Wo}))/(\mathrm{d})$$

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.

Total and differential haemocyte counts

Total haemocyte counts (THC) and differential haemocyte counts (DHC) were determined using Rose Bengal to stain haemocytes (Sritunyalucksana et al. 2005). One-tenth of a millilitre of haemolymph was withdrawn into a syringe containing 0.1 mL fixative (10 % formalin in 0.45 M NaCl) and transferred into an Eppendorf tube. After 10 min, the mixture was fixed using 20 μL Rose Bengal solution (1.2 % Rose Bengal in 50 % ethanol). The mixture was incubated at room temperature for 20 min and was used for determining THC with an improved Neubauer Bright-Line Haemocytometer, Sigma-Aldrich, USA, and to prepare smears on microscope slides for determining the DHC. THCs were determined and expressed as cell $\times 10^6$ cells mL^{-1} haemolymph. To calculate DHCs, smears were completely dried before counterstaining with haematoxylin solution for 8 min. The slide was then rinsed with tap water for 10 min, followed by dehydration with 95 % ethanol (10 dips) and 100 % ethanol (10 dips). After dehydration, the slide was submerged in xylene (thrice for 3 min each) before it was mounted with albumin and covered with a cover slip. The proportions of granular, semigranular, and hyaline cells from 200 haemocytes were recorded (Sritunyalucksana et al. 2011).

Antioxidant enzyme activity and LPO assay

The haemolymph from individual marron was diluted with physiological saline at a ratio of 1:1 and stored at 4 °C until used for determining GST, GPx, and LPO. GST was measured using an UV–visible spectrophotometer (Habig et al. 1974). A mixture containing 200 μL phosphate buffer (pH 6.5), 20 μL 1-chloro-2,4 dinitrobenzene (CDNB) in 95 % ethanol, and 730 μL distilled water was placed in a control tube, and 200 μL 0.5 M phosphate buffer, 20 μL of 25 mM CDNB, and 680 μL distilled water were placed in test tubes. Both the control and test tubes were incubated at 37 °C for 10 min. After incubation, 50 μL 20 mM GSH were added to both sets of tubes and mixed. After mixing, 50 μL haemolymph was added to the test tubes. An increase in absorbance was noted at 340 nm for 5 min. Values were expressed in nanomoles of CDNB conjugated per minute per milligram of protein. Protein was estimated using the method described by Lowry et al. (1951).

To calculate GPx activity, 0.2 mL of 0.4 M phosphate buffer (pH 7.0), 0.1 mL 10 mM sodium azide, 0.2 mL 0.2 mM reduced glutathione, and 0.1 mL 0.2 mM hydrogen peroxide were mixed. The mixtures were then incubated for 10 min at 37 °C after which 0.4 mL of 10 % trichloroacetic acid (TCA) was added to stop the reaction, and the mixtures were centrifuged at 3,200 rpm for 20 min. The supernatant was assayed for glutathione content using Ellman's reagent (19.8 mg 5,5′-dithiobis-[2-nitrobenzoic acid] [DTNB] in 100 mL 0.1 % sodium nitrate). The GPx activity was expressed as micrograms of GSH consumed per minute per milligram of protein (Rotruck et al. 1973; Lawrence et al. 1974).

LPO of marron haemolymph was determined by measuring the concentration of thiobarbituric acid reacting substances (Buege and Aust 1978). One hundred microlitres of marron haemolymph were mixed with 500 μL mixture solution containing 15 % (w/v) TCA, 0.375 % (w/v) thiobarbituric acid, and 80 % (v/v) hydrochloric acid 0.25 N. The mixture was heated to 100 °C for 15 min and cooled at room temperature. The mixture was then centrifuged at 1,500 rpm for 10 min. Absorbance in the supernatant was measured at 535 nm. The level of LPO was expressed as nmol MDA per mg protein. Protein in the haemolymph was estimated by the method of Lowry et al. (1951).

