Asian-Aust. J. Anim. Sci.
Vol. 21, No. 12 : 1794 - 1799
December 2008

www.ajas.info

A Preliminary Study on Effects of Different Dietary Selenium (Se) Levels on Growth Performance and Toxicity in Juvenile Black Seabream, *Acathopagrus schlegeli* (Bleeker)*

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ABSTRACT : This preliminary feeding trial was conducted to study the effects of different dietary selenium (Se) levels on growth performance and toxicity in juvenile black seabream, *Acanthopagrus schlegeli* (Bleeker). Fish averaging 7.0±0.1 g (mean±SD) were fed one of the five semi-purified diets containing 0.21, 0.30, 0.52, 1.29 and 12.3 mg sodium selenite (Na_2SeO_3)/kg diet (Se 0.21, Se 0.30, Se 0.52, Se 1.29 or Se 12.3) for 15 weeks. After the feeding trial, weight gain (WG), feed efficiency (FE), specific growth rate (SGR) and protein efficiency ratio (PER) of fish fed Se 0.21, Se 0.30, Se 0.52 and Se 1.29 diets were not significantly different, however fish fed Se 12.3 diet showed significantly lower WG, FE, SGR and PER than those of fish fed the other diets ($p<0.05$). Fish fed Se 0.21, Se 0.30, Se 0.52, Se 1.29 and Se 12.3 diets showed no significant differences in hematocrit (PCV), hemoglobin (Hb) and red blood cells (RBC), however fish fed Se 12.3 diet showed lower values of PCV, Hb and RBC than those of fish fed the other diets. Histopathological lesions such as tubular necrosis and polycystic dilation of tubules in the kidney tissues were observed in fish fed Se 12.3 diet. Se was accumulated in a dose-dependent manner in the liver, kidney, muscle and gill tissues. Based on the results of this preliminary feeding trial, a dietary Se level of 0.21 mg Na_2SeO_3/kg diet could be optimal for proper growth performances, and a dietary Se level of 12.3 mg Na_2SeO_3/kg diet may ultimately be toxic to juvenile black seabream, *Acanthopagrus schlegeli*. (**Key Words** : Selenium, Requirement, Growth Performance, Toxicity, Histopatholgy, Black Seabream)

INTRODUCTION

Selenium (Se) is the trace mineral which is a component of the enzyme glutathione peroxidase (GPx., EC 1.11.1.9) (Rotruck et al., 1973). This enzyme was discovered by Mill (1957), and the main biological role of the enzyme is to protect organism from oxidative damages. The Se requirement studies were carried out in three species, rainbow trout (Hilton et al., 1980), channel catfish (Gatlin and Wilson,1984) and grouper (Lin and Shiau, 2005), and optimum dietary Se levels were suggested as 0.38, 0.25 mg Na_2SeO_3/kg diet and 0.78 mg Se-Met/kg diet for each species, respectively.

* This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (No. R01-2007-000-11484-0) and Feeds and Foods Nutrition Research Center (FFNRC), Pukyong National University, Busan, Korea.
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Received April 22, 2008; Accepted June 16, 2008

Se is similar to sulfur with regard to its basic chemical and physical properties (has same valence states and forms analogs of hydrogen sulfide, thiosulfate, sulfite, and sulfate), and mammalian studies show that cells do not discriminate well between the two as proteins are being synthesized (it is assumed that the mechanistic features underlying toxicity are essentially the same for fish, since the resulting pathology and teratogenic features are the same). When present in excessive amounts, Se is erroneously substituted for sulfur, resulting in the formation of a triselenium linkage (Se-Se-Se) or a selenotrisulfide linkage (S-Se-S), either of which prevent the formation of the necessary disulfide chemical bonds (S-S). The end result is distorted, dysfunctional enzymes and protein molecules which impair normal cellular biochemistry (Ganther 1974; Stadtman 1974; Diplock and Hoekstra, 1976; Reddy and Massaro, 1983; Sunde, 1984).

None of researches have been carried out about a dietary Se requirement and/or toxicity in Korean aquaculture species. Black seabream is one of the

Table 1. Composition and proximate analysis of the basal diet (% of DM basis)

Ingredient	%
Casein (vitamin-free)[1]	70.0
Wheat flour[2]	7.0
Dextrin[1]	4.6
Fish oil[3]	10.0
Vitamin premix[4]	3.0
Mineral premix (selenium-free)[5]	3.0
Cellulose[1]	2.4
Proximate analysis (% of dry matter basis)	
Moisture	15.0
Crude protein	65.4
Crude lipid	10.3
Crude ash	5.0
Se (mg/kg)	0.21

[1] United States Biochemical, Cleveland, OH 44122.
[2] Young Nam Flour Mills Co., Busan, Korea.
[3] E-Wha oil Co., Ltd., Buasn Korea.
[4] Refer to Kim et al. (2002). [5] Refer to Kim et al. (2001).

commercially important aquaculture species, and the need for nutritional studies has increased, especially in the area of Se requirement and toxicity.

