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Bioavailability of selenium from different dietary sources in yellowtail kingfish (*Seriola lalandi*)

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ABSTRACT

Different forms of selenium (Se) were supplemented to a fishmeal based diet to investigate the digestibility and bioavailability of Se in yellowtail kingfish (*Seriola lalandi*). Five groups of fish in triplicate were fed a basal diet (containing 3.31 mg/kg Se) either un-supplemented or supplemented with 2 mg/kg Se from selenite, selenocystine (SeCys), selenomethionine (SeMet) or Se-yeast for six weeks. The basal un-supplemented diet resulted in significantly lower weight gain, red blood cell glutathione peroxidase (GPx) and bactericidal activities than the supplemented diets. Muscle Se concentration was increased by Se supplementation from SeCys, SeMet or Se-yeast, but not selenite. There was no difference in GPx activity of fish fed any supplemented diets. Bioavailability of Se from SeMet and Se-yeast was similar for all measurements. The most digestible sources of Se were from SeMet and Se-yeast, while the least was from fishmeal. Se from SeMet or Se-yeast produced more weight gain, higher Se accumulation in muscle tissues and bactericidal activity in yellowtail kingfish than Se from SeCys or selenite. This study shows that SeMet and Se-yeast are the most bioavailable sources of Se to yellowtail kingfish and are recommended to be supplemented to fishmeal based formulated diets for yellowtail kingfish.

Keywords: Bioavailability; Digestibility; Selenium; Yellowtail kingfish

1. Introduction

Yellowtail kingfish (*Seriola lalandi*) has excellent attributes for aquaculture including high growth rate, highly accepted taste and market acceptance, and their suitability to be grown in sea cages as well as in inland recirculating systems (Miegel et al., 2010; Abbink et al., 2012). Recently as the expansion and intensity of its aquaculture activity, the research in the area of nutrition of this species has been conducted to refine practical diet formulations. However, the research has focused mainly on requirements for protein, energy and various sources of lipid (Booth et al., 2010; Bowyer et al., 2012), and there is a lack of information on the mineral requirements.

One mineral that has been known as an essential trace element for normal growth and physiological function of animals, including fish is selenium (Se) (NRC, 1993). Se is a component of the enzyme glutathione peroxidase (GPx), which plays an important role in protecting cell membranes against oxidative damage (Rotruck et al., 1973). The GPx activity was demonstrated to decrease in rainbow trout (*Salmo gairdneri*) (Hilton et al., 1980), channel catfish (*Ictalurus punctatus*) (Gatlin et al., 1986; Wise et al., 1993) and Atlantic salmon (*Salmo salar*) (Bell et al., 1987) when fish were fed diets deficient in Se. Se in the form of selenomethionine (SeMet) has been reported to increase GPx activity, muscle Se concentration and growth of grouper (*Epinephelus malabaricus*) (Lin and Shiau, 2005) and cobia (*Rachycentron canadum*) (Liu et al., 2010). In addition, growth and immune responses

of channel catfish have been shown to be affected by dietary Se supplemented as SeMet, Se-yeast or selenite (Wang et al., 1997).

The optimal Se concentration in diet for yellowtail kingfish has now been studied by Le and Fotedar (2013). However, the requirement of dietary Se is not only met by its presence in the diet but also is met by its bioavailability which in turn depends on various sources of Se in the diet (Fairweather-Tait et al., 2010). Organic sources such as selenomethionine (SeMet) and Se-yeast are generally believed to be more bioavailable than inorganic sources such as selenite. For example, the digestibility of SeMet is higher than selenite in Atlantic salmon (Bell and Cowey, 1989). Further, Se derived from SeMet or Se-yeast is more efficiently incorporated into muscle tissues (Wang and Lovell, 1997) and has a greater bioavailability than selenite to provide antibody production and macrophage chemotactic response in channel catfish (Wang et al., 1997).

The information on the bioavailability of Se from different dietary sources to yellowtail kingfish is not known yet, and therefore, the aim of this study was to select the Se source which is highly bioavailable to juveniles of yellowtail kingfish. The fish were fed various sources of Se and digestibility, tissue accumulation, GPx activity and immune response were measured to assess the bioavailability of Se.