Neutral red retention time

NRRT was determined using assays based on previous protocols (Hauton and Smith 2004). To prepare a stock solution, 10 mg neutral red powder was dissolved in 1 mL dimethyl sulphoxide. A working solution (dye concentration 0.02 mg mL^{-1}) was prepared by mixing 10 mL stock solution and diluting with 5 mL artificial saline water. Then, 0.2 mL marron haemolymph sample was transferred to an Eppendorf tube containing 0.2 mL artificial saline water and gently mixed. A sample of the mixture was placed onto a microscope slide treated with a poly-L-lysine solution to increase cell adhesion. The slide was immediately placed in a 10 °C incubator for 15 min to allow the haemocytes to attach to the slide. The slide was then immediately removed from the incubator and the excess haemolymph was removed. Forty microlitres of neutral red working solution was added to the slide and covered with a cover slip. The slide was then returned to the incubator. The slide was removed from the incubator every 15 min, and the sample was examined under a light microscope (400× magnification, Olympus SC30 BH2; Olympus, UK) for 2 min. The time at which 50 % of the haemocytes had begun to lose dye from their lysosomes was recorded as the NRRT of the marron lysosomal membrane.

Determination of total soluble Se

At the end of the trial, 3 marron from each group were sacrificed to determine the total soluble Se in their haemolymph. Total soluble Se was determined using the spectrophotometric method according to Revanasiddappa and Dayananda (2006). Briefly, 4 mL concentrated HNO_3 was added to all samples and heated at 80 °C for 1 h. Thereafter, an additional 3 mL HNO_3 was added to the sample solution. The solution was heated for an additional 3 h until the samples were completely mineralized. The solution was then cooled and diluted to 10 mL with distilled water. Next, 1 mL of the resulting solution was transferred to a test tube, and 1 mL concentrated HCl was added to reduce Se^{6+} to Se^{4+}. This solution was then heated at 100 °C for 10 min in a thermostat bath and diluted to 10 mL with a 2 % HCl solution after it had cooled to room temperature. The final solution was used to analyse total soluble Se levels.

To measure total soluble Se, 1 mL final solution was transferred to a 10-mL flask, to which 1 mL 1 % potassium iodide and 0.5 mL 1 M hydrochloric acid were added. The flasks were gently shaken, 0.5 mL of 0.05 % leuco malachite green (LMG) was added, and the flasks were shaken again. After 2 min, 3 mL acetate buffer (pH 4.5) was added and the reaction mixture was kept in a water bath at 40 °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 mixed thoroughly and allowed to stand for 20 min. The absorbance was measured at 615 nm against the blank. The concentration of total soluble Se was established by reference to the calibration graph.

Statistical analysis

Results are expressed as mean ± standard error (SE), and data were analysed using SPSS version 17. All per cent data of survival was transformed to arcsine before prior to one-way ANOVA The data of initial weight, final weight, SGR, THC, DHC, antioxidant enzymes activity (GSH and GPx), LPO, NRRT, and total soluble Se accumulation on day 90 of feeding were subjected to one-way ANOVA, followed by the Duncan post hoc test to

evaluate significant differences among the groups of supplements. All significant tests were at $P < 0.05$ levels.

Results

All dietary supplements significantly improved ($P < 0.05$) the final weight, SGR, and survival and resulted in significant differences in mean THCs and the mean proportion of hyaline cells in the marron haemolymph. Supplementing with dietary MOS resulted in higher final weight and SGR of marron than any other supplement (Table 2). The highest survival (Fig. 1) and THC (Table 3) were found in marron fed dietary Sel-Plex®, whereas the lowest mean hyaline cells were found in the marron fed MOS. After 90 days of feeding, the mean proportion of granular cells was significantly higher ($P < 0.05$) in the marron fed MOS, but there were no significant differences ($P > 0.05$) in the marron fed either the control diet or any source of selenium. The proportion of semigranular cells were significantly different between the control group and marron fed either IS or MOS (Table 3).

