# Toxic effects of excessive levels of dietary selenium in juvenile yellowtail kingfish (*Seriola lalandi*)

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ABSTRACT

Selenomethionine (SeMet) was supplemented to a fishmeal-based diet to investigate the toxic effects of excessive levels of dietary selenium (Se) in juvenile yellowtail kingfish (*Seriola lalandi*). For 10 weeks, the fish were fed one of five experimental diets; a basal diet containing 2.31 mg/kg of inherent Se or diets supplemented with SeMet to provide 4.91, 9.58, 15.43 or 20.87 mg/kg of Se. The results showed that the fish muscle proximate composition, feed conversion ratio and survival were not sensitive to dietary Se treatments; and no histopathological lesions were observed in heart and intestine tissues of the fish. The Se concentrations in liver and muscle tissues showed a strong linear positive relationship with the levels of Se in diets. Fish when fed the basal diet exhibited Se deficiency symptoms including myopathy, reduced feed intake, glutathione peroxidase activity and growth; whereas those fed the diets containing ≥ 4.91 mg Se/kg did not. While fish fed the 15.43 mg Se/kg diet did not show any toxic effects, the 20.87 mg Se/kg diet caused histopathological changes in liver and spleen as well as reduced feed intake, growth, haematocrit and hepatosomatic index, indicating Se toxicity. In conclusion, Se levels in liver and muscle tissues can be used as effective indicators of dietary Se exposure and dietary Se level between 15.43 and 20.87 mg/kg may be a threshold level in juvenile yellowtail kingfish.

*Keywords:* Histopathology; Selenomethionine; Toxicity; Yellowtail kingfish

## 1. Introduction

As an essential trace element for normal growth and physiological function of animals including fish (NRC, 1993), selenium (Se) has gained the attention of many researchers in fish nutrition. This includes research in Se deficiency, requirement and bioavailability of Se in various fish species (Hilton et al., 1980; Gatlin and Wilson, 1984; Bell et al., 1987; Lin and Shiau, 2005; Liu et al., 2010; Le and Fotedar, 2013). In addition, due to its potential toxicity to terrestrial animals (Halverson et al., 1966; Mézes and Balogh, 2009), the toxic effects of dietary Se in fish has been also of interest. Signs of Se toxicity in fish include high mortalities, histopathological changes in liver tissues, diminished reproductive performance and reduced feed intake, growth response and haematocrit values (Hilton et al., 1980; Gatlin and Wilson, 1984; Sorensen et al., 1984; Lemly, 1997; Tashjian et al., 2006; Jaramillo et al., 2009). However, the toxic levels of dietary Se have been a controversial topic for many years. Hamilton et al. (1990) proposed that concentrations of dietary Se in the range of 3 to 5 mg/kg are toxic to chinook salmon *(Oncorhynchus tshawytscha*). Whereas, Tashjian et al. (2006) suggested the dietary Se toxicity threshold for white sturgeon (*Acipenser transmontanus*) is between 10 and 20 mg/kg. Interestingly, cutthroat trout (*Oncorhynchus clarki bouvieri*) fed

Se up to 11.2 mg/kg for 2.5 years showed no signs of Se toxicity (Hardy et al., 2010). The authors argued that cutthroat trout can regulate Se through excretion to maintain Se concentrations below toxic levels.

As the difference between beneficial and toxic effects of dietary Se is narrow (Watanabe et al., 1997), it is essential to map the beneficial and toxic concentrations of dietary Se in order to optimise its dietary inclusion level. The requirement and bioavailability of dietary Se for yellowtail kingfish (*Seriola lalandi*) has been studied (Le and Fotedar, 2013; 2014), in which the supplementation of Se from Se-yeast at 2 mg/kg to a fishmeal-based diet containing 3.35 mg Se/kg resulted in the maximal growth, and organic Se such as selenomethionine (SeMet) or Se-yeast appeared to be more bioavailable than an inorganic form, selenite. However, nothing is reported about its toxic effects to this species. Therefore, this study was carried out to investigate physiological responses of yellowtail kingfish to excessive levels of dietary Se and to set a threshold dietary Se level for yellowtail kingfish culture.