2. Materials and methods

All experimental work was approved by the Curtin University Animal Ethics Committee and performed according to the Australian Code of Practice for the care and use of animals for scientific purposes.

2.1. Experimental diets and design

A fishmeal basal diet (Table 1) was supplemented with 2 mg/kg of Se from sodium selenite, DL-selenocystine (SeCys), DL-selenomethionine (SeMet) (Sigma-Aldrich, St. Louis, MO, USA) or Se-yeast (Selplex®, Alltech, Nicholasville, KY, USA). The basal diet contained chromic oxide (0.5%) as a digestibility marker. The pre-determined quantities of chemicals containing Se were dissolved in water and added to the basal ingredients before pelleting the feeds through a 2.5-mm diameter die. The pellets were then air-dried at room temperature and stored at -20 ^{o}C until used.

The fishmeal contained 5.93 ± 0.12 mg Se/kg (mean ± SD, n=3), which gave a Se concentration in the basal diet of 3.31 ± 0.01 mg/kg (mean ± SD, n=3). The measured Se concentrations in selenite, SeCys, SeMet and Se-yeast supplemented diets were 5.34 ± 0.02, 5.37 ± 0.03, 5.36 ± 0.02 and 5.36 ± 0.02 mg/kg (mean ± SD, n=3), respectively. The selected inclusion of Se was based on the Se requirement of yellowtail kingfish (Le and Fotedar, 2013).

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 15 fish per tank. Total weight of fish in each tank was 146.72 ± 1.20 g (mean ± SD), with an average individual weight of 9.78 ± 0.08 g (mean ± SD). The tanks were filled with seawater at salinity of 35 ppt and were supplied with constant aeration and pure oxygen (oxygen compressed, BOC, Perth, WA, Australia). Each tank had an external bio-filter (Fluval 406, Hagen, Italy) running continuously to create a recirculating system and an automatic heater (HA-200, Sonpar®, China) to maintain water temperature. Half of the water was changed twice weekly in the first two weeks, and every two days afterwards. Water temperature, pH and dissolved oxygen were measured daily using

digital pH/mV/^{o}C 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). During the trial, water temperature, pH and dissolved oxygen averaged 21.9 ± 0.8 °C, 7.5 ± 0.2, and 6.6 ± 0.3 mg/L (mean ± SD), respectively. Total ammonia (NH_3/NH_4^+) was always ≤ 1.0 mg/L.

Three tanks of fish were randomly assigned to each dietary treatment. The fish were fed twice daily to satiation for six weeks. The food was proffered by hands to ensure no uneaten food. The amount of feed consumed was recorded daily by calculating the differences in the weight of feed before the first and after the last feeding to estimate feed intake. Mortality was recorded daily to calculate survival. Fish in each tank were group weighed at the end of the trial to estimate weight gain. Weight measurement and feed intake were used for estimation of feed conversion ratio (FCR, feed intake divided by the wet weight gain).

2.2. Digestibility study

Samples of faeces were collected from all fish in each tank at the end of week 4, 5 and at the end of the feeding trial. The fish were anaesthetized with tricaine methanessulfonate (MS-222, Sigma-Aldrich, Castle Hill, NSW, Australia) and faecal samples were collected by stripping from the ventral abdominal region to the anal region. Pooled samples of faeces from each tank were dried at 55 ^{o}C and kept at -20 ^{o}C prior to analysis of Se and chromic oxide (Cr_2O_3). Se and Cr_2O_3 in collected faeces from each tank were analysed in triplicate.

The Se digestibility coefficients (*DC*) in all diets were calculated by the formula:

DC (%) = 100 × [1 – (% *Cr_2O_3 in diet*) × (% *Se in faeces*) / (% *Cr_2O_3 in faeces*) × (% *Se in diet*)]

The digestibility coefficients of supplemented Se (DC_{suppl}) from different sources were corrected for residual Se in the basal diet and calculated as follows (Paripatananont and Lovell, 1997):

DC_{suppl} (%) = 100 × [(*DC of supplemented diet*) × (*Se in supplemented diet*) – (*DC of basal diet*) × (*Se in basal diet*)] / *amount of supplemented Se*

Digestible Se intake of the fish was calculated by multiplying the feed intake by Se content of the diet and its associated Se digestibility coefficient.