Inclusion of any types of feed supplement increased the antioxidant activity in the marron. No significant difference in GST activity was evident among any marron fed with supplements. Adding Sel-Plex® to the diet resulted in significantly higher ($P < 0.05$) GPx activity, reduction in LPO levels, and improvement in NRRT in the marron haemolymph (Table 4).

Marron fed IS or Sel-Plex® supplements showed significantly higher ($P < 0.05$) total soluble Se in the haemolymph than those fed the control and MOS supplements. The highest total Se in the haemolymph was found in marron fed Sel-Plex®.

Discussion

The present study indicated that the basal diet supplemented with either sodium selenate, Sel-Plex®, or Bio-Mos® could equally improve SGR (Table 2) and marron survival (Fig. 1). Current research also indicated that marron fed a supplement of MOS showed significantly higher SGR than those fed other supplements. This finding was in line with previous research that MOS inclusion in the diet significantly improved marron SGR (Sang and Fotedar 2010). According to Sang and Fotedar (2010), MOS can improve marron SGR

Table 2 Mean ± SE growth performance of marron fed different supplements

Parameters	Types of supplements			
	BD	IS	OS	MOS
Initial weight (g)	38.68 ± 0.18^{a}	43.17 ± 2.13^{a}	39.57 ± 1.72^{a}	38.80 ± 0.99^{a}
Final weight (g)	40.54 ± 0.28^{a}	45.90 ± 0.82^{b}	45.50 ± 0.91^{b}	47.33 ± 0.89^{b}
SGR (% day^{-1})	0.052 ± 0.006^{a}	0.070 ± 0.003^{ab}	0.157 ± 0.025^{bc}	0.22 ± 0.008^{c}

Different superscript alphabets in the same row indicate significantly different means $P < 0.05$. BD = basal diet; IS = 0.4 mg kg^{-1} Se (sodium selenate was used as a source of inorganic Se); OS = 0.2 g kg^{-1} Sel-Plex®, approximately equates to 0.4 mg kg^{-1} of organic Se; MOS = mannan oligosaccharide (0.4 % of Bio-MOS®)

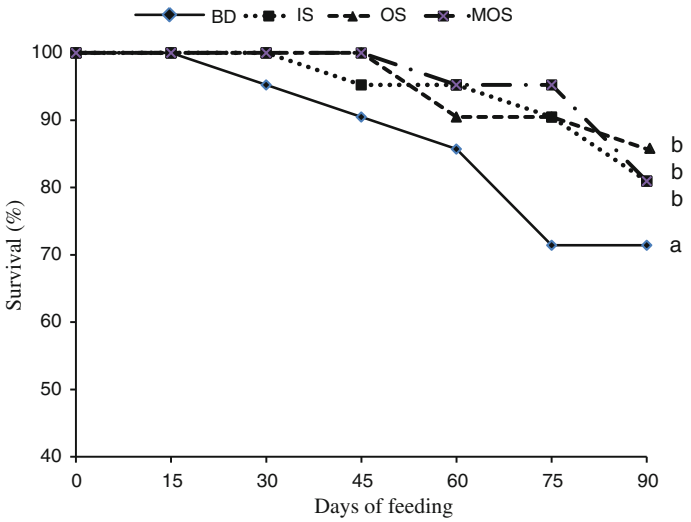

Fig. 1 Survival of marron fed different diet supplements BD = basal diet; IS = 0.4 mg kg^{-1} Se (sodium selenate was used as a source of inorganic Se); OS = 0.2 g kg^{-1} Sel-Plex®, approximately equates to 0.4 mg kg^{-1} of organic Se; MOS = mannan oligosaccharide (0.4 % of Bio-MOS®). Different alphabets denote significant difference ($P < 0.05$)