## 2. Materials and methods

### *2.1. Experimental diets and design*

A basal diet (Table 1) was supplemented with four graded levels of Se as DL-selenomethionine (SeMet; Sigma-Aldrich, St. Louis, MO, USA). Selenomethionine was chosen as it is the dominant form of Se present in food (Suzuki, 2005) and it has been shown to accumulate in yellowtail kingfish (Le and Fotedar, 2014). The pre-determined quantities of Se were dissolved in water and added to the basal ingredients before pelleting the feeds through a 3-mm diameter die. The pellets were then air-dried at room temperature and stored at -20 ºC until used. The Se concentrations in the basal diet and the Se supplemented diets were then analysed to be 2.31 and 4.91, 9.58, 15.43 and 20.87 mg/kg, respectively.

Juveniles of yellowtail kingfish were obtained from the Australian Centre for Applied Aquaculture Research, Fremantle, WA, Australia and brought to the Curtin Aquatic Research Laboratory (CARL), Curtin University. The fish were group weighed and stocked into each of 15 experimental 300-L tanks at a density of 12 fish per tank (0.78 kg/$m^3$). Total weight of fish in each tank was 234.62 ± 0.53 g (mean ± SE), with an average individual weight of 19.55 ± 0.04 g (mean ± SE). The tanks were filled with seawater at salinity of 35 ppt and Se concentration < 1µL, and were supplied with constant aeration and pure oxygen. Each tank had an external bio-filter running continuously to create a recirculating system and an automatic heater set at approximately 22.8 $^{o}$C to maintain water temperature. Faecal matter was removed daily and half of the water was changed every two days. Water temperature, pH and dissolved oxygen were measured daily using digital pH/mV/ºC and dissolved oxygen meters (CyberScan pH 300 and CyberScan DO 300, Eutech Instruments, Singapore). Total ammonia was monitored daily by an ammonia ($NH_3/NH_4^+$) test kit (Mars Fishcare, Chalfont, PA, USA).

Each dietary treatment was randomly assigned to three tanks. The fish were hand fed to satiation, twice a day at 08 am and 04 pm for 10 weeks. The fish were fed slowly to ensure no uneaten food. The amount of feed consumed was recorded daily to estimate feed intake. All of the fish from each tank were weighed every two weeks to monitor growth. Total feed intake and weight measurement at the end of the trial were used for the estimation of feed conversion ratio (FCR, feed intake divided by the wet weight gain).

### *2.2. Sample collection*

At the commencement of the trial, 18 additional fish were used to estimate initial Se content in the liver and muscle. Both liver and muscle tissue samples were pooled before the analysis.

At the end of the feeding trial, three fish from each tank were randomly selected and blood was sampled from the caudal vein with syringes and directly used for measurement of haematocrit. The remaining blood was allowed to clot for 2 h at 4 ºC and red blood cell pellets were separated by centrifugation of whole blood at 1500×*g* for 10 min at 4 ºC using a centrifuge (5804R, Eppendorf, Hamburg, Germany). The red blood cell pellets were stored at -80 ºC until used for glutathione peroxidase assay.

Following the blood sampling, liver, spleen, heart, left anterior dorsal muscle and anterior intestine were dissected from each fish and fixed in 10% buffered formalin for 24 h for histopathological examination. The remaining muscle tissues from each fish were used for estimation of Se content and proximate composition.

The remaining fish (nine per tank) and their livers were individually weighed to calculate hepatosomatic index (HSI = 100 × liver weight / body weight). The livers of the nine fish were pooled for estimation of Se content.

*2.3. Haematocrit assay*

Haematocrit (Ht) of each fish was determined in triplicate by the microhaematocrit method (Rey Vázquez and Guerrero, 2007). Blood was collected into heparin-coated microhaematocrit tubes and centrifuged at 13000×*g* for 5 min to determine Ht (the percent packed cell volume).