2.3. Collection of blood and muscle samples

After the collection of faecal samples at the end of the feeding trial, three fish from each tank were randomly selected and blood was sampled from the caudal vein with a 25-gauge needle attached to a 3-ml syringe. The blood was allowed to clot for 2 h at 4°C and serum was separated by centrifugation of whole blood at 1,500 × g for 10 min at 4 ^{o}C using a centrifuge (5804R, Eppendorf, Hamburg, Germany). Serum was used for bactericidal activity assay. The red blood cell pellets were used for glutathione peroxidase assay. Serum and red blood cell pellet samples were kept at -80°C until analysis.

Following the blood sampling, the fish were euthanized with MS-222 and filleted. Se content and proximate composition of muscle was determined for each fish.

2.4. Bactericidal activity assay

Serum bactericidal activity was performed in duplicate for each fish by the method of Ueda et al. (1999). *Vibrio anguillarum* stock culture was obtained from Department of Agriculture and Food, Perth, WA, Australia. Fifty µL suspension of *V. anguillarum* (1.6×10^4 CFU/mL) in phosphate buffered saline (PBS; 0.1 M, pH 7.2) was added to 50 µL serum, and the

mixture was reacted for 30 min at 25°C. The same volume of bacterial suspension was added to 50 µL of PBS as control, and was also reacted for 30 min at 25°C simultaneously. After reaction, 50 µL from the mixture was plated onto duplicate tryptone soya agar and incubated for 24 h at 25°C. Bactericidal activity was calculated as decrease in number of viable *V. anguillarum* cells, i.e. $\log_{10}$ CFU/mL in the control minus $\log_{10}$ CFU/mL in serum.

2.5. 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 ^{o}C. 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).

2.6. Chemical analysis

Protein, lipid, moisture, ash and Se were determined 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°C to a constant weight and ash by combustion at 550°C for 24 h. Se was estimated using an atomic absorption spectrometer equipped with vapour generation assembly (AA280 FS and VGA 77, Varian, Mulgrave, Vic, Australia). Chromic oxide was measured by the procedure described in Bolin et al. (1952) using a spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan). 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. Percentage data were arcsine transformed prior to 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 to plot digestible Se intake of the fish against fish weight gain and Se concentration in muscle tissues. The statistical significance was set at $P < 0.05$.

3. Results

Dietary Se treatments did not influence feed intake, FCR and survival of yellowtail kingfish (Table 2). However, weight gain was affected by the dietary treatments, fish fed the basal diet gained significantly ($P < 0.05$) less weight than fish fed Se supplements (Table 2). Weight gains of fish fed SeMet and Se-yeast did not differ but were significantly ($P < 0.05$) higher than that of fish fed selenite. SeMet and Se-yeast resulted in significantly ($P < 0.05$) higher digestible Se intake of the fish than SeCys and selenite (Table 2). Linear regression analysis of fish weight gain showed linear response to the digestible Se intake of the fish ($y = 0.0696x + 24.014$, $R^2 = 0.8238$, Fig. 1).

Proximate composition and gross energy of muscles were not affected by the different dietary treatments (Table 3). In contrast, the sources of Se had significant effects on Se digestibility, Se concentration in muscle tissues and bactericidal activity (Table 4). Se derived from SeMet and Se-yeast showed the highest digestibility and bactericidal activities, significantly higher ($P < 0.05$) than Se from selenite and SeCys, while Se from the fishmeal (basal diet) was the lowest. Similarly, the highest muscle Se concentrations were in fish fed SeMet and Se-yeast, while the lowest was found in fish fed the basal diet. Se accumulation in muscle of fish fed SeCys was significantly higher ($P < 0.05$) than fish fed selenite, but significantly lower ($P <$

183 0.05) than fish fed SeMet or Se-yeast. Similar to the weight gain of fish, there was a positive
184 linear regression between muscle Se accumulation and the digestible Se intake of the fish (y
185 = 0.005x - 0.0908, R^2 = 0.6394, Fig. 2). Red blood cell glutathione peroxidase (GPx) activity
186 was the same for fish fed Se supplemented diets, but was significantly higher ($P < 0.05$) than
187 that in fish fed the basal diet (Table 4).