Therefore, this preliminary study was carried out to investigate the effects of different dietary Se levels on growth performance and toxicity in juvenile black seabream, *Acanthopagrus schlegeli*.

MATERIALS AND METHODS

Experimental design and diets

The composition and proximate analysis of the semi-purified basal diet are shown in Table 1. The diet was supplemented with graded levels of Se as sodium selenite (Na_2SeO_3, Sigma Chemical Co., St. Louis, MO, USA). The resulting dietary Se concentrations were 0.21, 0.30, 0.52, 1.29 or 12.3 mg Se/kg diet (Se 0.21, Se 0.30, Se 0.52, Se 1.29 and Se 12.3) based on the analysis of the diets by ICP-MS determination (AOAC, 2000). The experimental diets were formulated to contain 65.4% crude protein and available energy level of 23.3 kJ/g (Om et al., 2001). Vitamin-free casein was used as the main protein source. Experimental diets were prepared as described previously (Lee et al., 1998). After processing, all the experimental diets were stored at -20°C until use.

Fish and feeding trial

Juvenile black seabream were obtained from Goseong Fisheries Science Center (a research station branch of Pukyong National University) in Goseong district, Gyeongsang province, Republic of Korea. Prior to the start of the feeding trial, fish were fed the basal diet for 2 weeks to acclimate them to the experimental diets and conditions. The feeding trial was conducted using in a 30 L rectangular aquaria in an indoor semi-recirculation system. Supplemental aeration was provided to maintain the dissolved oxygen near air saturation. The water temperature was maintained at 20±1°C during whole-experiment periods. Experimental fish averaging 7.0±0.1 g (Mean±SD) were randomly distributed in each aquarium as a group of 10 fish. The feeding trial was conducted for 15 weeks. The experimental diets were fed to triplicate groups of fish at a fixed rate of 2-2.5% (2.5% of wet body weight in the beginning and 2.0% at the end of the feeding trial) per day on a dry-matter basis. The fish were fed twice a day at 10:00 and 16:00 h during the feeding trial. The total fish weight in each aquarium was determined every 2-week, and the feeding rate was adjusted accordingly.

Sample collection and analyses

At the end of the feeding trial, all of the fish were weighed and counted for calculation of weight gain, feed efficiency, specific growth rate, protein efficiency ratio and survival rate. Blood samples were obtained from the caudal vein with a 1 ml syringe. Hematocrit was determined on three individual fish randomly selected per aquarium by the microhematocrit method (Brown, 1980), and hemoglobin was measured with the same fish by the cyan-methemoglobin procedure using Drabkin's solution. Hemoglobin standard prepared from human blood (Sigma Chemical Co., St Louis, MO, USA) was used. Three randomly selected fish per aquarium were used for whole-body proximate analyses. Proximate composition analyses of experimental diets and fish body were performed by the standard methods of AOAC (1995). Samples of diets and fish were dried to a constant weight at 105°C to determine moisture content. Ash was determined by incineration at 550°C; protein by Kjeldahl method (N×6.25), after acid digestion; and crude lipid by soxhlet extraction using Soxtec system 1046 (Tacator AB, Sweden) after freeze-drying samples for 20 h.

Se analysis

Tissue and water Se levels were assessed using standard methods (AOAC, 2000). For each treatment three fish were randomly selected and their gill, kidney, liver and muscle employed for tissue analysis at the end of the feeding trial. Water samples were collected at the beginning, middle and end of this study and frozen until analysis. A Perkin-Elmer 3300 Inductively Coupled Plasma Mass Spectrometer (Perkin-Elmer, Waltham, MA, USA) was utilized for all Se analysis.