*2.4. Glutathione peroxidase assay*

Glutathione peroxidase (GPx) activity in red blood cells from each fish was assayed using the Ransel RS-505 kit (Randox, Crumlin, County Antrim**,** UK) and a chemistry immune analyser (AU400, Olympus, Tokyo, Japan) at 340 nm and 37 ºC. The results were expressed as units of GPx/g of haemoglobin (Hb). Haemoglobin was measured using the Hb HG-1539 kit (Randox, Crumlin, County Antrim, UK).

*2.5. Histopathological examination*

The histological samples were routinely processed, dehydrated in ethanol before equilibration in xylene and embedded in paraffin wax. Sections of approximately 5 µm were cut and stained with haematoxylin and eosin and observed by a light microscope (BX40F4, Olympus, Tokyo, Japan) under 100× and 400× magnifications. Numbers of macrophage aggregates (MAs) per sections of entire spleens were counted.

*2.6. Chemical analysis*

Protein, lipid, moisture, ash and Se were analysed according to the standard methods of the Association of Official Analytical Chemists (1990): crude protein by analysis of nitrogen using the Kjeldahl method; crude lipid by petroleum ether extraction using the Soxhlet method; moisture by drying at 105 ºC to a constant weight and ash by combustion at 550 ºC for 24 h. Selenium was estimated using an atomic absorption spectrometer equipped with vapour generation assembly (AA280 FS and VGA 77, Varian, Mulgrave, Vic, Australia). Gross energy was determined using a bomb calorimeter (C2000, IKA, Staufen, Germany).

*2.7. Statistical analysis*

Data were analysed using PASW Statistics 18.0 (IBM Corporation, New York, US). All data were subjected to Levene's test for homogeneity of variance and one-way ANOVA. Macrophage aggregate data were square-root transformed before analysis. When a significant treatment effect was observed, Tukey's Honest Significant Difference test was used for multiple mean comparisons. Linear regression analyses were performed on tissue Se

concentrations against dietary Se concentrations. The statistical significance was set at $P < 0.05$.

## 3. Results

The measured water quality parameters were not significantly different ($P > 0.05$) among the dietary treatments. During the trial, water temperature, pH and dissolved oxygen averaged 21.7 ± 0.7 ºC, 7.6 ± 0.2, and 6.9 ± 0.4 mg/L (mean ± SD), respectively. Total ammonia ($NH_3/NH_4^+$) was always ≤ 1.0 mg/L.

During the first four weeks, no dietary treatment resulted in any significant differences ($P > 0.05$) in fish growth. However, from week 6 the dietary Se supplementations resulted in significantly ($P < 0.05$) higher weight gains than the basal diet (Table 2). At week 8, the fish fed 20.87 mg Se/kg diet started to show decrease in weight gain which became similar to the basal diet at week 10.

Dietary Se had no effects on proximate composition and gross energy of muscle tissues (Table 3). Similarly, feed conversion ratio and survival of fish were not affected by dietary Se levels, but feed intakes were significantly influenced by the dietary Se levels (Table 4). Significantly ($P < 0.05$) lower feed intakes were found in fish fed the lowest and highest levels of Se.

There were significant ($P < 0.05$) increases in yellowtail kingfish liver and muscle Se concentrations which corresponded with increasing dietary Se levels (Table 4). Linear regression analysis of tissue Se accumulation showed linear responses to dietary Se levels ($y = 0.2888x - 0.0092$, $R^2 = 0.964$ and $P < 0.001$ for liver; $y = 0.0701x + 0.237$, $R^2 = 0.920$ and $P < 0.001$ for muscle; Fig.1).

Significant differences in Ht, HSI and GPx activities between dietary treatments were observed (Table 5). Haematocrit values and HSI were significantly ($P < 0.05$) lower in the fish fed the diet containing the highest level of Se. The fish fed the basal diet had significantly ($P < 0.05$) lower GPx activity than other fish. The highest GPx activity was found in fish fed the highest Se level.