188 **4. Discussion**

189 A fishmeal based diet containing 1.2 mg/kg Se has been reported to meet the Se requirement
190 of Atlantic salmon (Lorentzen et al., 1994). This is in contrast to the findings of the present
191 study, in which yellowtail kingfish fed the fishmeal based un-supplemented Se diet showed
192 lower growth and GPx activity than those fed Se supplemented diets. The reduced growth
193 and GPx activity are signs of Se deficiency (Poston et al., 1976; Bell et al., 1986). This
194 demonstrates that the basal diet was not met the Se requirement in yellowtail kingfish. The
195 relatively high Se requirement by yellowtail kingfish has discussed previously in Le and
196 Fotedar (2013). The current study was performed to compare the bioavailability of Se from
197 different dietary sources in yellowtail kingfish.

198 Se bioavailability depends on its chemical forms, which are absorbed and metabolized
199 differently (Fairweather-Tait et al., 2010). Organic Se appears to be more bioavailable than
200 inorganic sources to fish (Wang and Lovell, 1997; Jaramillo et al., 2009) as the former is
201 better absorbed (Paripatananont and Lovell, 1997) and has higher retention (Rider et al.,
202 2009). In the present study, bioavailability of Se from SeMet and Se-yeast was similar for all
203 the tools used to measure physiological performance of yellowtail kingfish. This similarity
204 can be attributed to the fact that Se-yeast contains more than 90% of its Se in the form of
205 SeMet (Schrauzer, 2006). Se from both sources, SeMet and Se-yeast, is well digested by
206 yellowtail kingfish. The absorption of Se from these two organic sources was one and a half
207 times more than that of Se from SeCys and selenite, and over twice that of Se from fishmeal.
208 In fish and other higher vertebrates, ingested Se is absorbed in the anterior intestine, while
209 uptake of selenite is by passive diffusion (Daniels, 1996), the absorption of SeMet is more
210 efficient via the Na^+ dependent neutral amino acid transport system (Schrauzer, 2003).
211 Furthermore, the study on the movement of Se in intestinal sacs of hamsters by McConnell
212 and Cho (1965) showed that there is an active transport of SeMet, but not SeCys or selenite,
213 and that SeMet is transported intact across the intestinal membrane. The absorption of Se
214 from the fishmeal in the basal diet is low as Se is bound to heavy metals (Webster and Lim,
215 2002), for example, the insoluble copper–Se compound may be one of the contributing
216 factors in reduced Se absorption from fishmeal (Lorentzen et al., 1998).

217 Se from fishmeal has been reported to have lower absorption than selenite and SeMet in
218 Atlantic salmon (Bell and Cowey, 1989). The absorption of SeMet by Atlantic salmon is
219 similar to the present research; but, the absorption of selenite is higher for Atlantic salmon
220 than yellowtail kingfish. Apart from the dependence on species, different Se absorption could
221 be due to differences in other feed ingredients present in the basal formulated diets. The
222 interaction between minerals and other nutrients in yellowtail kingfish diet may decrease
223 absorption of selenite. The reduced absorption of inorganic minerals by interaction with other
224 nutrients has been reviewed by Paripatananont and Lovell (1997).