Table 3 Mean ± SE immunocompetence of marron fed different supplements

Parameters	Types of supplements			
	BD	IS	OS	MOS
THC (cells x 10^6 mL)	1.76 ± 0.06^a	2.41 ± 0.05^b	3.13 ± 0.08^c	2.28 ± 0.03^b
Granular (%)	34.33 ± 2.02^a	23.66 ± 0.33^a	23.00 ± 1.52^a	59.00 ± 1.15^b
Semigranular (%)	35.00 ± 3.05^a	20.66 ± 1.76^b	27.33 ± 2.02^{ab}	19.66 ± 0.66^b
Hyaline (%)	34.33 ± 1.45^a	55.66 ± 1.45^b	50.33 ± 1.85^b	20.66 ± 0.88^c

Different superscript alphabets in the same row indicate significantly different means $P < 0.05$. BD = basal diet; IS = 0.4 mg kg^{-1} Se (sodium selenate was used as a source of inorganic Se); OS = 0.2 g kg^{-1} Sel-Plex®, approximately equates to 0.4 mg kg^{-1} of organic Se; MOS = mannan oligosaccharide (0.4 % of Bio-MOS®)

by improving digestive-tract health, such as increasing the number of beneficial bacteria and villi density in the gut as well as enhancing epithelium thickness.

In addition to improving SGR, the current study showed that any source of Se supplementation and MOS inclusion in the diet enhanced marron survival rate; however, there was no differential effect of Se and MOS supplementation on survival. These results were concomitant with previous research on prawns fed an Se-enriched diet (Torrecillas et al. 2007) and cobia (*Rachycentron canadum*) larvae fed MOS that showed similar increases in survival; however, organic Se had a greater impact than inorganic Se on the survival of cultured channel catfish (*Ictalurus punctatus*) (Wang and Lovell 1997) and rainbow trout (Kucukbay et al. 2009).

The results of the present study indicate that, because of their positive influence on the immune system, supplementation of diet with both IS and Sel-Plex® can increase THCs in marron. Supplementing the diet with both Se forms can affect the proportion of circulating

Table 4 GST, GPx, LPO activity, NRRT, and total soluble selenium in the haemolymph of marron fed different supplements for 90 days of feeding trial

Parameters	Types of supplements			
	BD	IS	OS	MOS
GST	159.70 ± 49.46[a]	319.55 ± 7.64[b]	329.49 ± 15.24[b]	277.22 ± 47.01[b]
GPx	198.04 ± 65.35[a]	408.34 ± 8.62[b]	616.61 ± 18.76[c]	213.48 ± 9.74[d]
LPO	0.126 ± 0.002[a]	0.095 ± 0.006[b]	0.067 ± 0.004[c]	0.110 ± 0.004[ab]
NRRT	65.00 ± 5.00[a]	115.00 ± 5.00[b]	150.00 ± 8.66[c]	115.00 ± 5.00[b]
Total soluble selenium	0.08 ± 0.01[a]	0.41 ± 0.04[b]	0.54 ± 0.05[c]	0.17 ± 0.05[a]

Different superscript numericals in the same row indicate significantly different means $P < 0.05$. BD = basal diet; IS = 0.4 mg kg^{-1} Se (sodium selenate was used as a source of inorganic Se); OS = 0.2 g kg^{-1} Sel-Plex, approximately equates to 0.4 mg kg^{-1} of organic Se; MOS = mannan oligosaccharide (0.4 % of Bio-MOS®). GST = glutathione-*S*-transferase (nano moles of CDNB conjugated min^{-1} mg $protein^{-1}$); GPx = glutathione peroxidase (μg GSH consumed min^{-1} mg $protein^{-1}$); LPO = lipid peroxidase (nmol malondialdehyde-MDA mg^{-1} of protein); NRRT = neutral red retention time (s = second). Total selenium soluble in the haemolymph expressed as μg mL^{-1}