Yellowtail kingfish fed different dietary Se concentrations did not show any histopathological lesions or degeneration in heart and intestine tissues. However, the number of splenic macrophage aggregates was four times significantly ($P < 0.05$) higher in fish fed the highest Se diet than those fed the lower Se diets (Table 5; Fig. 2). The highest Se diet also resulted in hepatocyte atrophy (Fig. 3). In contrast, necrotic muscle tissues were only observed in the fish fed the lowest Se diet, 2.31 mg/kg (Fig. 4).

## 4. Discussion

The dietary Se concentration required to prevent yellowtail kingfish from Se deficiency has been reported between 3.35 and 4.86 mg/kg diet (Le and Fotedar, 2013). In agreement with this, in the present study the basal diet containing 2.31 mg Se/kg resulted in muscle tissue myopathy, reduced feed intake, GPx activity and growth, which are typical Se deficiency symptoms in fish (Poston et al., 1976; Hilton et al., 1980; Gatlin et al., 1986; Le and Fotedar, 2014), while no sign of Se deficiency was observed in the fish fed the diets containing ≥ 4.91 mg Se/kg. On the other hand, the highest dietary Se level of 20.87 mg/kg caused atrophic hepatocytes, increased number of splenic macrophage aggregates, and reduction in feed intake, weight gain, Ht and HSI, which are indications of Se toxicity. The reason for Se toxicity is attributed to indiscriminate substitution of Se for sulphur when present in excessive amounts (Lemly, 2002a). Due to its higher reactivity and lower stability compared to sulphur, Se can cause metabolic problems (Stadtman, 1974; Sunde, 1984).

The toxic dietary Se concentration to fish in the present study is relatively higher than those reported previously. For example, 15.43 mg of dietary Se/kg did not cause any toxic effects in yellowtail kingfish, whereas the diet containing 13 mg Se/kg appeared to be toxic to 1.3-g rainbow trout (*Salmo gairdneri*) after four weeks of feeding (Hilton et al., 1980). Selenium concentrations of ≥ 4.6 mg/kg in food was toxic to razorback sucker (*Xyrauchen texanus*) larvae, the mortality occurred after one-week exposure (Hamilton et al., 2005). With regard to survival, yellowtail kingfish are relatively less sensitive to Se toxicity than bluegill (*Lepomis macrochirus*) and chinook salmon (*O. tshawytscha*). Dietary Se as SeMet at 6.5 and 9.6 mg/kg caused significant decreases in survival of 3-month-old bluegill (0.2 g) (Cleveland et al., 1993) and 70-mm fingerling chinook salmon (Hamilton et al., 1990) after being fed for 8.6 and 12.8 weeks respectively, while the survival of 19.55-g yellowtail kingfish fed up to 20.87 mg/kg of Se remained 100% even after 10 weeks. Toxicity of Se can be influenced by the duration of the Se exposure and life stages of the host animal (Lemly, 2002b). The earliest life stages of fish are the most sensitive to Se toxicity (Lemly, 2002a; Teh et al., 2002). The bigger fish used in the present study than other studies mentioned above may explain the less sensitive to Se toxicity of yellowtail kingfish than other fish species.

As Se concentrations in liver and muscle showed linear response to the dietary Se with no sign of plateauing, the levels of Se in these tissues can be used as bio-indicators of dietary Se exposure. Similarly, Se concentration in kidney, muscle, liver, gill, and plasma tissues of white sturgeon (*A. transmontanus*) increased as dietary Se (SeMet) increased and no plateau was reached after being fed up to 191.1 mg/kg diet for 8 weeks (Tashjian et al., 2006). The histopathological alterations in liver have been reported in green sunfish (*Lepomis cyanellus*) (Sorensen et al., 1984) and white sturgeon (Tashjian et al., 2006) with liver Se concentrations of 21.4 and ≥ 37.4 mg/kg dry weight, respectively. For yellowtail kingfish in the present study, those having liver Se concentration of 6.45 mg/kg wet weight or 20.82 mg/kg dry weight showed histopathological changes in their livers. Whereas, Lemly (2002b) recommended that the toxic level of Se in liver of freshwater and anadromous fish is only 12 mg/kg dry weight.