225 Absorption has been used to measure the bioavailability of Se in various food items
226 (Fairweather-Tait et al., 2010). However, absorption alone cannot explain all the differences
227 in bioavailability of different Se compounds as the metabolism and storage of Se varies
228 depending on its chemical form after being absorbed. SeMet is probably more bioavailable
229 for metabolic processes than other Se forms as it is readily incorporated into protein in place
230 of methionine (Daniels, 1996). In the present study more Se from SeCys retained in muscle

tissues than from selenite although both Se forms had the same digestibility coefficients. This could be due to the extensive recycling of organic Se (Swanson et al., 1991) and/or the difference in metabolic pathways of different Se compounds in different tissues. Inorganic form increases Se in liver but not muscle tissues of rainbow trout (*Oncorhynchus mykiss*) whereas organic can increase both hepatic and muscle Se reserves (Rider et al., 2010). There was almost no increase in Se concentration in muscle tissues of Atlantic salmon (Lorentzen et al., 1994) and yellowtail kingfish when selenite was supplemented to the fish diets. In contrast, muscle Se concentration in channel catfish increased by supplementing the diet with selenite as well as SeMet or Se-yeast, however, SeMet or Se-yeast as a source of Se was more effectively incorporated into muscular tissues of fish than selenite (Wang and Lovell, 1997). The higher bioavailability of SeMet than selenite for whole body Se accumulation was also reported for hybrid striped bass (*Morone chrysops* × *M. saxatilis*) (Jaramillo et al., 2009). The high muscle Se content in yellowtail kingfish fed SeMet or Se-yeast can be partially attributed to the high absorption of SeMet. In addition, the main protein concentration rests in fish muscle tissues, therefore when SeMet is incorporated directly into proteins (Waschulewski and Sunde, 1988), it leads to an increase in Se concentration in fish muscles.

GPx is one of the most important antioxidant defence enzymes in fish (Ross et al., 2001) and its activity is dependent on the dietary Se intake (Ganther et al., 1976), thus, the GPx activity is frequently used to estimate Se bioavailability in fish. Organic Se has been reported to be more efficacious than inorganic Se in raising hepatic GPx activity in common carp (*Cyprinus carpio*) (Jovanovic et al., 1997) and channel catfish (Wang and Lovell, 1997). However, this is not consistent with other studies on other fish species. For example, Cotter et al. (2008) showed that selenite gives higher hepatic GPx activity in hybrid striped bass than Se-yeast when supplemented at 0.4 mg/kg. Another study on Atlantic salmon suggested that selenite or SeCys was a better source of Se for plasma GPx activity than SeMet, more Se from selenite and SeCys was incorporated into plasma GPx than Se from SeMet (Bell and Cowey, 1989). In the present study, GPx activity in red blood cells showed no correlation with the different sources of supplemented Se. This indicates no direct relationship between GPx activity and Se form, probably because the metabolic role of Se from different forms and sources may be the same in red blood cell GPx. Similar effect of organic and inorganic Se on red blood cell and hepatic GPx activity has been observed in domestic animals (Kumar et al., 2009) and rainbow trout (Rider et al., 2010), respectively.

Se exerts its effect on the immune system principally via selenoproteins (Arthur et al., 2003). For example, Se-containing proteins, glutathione peroxidases, protect neutrophils from superoxide- derived radicals, which are produced by neutrophils to kill foreign microbes. Bactericidal activity is a natural defence factor for protection against invading microorganisms, and directly killing bacterial cells (Ueda et al., 1999). The bactericidal activity has been found in serum of fish and is reported to be affected by dietary Se (Le et al., 2013). Therefore, the measurement of immune competence, such as bactericidal activity, can partially reflect the bioavailability of Se. Unlike GPx activity, serum bactericidal activity in yellowtail kingfish was responsive to the sources of Se. Se supplemented as SeMet or Se-yeast was more available for bactericidal activity than Se from selenite or SeCys. This corresponded with the higher absorption of Se from SeMet and Se-yeast in comparison to selenite and SeCys. The higher bioavailability of SeMet and Se-yeast than selenite in improving immune capacity has been also demonstrated in channel catfish (Wang et al., 1997) as channel catfish fed SeMet or Se-yeast had higher antibody production and macrophage chemotactic activity than those fed selenite.