Histopathology

Liver, kidney, gill, and muscle tissues fixed in 10% neutral buffered formalin were dehydrated in a graded ethanol series and embedded in paraffin. Tissue blocks were

Table 2. Growth performance and haematological characteristics determination of fish fed the experimental diets for 15 weeks[1]

Diets	WG[2] (%)	FE[3] (%)	SGR[4] (%)	PER[5]	PCV[6] (%)	Hb[7] (g/100 ml)	RBC[8] ($\times10^6$ cell/μl)
Se 0.21	327.4[a]	93.3[a]	2.70[a]	1.72[a]	42.2	15.3	3.34
Se 0.30	357.5[a]	94.9[a]	2.88[a]	1.79[a]	38.2	16.5	3.69
Se 0.52	325.6[a]	91.9[a]	2.69[a]	1.72[a]	38.6	16.1	3.59
Se 1.29	349.7[a]	94.3[a]	2.80[a]	1.78[a]	32.5	17.0	3.12
Se 12.3	254.4[b]	84.2[b]	2.23[b]	1.53[b]	26.6	13.1	2.90
Pooled SEM[9]	13.0	1.40	0.08	0.03	2.46	0.72	0.16

[1] Values are means from groups (n = 3) of fish where the means in each column with a different superscript are significantly different ($p<0.05$).
[2] Weight gain (%) = (final weight-initial weight)×100/initial weight
[3] Feed Efficiency (%) = wet weight gain (g)×100/dry feed intake (g)
[4] Specific growth rate (%) = ($\log_e$ final wt.-$\log_e$ initial wt.)/days
[5] Protein efficiency ratio: wet weight gain/protein intake. [6] PCV = Hematocrit. [7] Hb = hemoglobin. [8] RBC = Red blood cell.
[9] Pooled standard error of mean: SD/$\sqrt{n}$.

sectioned (4 μm thick) and stained with hematoxylin and eosin (H & E). Tissue sections were examined under a CX31 Olympus microscope for common and/or significant lesions.

Statistical analysis

All data were subjected to one-way ANOVA test using SAS Version 9.0 (SAS Institute, Cary, NC, USA). When a significant treatment effect was observed, a Least Significant Difference (LSD) test was used to compare means. Treatment effects were considered with the significant level at $p<0.05$.

RESULTS AND DISCUSSION

Data on weight gain (WG), feed efficiency (FE), specific growth rate (SGR), protein efficiency ratio (PER) and haematological characteristics determination are summarized in Table 2. Fish fed Se 0.21, Se 0.30, Se 0.52 and Se 1.29 diets showed no significant differences in WG, FE, SGR and PER, however fish fed Se 12.3 diet showed significantly lower WG, FE, SGR and PER than did fish fed the other diets ($p<0.05$). Hematocrit (PCV), hemoglobin (Hb) and red blood cell (RBC) of fish fed the experimental diets were not significantly different, however fish fed Se 12.3 diet showed the lower values of PCV (26.6±2.0%), Hb (13.1±1.0 g/100 ml) and RBC ($2.9\pm0.3\times10^6$ cell/μl) than did fish fed the other diets (PCV, 37.5±7.2%; Hb, 15.9± 2.2 g/100 ml; RBC, $3.4\pm0.5\times10^6$ cell/μl). Juvenile black seabream exposed to dietary concentrations ranging between 0.21 and 12.3 mg Na_2SeO_3/kg diet for 15 weeks showed no significant difference in survival rate. Hilton et al. (1980) reported that rainbow trout, *Salmo gairdneri* fed diets containing 0.07, 0.15, 0.38, 1.25 and 3.67 μg Na_2SeO_3/g diet for 20 weeks showed no significant differences in body weight gain, a feed:gain ratio and mortalities, however rainbow trout fed a diet containing 13.1 μg Na_2SeO_3/g diet showed significantly lower body weight increase, a feed:gain ratio and mortalities than those of fish fed the other diets. Gatlin and Wilson (1984) showed that channel catfish, *Ictalurus punctatus* fed diets containing different supplemental Se levels as 0.2, 0.3, 0.4, 0.5, 1.0 and 5.0 mg Na_2SeO_3/kg diet for 15 weeks showed no significant differences in WG and FE, however a supplemental Se level of 15 mg Na_2SeO_3/kg diet caused a reduced growth response which indicated Se toxicity in catfish. Lin and Shiau (2005) reported that grouper, *Epinephelus malabaricus* fed a diet containing 0.79 mg Se-Met/kg diet for 8 weeks showed significantly higher WG than that of grouper fed diets containing 0.17, 0.25, 0.39 and 0.55 mg Se-Met/kg diet, however there was no significant difference in FE among fish fed all the diets. The amount of Se for proper growth performance in black seabream diet obtained from this study was 0.21 mg Na_2SeO_3/kg diet. This result is similar to that the dietary Se requirement in rainbow trout and channel catfish is 0.15-0.35 mg and 0.25 mg Na_2SeO_3/kg diet, respectively, however the dietary Se requirement in grouper is 0.79 mg Se-Met/kg diet which is higher than black seabream. The bioavailability between inorganic and organic Se is different (Wang and Lovell, 1997; Lyons et al., 2007), and minimum level for proper growth performance in juvenile black seabream could be different if organic Se sources such as Se-Met or selenoyeast (Se-Y) were used. Also, the dietary Se requirement in fish could be affected by waterborne Se in rearing water. Hilton et al. (1980) reported that trout can readily take up waterborne Se at such low concentrations, and Se concentrations in the rearing water (freshwater) of the above two studies (rainbow trout and channel catfish) were ranging from 0.4 to 2.5 μg Se/L. The waterborne Se concentration in the rearing sea water from this study was ranging from 3.42 to 8.25 μg Se/L which was much higher concentrations than above two studies. However, it is difficult to suppose that such higher Se concentration could prevent Se deficiency for proper growth performance in this species. Based on the results of preliminary feeding trial,