Splenic macrophage aggregates in fish play an important role in the storage of damaged cells (Wolke, 1992) and have been used as a bio-indicator for assessment of degraded environments (Fournie et al., 2001). The number of splenic macrophage aggregates may increase as fish are exposed to toxic chemicals. Exposure of plaice (*Pleuronectes platessa*) to 0.5 mg/L potassium dichromate resulted in an increase in density of splenic macrophage aggregates (Kranz and Gercken, 1987). In the present study, dietary Se at 20.87 mg/kg caused a significant increase in numbers of macrophage aggregates in the spleen, suggesting that splenic macrophage aggregates are sensitive to Se toxicity and can serve as a biomarker for the measurement of toxic effects of high dietary Se concentrations in yellowtail kingfish.

A reduction in Ht caused by waterborne Se poisoning has been reported in green sunfish (*L. cyanellus*) (Sorensen et al., 1984). The decreased Ht induced by the toxic effect of dietary Se was also seen in yellowtail kingfish fed the 20.87 mg Se/kg diet. Changes in Ht reflect the changes in the overall health of the fish. For example, Japanese yellowtail (*Seriola quinqueradiata*) is considered in an anaemic state when Ht is lower than 27.00% and in a healthy status when Ht is higher than 38.20% (Watanabe et al., 1998). Reductions in Ht are associated with decreased respiratory capacity, which causes metabolic stress and in turn leads to reduced fish health (Lemly, 1993).

The results of HSI indicated that the liver of fish fed the highest Se level was smaller compared to fish fed the lower levels. This may be as a result of liver atrophy caused by Se toxicity. Liver necrosis and reduced HSI have also been observed in white sturgeon exposed

to 191.1 mg Se/kg diet for 8 weeks (Tashjian et al., 2006). However, Sorensen et al. (1984) found that green sunfish with higher Se concentrations in livers (21.4 mg/kg compared to 7.0 mg/kg) had higher HSI. The authors reasoned that larger livers in fish having higher Se levels were due to edema caused by waterborne Se toxicity (Sorensen et al., 1984). Whereas, rainbow trout (*S. gairdneri*) fed various dietary Se levels from 0.38 to 13.06 mg/kg for four weeks showed no significant differences in HSI (Hilton et al., 1980), probably due to the brief duration of the exposure.

Glutathione peroxidase is considered as an indicator of Se status as its activity is dependent on the dietary Se intake (Ganther et al., 1976). Red blood cell GPx activity in yellowtail kingfish plateaued when dietary Se levels were between 4.91 and 15.43 mg/kg and continued to increase at the toxic dietary Se concentration of 20.87 mg/kg. A similar pattern has been reported for channel catfish (*Ictalurus punctatus*), in which plasma GPx activity levelled off above a Se level of 0.56 mg/kg and then increased at a Se level of 15.06 mg/kg, which was recommended as a toxic concentration to the fish (Gatlin and Wilson, 1984). An increase in GPx activity caused by toxic concentrations of Se has been found in algae (Vítová et al., 2011).

The fish in the present study showed more than two times slower growth compared to yellowtail kingfish cultured in a recirculating aquaculture system in a previous study (Abbink et al., 2012). Apart from differences in culture conditions, the lower feeding frequency is one of the main reasons for the slower growth of the fish in the present study. The fish in both studies had similar life stages, however, in the study of Abbink et al., the fish were fed to satiety eight times a day, whereas the fish in the present study were fed two times. This led to three times higher in feed intake in the study of Abbink et al. than in the present study. The higher feed intake in turn could result in faster growth.

In summary, Se deficiency symptoms were observed in yellowtail kingfish fed the basal diet containing 2.31 mg Se/kg while fish fed 20.87 mg Se/kg diet showed Se toxicity. Signs of Se toxicity included reduced feed intake, growth, Ht and HIS, increased splenic macrophage aggregates and liver atrophy. There were no detectable toxic effects in fish fed up to 15.43 mg Se/kg diet. Therefore, the toxic effect threshold of dietary Se for yellowtail kingfish appears to be between 15.43 and 20.87 mg/kg. It is recommended that further studies on toxic effects of dietary Se on different life stages of the fish are needed to determine whether the thresholds differ.