Wang and Lovell (1997) reported that Se from SeMet and Se-yeast had 336 and 269% respectively more availability than Se derived from selenite for growth of channel catfish. Similarly, in the present study SeMet and Se-yeast appeared to be more bioavailable than selenite for the growth of yellowtail kingfish. This could be explained by the higher digestible Se intake of the fish fed SeMet or Se-yeast than those fed selenite. The effects of Se on fish growth might be associated with its biological functions and probably mediated by selenoproteins (McKenzie et al., 2002). Further research is needed to elucidate a mechanism of action by which Se enhances growth of fish.

In conclusion, different forms of Se supplemented to diets are digested and utilized differently by yellowtail kingfish. Se supplied as SeMet or Se-yeast was relatively more absorbed and was more bioavailable than SeCys or selenite. As Se-yeast had the same bioavailability as SeMet, it is recommended to use Se-yeast or SeMet as Se supplement in yellowtail kingfish feed.

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Table 1

Ingredient formulation and proximate composition of the basal diet.

Ingredient[a]	(g/kg)	Proximate composition[c]	(%)
Fishmeal	550	Protein	53.04 ± 0.22
Fish oil	125	Lipid	15.23 ± 0.31
Wheat flour	100	Moisture	7.61 ± 0.24
Wheat gluten	100	Ash	9.64 ± 0.10
Shrimp meal	70	Gross energy (MJ/kg)	22.04 ± 0.10
Starch	40		
Se-free premix[b]	10		
Chromic oxide	5		

[a] Supplied by Specialty Feeds, Perth, WA, Australia, except chromic oxide obtained from Thermo Fisher Scientific, Scoresby, Vic, Australia.

[b] Contains (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; ethoxyquin, 0.125.

[c] Values are means ± SD, n=3.

Table 2

Weight gain, digestible Se intake, feed intake, feed conversion ratio and survival of yellowtail kingfish fed different Se sources.[1]

Se source	Weight gain (g/fish)	Digestible Se intake (μg/fish)	Feed intake (g/fish)	FCR	Survival (%)
Basal diet	27.46 ± 0.46[a]	48.02 ± 0.43[a]	37.73 ± 0.68	1.38 ± 0.05	100
Selenite	30.24 ± 0.80[b]	100.87 ± 1.37[b]	41.12 ± 0.84	1.36 ± 0.04	100
SeCys	31.19 ± 0.64[bc]	100.52 ± 2.81[b]	40.19 ± 1.35	1.29 ± 0.03	100
SeMet	33.02 ± 0.44[c]	126.83 ± 3.92[c]	41.19 ± 1.51	1.25 ± 0.05	100
Se-yeast	32.95 ± 0.41[c]	123.64 ± 6.96[c]	40.33 ± 1.85	1.22 ± 0.05	100
P value	*0.000*	*0.000*	*0.394*	*0.138*	

[1] Values represent means ± SE of three replicates per treatment.

SeCys, selenocystine; SeMet, selenomethionine; FCR, feed conversion ratio.

Means in the same column with different superscript letters are significantly different ($P < 0.05$).

435 **Table 3**
436 Proximate composition and gross energy of muscles of yellowtail kingfish fed different Se
437 sources.[1]

Se source	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)	GE (MJ/kg)
Basal diet	19.88 ± 0.12	2.49 ± 0.06	77.04 ± 0.25	1.32 ± 0.00	5.30 ± 0.11
Selenite	20.04 ± 0.04	2.49 ± 0.04	76.87 ± 0.14	1.35 ± 0.03	5.42 ± 0.02
SeCys	20.17 ± 0.13	2.53 ± 0.04	76.84 ± 0.19	1.34 ± 0.03	5.41 ± 0.08
SeMet	20.17 ± 0.11	2.46 ± 0.04	76.77 ± 0.15	1.34 ± 0.01	5.45 ± 0.09
Se-yeast	20.22 ± 0.15	2.50 ± 0.01	76.61 ± 0.20	1.35 ± 0.02	5.51 ± 0.03
P value	*0.318*	*0.825*	*0.632*	*0.887*	*0.449*

438 [1] Value are means ± SE of one determination per fish, three fish per tank and three tanks per
439 treatment.
440 SeCys, selenocystine; SeMet, selenomethionine; GE, gross energy.
441

Table 4

Se digestibility of diets, digestibility of Se sources, muscle Se, glutathione peroxidase and bactericidal activities in yellowtail kingfish fed different Se sources.