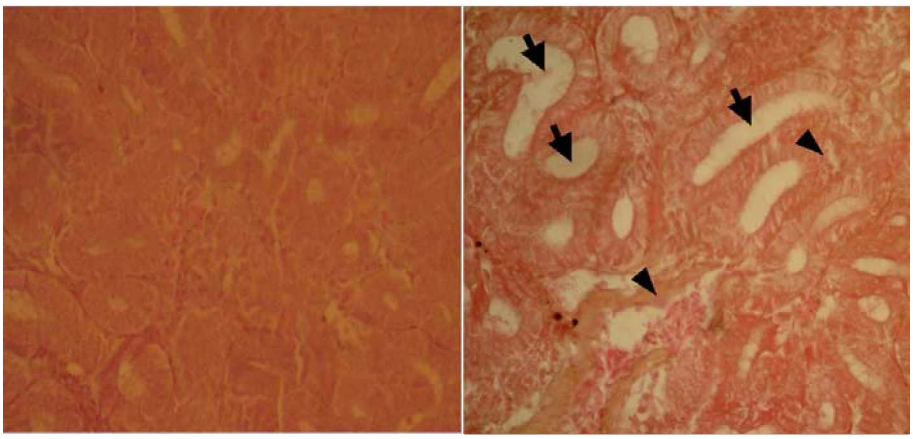

Figure 1. (Left) Kidney of black seabream exposed to 0.21 mg Na_2SeO_3/kg diet for 15 weeks. (Right) Polycystic dilation of tubules (arrow) and tubular necrosis (arrowhead) in the kidney of black seabream exposed to 12.3 mg Na_2SeO_3/kg diet for 15 weeks.

therefore, the minimum level of dietary Se for proper growth performance in juvenile black seabream could be 0.21 mg Na_2SeO_3/kg diet, however the dietary Se level might be changed if further researches were carried out with analysis of a Se-dependent enzyme such as glutathione peroxidase or basal diet containing much lower Se level of this present study (0.21 mg Na_2SeO_3/kg diet).

Chronic dietary Se toxicity was found to occur in black seabream fed a diet containing 12.3 mg Na_2SeO_3/kg diet which compares favorably with the results of rainbow trout (13.1 μg Na_2SeO_3/g dry feed) and channel catfish (15 mg Na_2SeO_3/kg diet). The main effects of Se toxicity in black seabream were reduced growth performances similar to other studies (Hilton et al., 1980; Gatlin and Wilson, 1984; Teh et al., 2004; Tashjian et al., 2006). Lemly (2002) mentioned that shifts in hematological parameters reflect important changes in the overall health of fish, and reported that green sunfish exposed to higher level of Se showed significantly reduced hematocrit values. In this study, black seabream fed the experimental diets containing 0.21-12.3 mg Na_2SeO_3/kg diet showed no significant difference in hematocrit, however fish fed a diet containing 12.3 mg Na_2SeO_3/kg diet exhibited the lower values as compared with fish fed diets containing 0.21-1.29 mg Na_2SeO_3/kg diet. According to Lemly (2002), reductions in hematocrit are associated with anemia and lowered mean corpuscular hemoglobin concentration (MCHC; Lemly, 1993). Reduced MCHC causes impaired respiratory capacity because Se can bind to hemoglobin, rendering it incapable of carrying oxygen.