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**Table 1**

Ingredient formulation and proximate composition of the basal diet.

| Ingredient[a] | (g/kg) | Proximate composition[c] | (%) |
|---|---|---|---|
| Fishmeal | 500 | Protein | 49.18 ± 0.39 |
| Fish oil | 150 | Lipid | 18.56 ± 0.30 |
| Wheat flour | 130 | Moisture | 8.04 ± 0.03 |
| Wheat gluten | 100 | Ash | 8.27 ± 0.01 |
| Shrimp meal | 70 | Gross energy (MJ/kg) | 21.18 ± 0.16 |
| Starch | 40 | | |
| Se-free premix[b] | 10 | | |

[a] Supplied by Specialty Feeds, Perth, WA, Australia.

[b] Contains the following (as g/kg of premix): iron, 10; copper, 1.5; iodine, 0.15; manganese, 9.5; zinc, 25; vitamin A retinol, 100 IU; vitamin D3, 100 IU; vitamin E, 6.25; vitamin K, 1.6; vitamin B1, 1; vitamin B2, 2.5; niacin, 20; vitamin B6, 1.5; calcium, 5.5; biotin, 0.1; folic acid, 0.4; inositol, 60; vitamin B12, 0.002; choline, 150; and ethoxyquin, 0.125.

[c] Values are presented as means ± SD, n=3.

**Table 2**

Weight gain of yellowtail kingfish fed different Se levels during the feeding trial.

| Dietary Se (mg/kg) | wk 2 | wk 4 | wk 6 | wk 8 | wk 10 |
|---|---|---|---|---|---|
| 2.31 | 8.59 ± 0.33 | 20.54 ± 0.29 | 32.95 ± 0.36$^{a}$ | 46.06 ± 0.51$^{a}$ | 60.04 ± 0.73$^{a}$ |
| 4.91 | 9.48 ± 0.20 | 22.10 ± 0.38 | 37.38 ± 0.47$^{b}$ | 54.94 ± 0.82$^{c}$ | 70.96 ± 0.55$^{b}$ |
| 9.58 | 9.14 ± 0.19 | 22.28 ± 0.39 | 37.45 ± 0.62$^{b}$ | 52.48 ± 0.81$^{bc}$ | 68.64 ± 0.87$^{b}$ |
| 15.43 | 9.30 ± 0.13 | 21.71 ± 0.38 | 37.25 ± 0.39$^{b}$ | 52.39 ± 0.52$^{bc}$ | 68.27 ± 0.97$^{b}$ |
| 20.87 | 9.09 ± 0.40 | 21.79 ± 0.52 | 36.31 ± 0.82$^{b}$ | 51.04 ± 0.75$^{b}$ | 63.12 ± 0.57$^{a}$ |
| *P* value | 0.264 | 0.077 | 0.001 | < 0.001 | < 0.001 |

In a column, means not sharing a common superscript letter are significantly different ($P < 0.05$).

Values are presented as the mean ± SE of three replicate groups.

**Table 3**

Muscle proximate composition and gross energy of yellowtail kingfish fed graded dietary Se for 10 weeks.

| Dietary Se (mg/kg) | Protein (%) | Lipid (%) | Moisture (%) | Ash (%) | GE (MJ/kg) |
|---|---|---|---|---|---|
| 2.31 | 18.91 ± 0.13 | 2.15 ± 0.05 | 77.37 ± 0.14 | 1.59 ± 0.05 | 5.04 ± 0.03 |
| 4.91 | 18.60 ± 0.11 | 2.05 ± 0.12 | 77.71 ± 0.24 | 1.43 ± 0.03 | 4.99 ± 0.06 |
| 9.58 | 18.61 ± 0.22 | 1.90 ± 0.04 | 77.73 ± 0.32 | 1.48 ± 0.05 | 4.96 ± 0.08 |
| 15.43 | 19.08 ± 0.15 | 1.96 ± 0.17 | 77.27 ± 0.27 | 1.54 ± 0.06 | 5.09 ± 0.06 |
| 20.87 | 18.98 ± 0.21 | 2.26 ± 0.15 | 77.12 ± 0.35 | 1.47 ± 0.06 | 5.12 ± 0.10 |
| *P* value | 0.225 | 0.285 | 0.471 | 0.277 | 0.539 |

GE, gross energy.