Se source	Se digestibility of diet (%)[1]	Digestibility of Se source (%)[1]	Muscle Se (mg/kg)[2]	GPx activity (units/g Hb)[2]	Bactericidal activity (log_{10})[3]
Basal diet	38.48 ± 0.82[a]	38.48 ± 0.82[a]	0.21 ± 0.01[a]	67.25 ± 1.72[a]	3.24 ± 0.01[a]
Selenite	45.95 ± 0.43[b]	59.01 ± 1.15[b]	0.24 ± 0.01[a]	85.97 ± 1.32[b]	3.47 ± 0.02[b]
SeCys	46.56 ± 0.21[b]	61.41 ± 0.56[b]	0.35 ± 0.00[b]	80.80 ± 2.25[b]	3.46 ± 0.01[b]
SeMet	57.47 ± 0.43[c]	90.35 ± 1.16[c]	0.61 ± 0.01[c]	91.54 ± 2.34[b]	3.56 ± 0.01[c]
Se-yeast	57.12 ± 0.74[c]	89.48 ± 1.99[c]	0.62 ± 0.01[c]	90.71 ± 3.96[b]	3.56 ± 0.01[c]
P value	*0.000*	*0.000*	*0.000*	*0.000*	*0.000*

[1] Values are means ± SE of three determinations of pooled samples of 15 fish per tank and three tanks per treatment.

[2] Value are means ± SE of one determination per fish, three fish per tank and three tanks per treatment.

[3] Value are means ± SE of two determinations per fish, three fish per tank and three tanks per treatment.

SeCys, selenocystine; SeMet, selenomethionine; GPx, glutathione peroxidase; Hb, haemoglobin.

Means in the same column with different superscript letters are significantly different ($P < 0.05$).

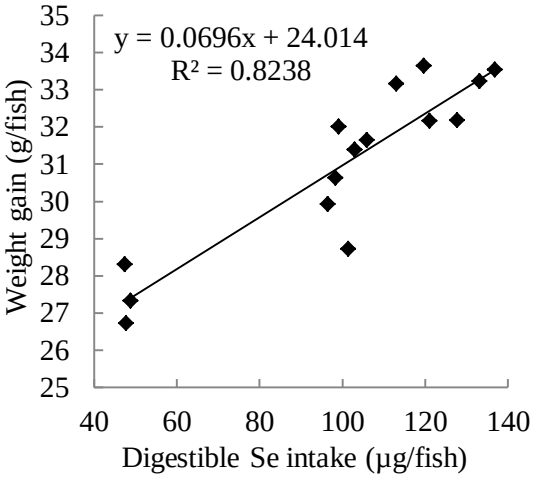

Fig. 1. Relationship between digestible Se intake of fish and fish weight gain. Each point represents one of three replicates of each treatment.

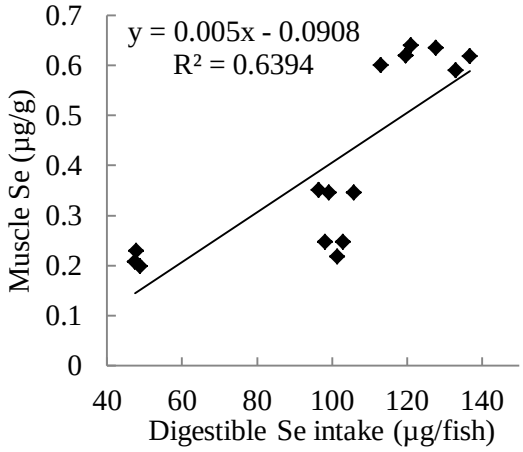

Fig. 2. Relationship between digestible Se intake of fish and muscle Se accumulation. Each point represents mean of one group of fish with three fish per group and one determination per fish.