Histopathological lesions were not observed in gill, liver and muscle tissues, but severe histopathological lesions were observed in kidney tissues of black seabream fed 12.3 mg Na_2SeO_3/kg diet for 15 weeks (Figure 1). Kidney lesions included tubular necrosis and polycystic dilation of tubules. These results observed in other species exposed to waterborne or dietary Se (Lemly, 2002; Tashjian et al., 2006). Lemly (2002) reported that Belews lake green sunfish that had accumulated high levels of Se showed that numerous tubular casts were present, and tubular epithelium was desquamated, vacuolated, and often destroyed (which can render the tubular system of the mesonephros incapable of functioning properly). Tashjian et al. (2006) showed that significant histopathological lesions were observed in kidney tissues of white sturgeon fed 20.5 μg Se-Met/g diet and above for 8 weeks. Lesions in the kidneys included tubular dilation, tubular cell hydropic degeneration and necrosis, and tubular inclusions with either eosinophilic or basophilic cast materials in the lumen. Histopathological changes in the kidneys of Chinook salmon were observed in fish fed 13 and 26 μg Se/g diet for 4.2 weeks (Hamilton et al., 1986). Contrary to this study, rainbow trout did not exhibit any histopathological lesions when fed up to 13 μg Se/g diet in the form of sodium selenite for 16 weeks (Hilton et al., 1980). These results are similar to the finding of this study that the dietary Se level of 12.3 mg Na_2SeO_3/kg diet induced histopathological lesions in liver tissues of black seabream. Otherwise, Teh et al. (2004) reported that liver section of Sacramento splittail fed 6.6 mg Se-yeast/kg diet showed that severe glycogen depletion (basophilic cytoplasm) and moderate fatty vacuolar degeneration at the end of 9 months exposure. Tashjian et al. (2006) showed that significant histopathological lesions were observed in liver tissues of white sturgeon fed 41.7 μg Se-Met/g diet and above. From these results, we could infer that histopathologcial lesions in liver tissues may be exhibited if black seabream were exposed to higher level of Se above 12.3 mg Na_2SeO_3/kg diet. Based on the results a dietary Se level of 12.3 mg Na_2SeO_3/kg could cause depressed growth performance and severe histopathological lesions in the kidney tissues in juvenile black seabream, and the toxic level of Se could be different with further

Table 3. Tissue Se concentrations (μg/g of wet matter basis) in juvenile black seabream exposed to dietary Na_2SeO_3 for 15 weeks[1]

Tissue	Treatment (mg Se/kg diet)	Se concentration (μg/g)
Liver	0.21	0.40[d]
	0.30	0.52[cd]
	0.52	0.64[c]
	1.29	0.86[b]
	12.3	1.55[a]
Pooled SEM		0.20
Kidney	0.21	0.16[b]
	0.30	0.27[b]
	0.52	0.31[b]
	1.29	0.45[b]
	12.3	1.48[a]
Pooled SEM		0.24
Gill	0.21	0.18[c]
	0.30	0.19[bc]
	0.52	0.23[bc]
	1.29	0.24[b]
	12.3	0.41[a]
Pooled SEM		0.04
Muscle	0.21	0.08[b]
	0.30	0.09[b]
	0.52	0.10[b]
	1.29	0.11[b]
	12.3	0.40[a]
Pooled SEM		0.06

[1] Values are means from triplicate groups of fish where the means in each column with a different superscript are significantly different ($p<0.05$).

researches designing experimental diets to have dietary Se concentrations lower than 12.3 mg Na_2SeO_3/kg diet.