Values are presented as the mean ± SE of three fish in each of three replicate groups.

**Table 4**

Feed intake, feed conversion ratio, liver Se, muscle Se and survival of yellowtail kingfish fed graded dietary Se for 10 weeks.

| Dietary Se (mg/kg) | Feed intake (g/fish)[1] | FCR[1] | Liver Se (mg/kg)[2] | Muscle Se (mg/kg)[3] | Survival (%) |
|---|---|---|---|---|---|
| 2.31 | 76.33 ± 1.59[a] | 1.27 ± 0.01 | 0.72 ± 0.06[a] | 0.20 ± 0.01[a] | 100 |
| 4.91 | 88.90 ± 1.33[b] | 1.25 ± 0.01 | 1.71 ± 0.08[b] | 0.68 ± 0.01[b] | 100 |
| 9.58 | 86.71 ± 0.97[b] | 1.26 ± 0.01 | 2.48 ± 0.11[c] | 1.10 ± 0.01[c] | 100 |
| 15.43 | 85.65 ± 1.33[b] | 1.25 ± 0.01 | 3.93 ± 0.02[d] | 1.33 ± 0.02[d] | 100 |
| 20.87 | 79.60 ± 0.44[a] | 1.26 ± 0.01 | 6.45 ± 0.17[e] | 1.61 ± 0.03[e] | 100 |
| *P* value | 0.000 | 0.793 | < 0.001 | < 0.001 | |

FCR, feed conversion ratio.

Means not sharing a common superscript letter are significantly different ($P < 0.05$).

Initial Se concentrations in liver and muscle were 0.84 and 0.06 mg/kg, respectively.

[1] Values are presented as the mean ± SE of three replicate groups.

[2] Values are presented as the mean ± SE of pooled samples of nine fish in each of three replicate groups.

[3] Values are presented as the mean ± SE of three fish in each of three replicate groups.

**Table 5**

Haematocrit, hepatosomatic index, glutathione peroxidase activity and splenic macrophage aggregates of yellowtail kingfish fed graded dietary Se for 10 weeks.

| Dietary Se (mg/kg) | Haematocrit (%)[1] | HSI (%)[2] | GPx activity (units/g Hb)[3] | Number of MAs per spleen[3] |
|---|---|---|---|---|
| 2.31 | 38.74 ± 0.61[a] | 0.99 ± 0.04[a] | 50.00 ± 3.25[a] | 24.89 ± 2.41[a] |
| 4.91 | 39.89 ± 1.00[a] | 1.01 ± 0.03[a] | 87.17 ± 3.61[b] | 21.44 ± 1.46[a] |
| 9.58 | 39.32 ± 0.67[a] | 0.96 ± 0.03[a] | 86.17 ± 3.61[b] | 24.11 ± 1.31[a] |
| 15.43 | 39.93 ± 0.91[a] | 1.01 ± 0.04[a] | 98.83 ± 5.92[bc] | 25.89 ± 0.99[a] |
| 20.87 | 34.85 ± 0.83[b] | 0.79 ± 0.02[b] | 109.50 ± 1.89[c] | 105.89 ± 4.28[b] |
| *P* value | 0.007 | 0.003 | < 0.001 | < 0.001 |

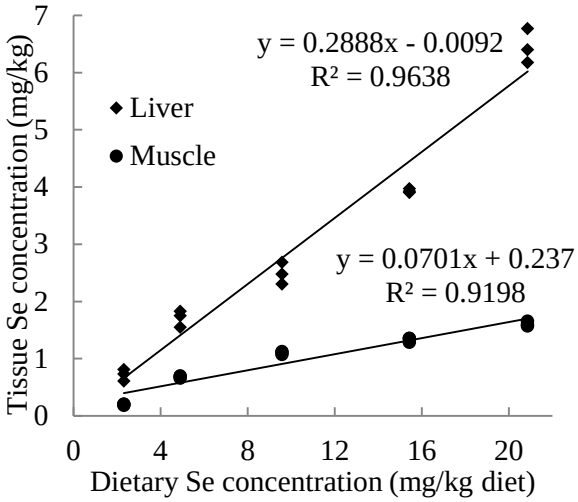
HSI, hepatosomatic index; GPx, glutathione peroxidase; Hb, haemoglobin; MA, macrophage aggregate.