haemocytes without affecting the proportions of granular cells. The mechanism by which dietary inclusion of Se alters the haemocyte profile of marron is not yet fully understood; however, Alina et al. (2009) stated that a Se-enriched diet can be assimilated into enzymes and antioxidants, which are important in body development and immunity. Meanwhile, Bio-Mos®, which is derived from the outer wall of *S. cerevisiae,* has also been proven to enhance an animal's performance, reinforce the natural defences, and stimulate immune response (Sang et al. 2011). Current results showed that MOS supplementation increases THCs and the mean proportion of granular cells of marron, but it reduces the proportion of hyaline cells. Dietary MOS can increase phagocytic cell activity (Yoshida et al. 1995; Savage et al. 1996) and inhibit the attachment and penetration of bacteria into cells (Swanson et al. 2002), thereby confirming the mode of action to improve immune function and antioxidant activity in marron.

In addition to enhancing immunity, inclusion of any type of dietary supplement in the marron's diet resulted in higher GST and GPx. Present findings showed that supplementing the diet with Sel-Plex® had the highest impact on the GPx activity. Dietary Sel-Plex® also had a beneficial effect on membrane stability compared to IS or MOS supplements. These findings were in agreement with a previous study that Sel-Plex® has more positive effects on GPx activity than does IS (Cotter 2006). GPx makes cellular and subcellular membranes less sensitive to oxidative damage and reduces free radicals by scavenging, binding, and inactivating free radicals to prevent damage to the cells (Winston and Di Giulio 1991; Michiels et al. 1994; Felton et al. 1996). Furthermore, GPx makes cellular and subcellular membranes less sensitive to oxidative damage, which is related to the LPO level.

The present results showed that Sel-Plex®-treated marron had significantly lower LPO than any other marron group. This result was supported by Han et al. (2011), who stated that GPx activities of fish fed diets supplemented with OS were significantly higher than fish fed the control diet. In contrast, Chiu et al. (2010) revealed that GPx activities of prawns fed OS-enriched diets did not significantly differ from prawns fed the control diet. The activity of GPx of marron fed Se could contribute to the maintenance of LPO levels (Monteiro et al. 2009). LPO, specifically polyunsaturated fatty acid (PUFA) oxidation, is highly unstable and can cause damage to the cellular biomembranes as a consequence of

oxidative deterioration of bipolar lipid membranes (Kanazawa 1991, 1993). Furthermore, increased levels of LPO may lead to decreased membrane fluidity and membrane disorganization (Mohankumar and Ramasamy 2006).

It is possible that adding Se to the diet might induce the lysosomal membrane stability and minimize the number of unhealthy cells (Ursini and Bindoli 1987). The unhealthy cells, caused by decreasing lipid membrane integrity, can lose neutral red dye at a faster rate than healthy cells. Results of the present study show that lysosomal membrane integrity was affected by both forms of Se supplementation and Bio-Mos®, as shown by the increase in NRRT. Furthermore, marron fed OS had the longest NRRT compared to those fed IS or MOS supplements.

The ability of animals to accumulate Se from dietary sources has been studied in several aquatic animals such as rainbow trout (Vidal et al. 2005) and juvenile grouper (*Epinephelus malabaricus*) (Lin and Shiau 2007). Current research showed that both organic and inorganic forms of Se in the test diet significantly increased the total soluble Se in marron haemolymph; however, total soluble Se in the haemolymph of OS-fed marron was significantly higher than total soluble Se in the haemolymph of IS- and MOS-fed marron. Similar research stated that selenomethionine has been proved to have a higher bioavailability than sodium selenite in Atlantic salmon (*Salmo gairdneri*) (Lorentzen et al. 1994) and channel catfish (Wang and Lovell 1997) and can be deposited as selenoprotein in the muscle and hepatopancreatic tissues of animals (Aguilar et al. 2009).

In conclusion, supplementing the marron's basal diet with IS and Sel-Plex® along with MOS has beneficial effects on growth performance, survival, THC, DHC, and antioxidant activity. Further research needs to be conducted to trace the biological pathways of Se and MOS absorption into the marron's body tissues.

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