Se was accumulated in a dose-dependent manner in the liver, kidney, muscle and gill tissues of black seabream fed the experimental diets for 15 weeks (Table 3). Fish fed 12.3 mg Na_2SeO_3/kg diet had a significantly higher liver, kidney, gill and muscle tissue concentrations than those of fish fed 0.21-1.29 mg Na_2SeO_3/kg diet. The Se concentrations of liver tissues were significantly increased in fish fed ≥0.64 mg Na_2SeO_3/kg diet. The Se concentrations of kidney and muscle tissues were not significantly increased in fish fed 0.21-1.29 mg Na_2SeO_3/kg diet, however it is likely that a break point of significant increase in Se concentrations of kidney and muscle tissue could lie between 1.29 and 12.6 mg Na_2SeO_3/kg diet. The Se concentrations of gill tissues were significantly increased in fish fed ≥1.29 mg Na_2SeO_3/kg diet. Liver Se concentrations significantly increased at 0.52 mg Na_2SeO_3/kg diet and continued increasing significantly at every treatment beyond this level. Liver Se concentrations of fish fed diets containing 0.21-1.29 mg Na_2SeO_3/kg diet were significantly higher than kidney, gill and muscle tissues, however liver and kidney Se concentrations of fish fed a diet containing 12.3 mg Na_2SeO_3/kg diet were not significantly different (Figure 2). These results indicated that liver Se concentrations reflected a more distinct dose-response to dietary Se concentrations than that of kidney, gill and muscle Se concentrations. On the contrary to the results of this study, Teh et al. (2004) reported that liver Se concentrations of Sacramento splittail, *Pogonichthys macrolepidotus* fed diets containing 0.4-12.6 mg Se-yeast/kg diet for 9 months were not significantly different and increased, however the muscle Se concentrations of fish fed diets containing ≥1.4 mg Se-yeast/kg diet significantly increased. Tashjian et al. (2006) showed that the Se concentrations of liver tissues were not significantly increased in white sturgeon, *Acipenser transmontanus* fed 0.4-20.5 μg Se-Met/g diet for 8 weeks, however the kidney and muscle Se concentrations of fish fed diets containing 0.4-20.5 μg Se-Met/g diet were significantly different and increased. Therefore, in a black seabream a liver may be better suited for evaluating Se bioaccumulation than kidney, gill and muscle tissues.

Whole-body proximate composition of fish fed the

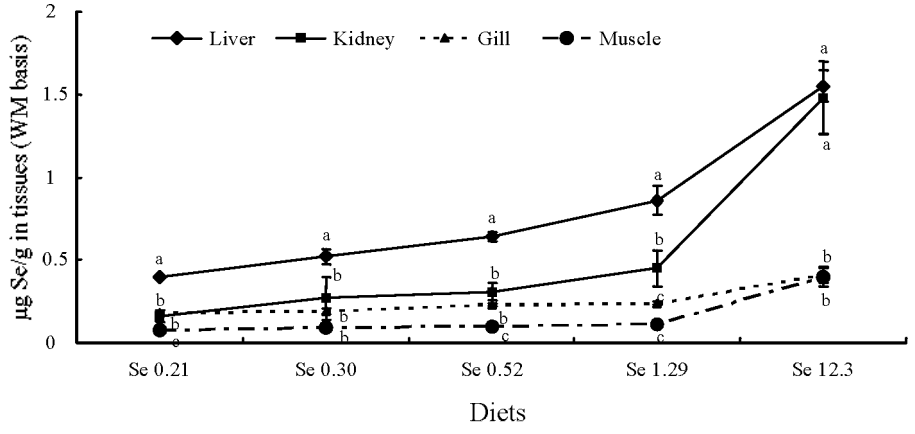

Figure 2. Se concentrations of black seabream (mean±SD, n = 3) fed the experimental diets for 15 weeks in liver, kidney, gill and muscle tissues. Note that vertical bars represent the SD and different letter above or below the bars indicate significant difference among treatments, $p<0.05$.

experimental diets containing 0.21-12.3 mg Na_2SeO_3/kg were not significantly different. Values of moisture, crude protein, crude lipid and ash as dry-matter basis were 70.8± 0.47% (Mean±SD), 54.9±1.40%, 21.5±1.25% and 15.9±0.93%, respectively. Tashjian et al. (2006) showed similar results that white sturgeon fed diets containing 0.4-89.8 μg Se-Met/g diet showed no significant differences in whole-body proximate composition.

In summary, black seabream fed diets containing 0.21-1.29 mg Na_2SeO_3/kg diet showed no significant differences in growth performance and hematological characteristics for 15 weeks, however black seabream fed a diet containing 12.3 mg Na_2SeO_3/kg diet showed significantly lower growth performances. Histopathological lesions in kidney tissues were observed in black seabream fed a diet containing 12.3 mg Na_2SeO_3/kg diet, however no histopathological lesions observed in liver, muscle and gill tissues of black seabream fed the experimental diets. Se was accumulated in a dose-dependent manner in the liver, kidney, muscle and gill tissues of black seabream fed the experimental diets, and liver tissues of black seabream were more sensitive to dietary Se concentrations than other tissues. Based on the results of this preliminary feeding trial, a dietary Se level of 0.21 mg Na_2SeO_3/kg diet could be optimal for proper growth performance, and a dietary Se level of 12.3 mg Na_2SeO_3/kg diet may ultimately be toxic to juvenile black seabream, *Acanthopagrus schlegeli*.

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