Means not sharing a common superscript letter are significantly different ($P < 0.05$).

[1] Value are presented as the mean ± SE of three fish with three determinations per fish in each of three replicate groups.

[2] Values are presented as the mean ± SE of nine fish in each of three replicate groups.

[3] Values are presented as the mean ± SE of three fish in each of three replicate groups.




403

404 **Fig. 1.** Relationship between Se concentrations in diets and tissues. For liver tissues, each
405 point presents mean of pooled samples of nine fish from each replicate group. For muscle
406 tissues, each point represents mean of three fish from each replicate group.

407

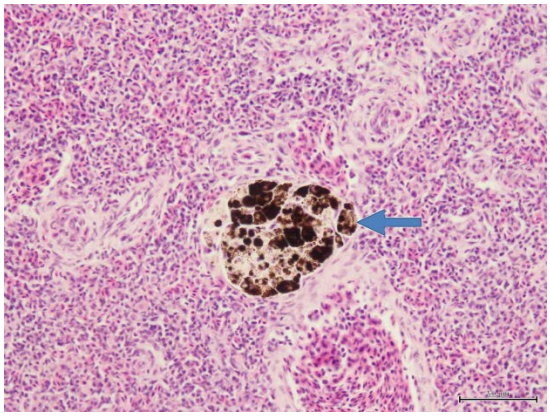


**Figure 2.** A macrophage aggregate (arrow) in a section of spleen of yellowtail kingfish fed the diet containing 20.87 mg/kg Se for 10 weeks. (Haematoxylin and eosin, scale bar = 50 µm).

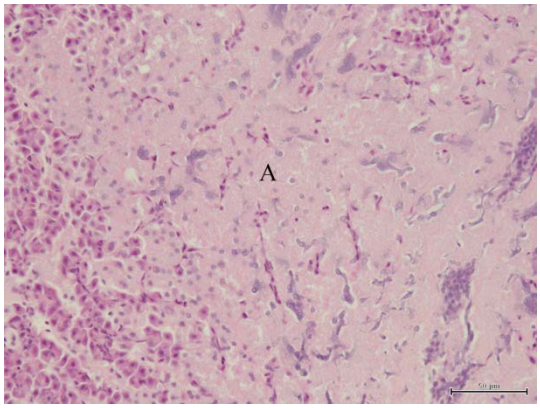



**Figure 3.** Section of liver of yellowtail kingfish fed the diet containing 20.87 mg/kg Se for 10 weeks showing atrophic hepatocytes (A). (Haematoxylin and eosin, scale bar = 50 µm).

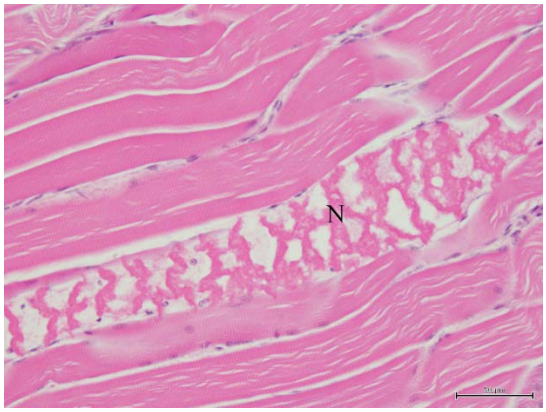



**Fig. 4.** Section of muscle of yellowtail kingfish fed the basal diet containing 2.31 mg/kg Se for 10 weeks resulting in necrotic fibres (N). (Haematoxylin and eosin, scale bar = 50 µm).