Effects of Dietary Selenium Source and Level on Growth Performance, Liver and Serum Antioxidant Indices, and Tissue Selenium Content in Juvenile Cobia (Rachycentron canadum) Postprint

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Abstract

This study aimed to investigate the effects of selenium source and level on growth performance, liver and serum antioxidant indices, and tissue selenium content in juvenile cobia, in order to determine the optimal dietary requirement for different selenium sources. Sodium selenite (Se-S) and selenomethionine (Se-Met) were supplemented to basal diets at 0 (control), 0.3, 0.6, 0.9, and 1.2 mg/kg (as selenium) to formulate nine experimental diets (sharing a common control diet), which were fed ad libitum to juvenile cobia with an initial body weight of (22.18$±$0.35) g for 10 weeks. Each experimental diet was fed to three net cages (replicates), with 30 fish per cage. The results showed: 1) Selenium level had a highly significant effect on specific growth rate (SGR) and weight gain rate (WGR) ($P<0.01$), but no significant effect on survival rate (SR) and feed conversion ratio (FCR) ($P>0.05$); neither selenium source nor the interaction between selenium source and level had significant effects on SGR, WGR, FCR, or SR ($P>0.05$). SGR and WGR increased initially and then decreased with increasing selenium levels. 2) Selenium level had a highly significant effect on liver glutathione peroxidase (GSH-Px) and glutathione reductase (GR) activities, malondialdehyde (MDA) content, and serum GSH-Px activity ($P<0.01$), and a significant effect on serum GR activity ($P<0.05$); selenium source had a highly significant effect on liver GR, total superoxide dismutase (T-SOD), and catalase (CAT) activities, MDA content, and serum T-SOD activity ($P<0.01$); the interaction between selenium source and level significantly affected liver CAT activity and MDA content, and serum T-SOD activity ($P<0.05$). Liver and serum GSH-Px activities increased initially and then stabilized with increasing selenium levels, while GR activity decreased initially and then stabilized. Both selenium sources produced the highest liver GSH-Px activity and lowest

liver GR activity at the 0.9 mg/kg supplementation level. Liver CAT activity increased gradually with increasing selenium levels. 3) Selenium content in vertebrae, whole fish, and liver increased with increasing selenium levels. Selenium source had a highly significant effect on vertebrae selenium content (P<0.01), and the interaction between selenium source and level had a highly significant effect on liver and vertebrae selenium content (P<0.01). Based on quadratic regression analysis, dietary selenium levels of 1.29 and 1.46 mg/kg were required to achieve maximum SGR in juvenile cobia fed selenomethionine and sodium selenite, respectively. Using SGR and whole-fish selenium content as criteria, the bioavailability of selenomethionine for juvenile cobia was 1.20 and 2.90 times that of sodium selenite, respectively.

Full Text

Effects of Selenium Source and Selenium Level on Growth Performance, Liver and Serum Antioxidant Indices, and Tissue Selenium Content in Juvenile Cobia (Rachycentron canadum)

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Abstract: This study investigated the effects of selenium (Se) source and dietary Se level on growth performance, liver and serum antioxidant indices, and tissue Se content in juvenile cobia (*Rachycentron canadum*) to determine the optimal dietary Se requirement from different sources. Nine experimental diets (with a shared control) were formulated by supplementing a basal diet with 0 (control), 0.3, 0.6, 0.9, and 1.2 mg/kg Se from either sodium selenite (Se-S) or selenomethionine (Se-Met). Juvenile cobia with an initial body weight of (22.18$±$0.35) g were fed to satiation for 10 weeks. Each experimental diet was fed to three replicate cages, with 30 fish per cage. The results showed: 1) Se level had an extremely significant effect on specific growth rate (SGR) and weight gain rate (WGR) (P<0.01), but no significant effect on survival rate (SR) or feed conversion ratio (FCR) (P>0.05). Neither Se source nor the interaction between Se source and Se level significantly affected SGR, WGR, FCR, or SR (P>0.05). Both SGR and WGR increased initially and then decreased with rising Se levels. 2) Se level extremely significantly affected liver glutathione peroxidase (GSH-Px) and glutathione reductase (GR) activities and malondialdehyde (MDA) content, as well as serum GSH-Px activity (P<0.01), and significantly affected serum GR activity (P<0.05). Se source extremely significantly affected liver GR, total superoxide dismutase (T-SOD), catalase

(CAT) activities and MDA content, and serum T-SOD activity ($P<0.01$). The interaction between Se source and Se level significantly affected liver CAT activity and MDA content and serum T-SOD activity ($P<0.05$). Liver and serum GSH-Px activities increased initially and then stabilized with increasing Se levels, while GR activities decreased initially and then stabilized. Both Se sources produced the highest liver GSH-Px activity and lowest liver GR activity at the 0.9 mg/kg supplementation level. Liver CAT activity increased progressively with Se level. 3) Se content in vertebrae, whole body, and liver increased with dietary Se level. Se source extremely significantly affected vertebrae Se content ($P<0.01$). The interaction between Se source and Se level extremely significantly affected liver and vertebrae Se content ($P<0.01$). Quadratic regression analysis indicated that maximal SGR was achieved at dietary Se levels of 1.29 mg/kg and 1.46 mg/kg for Se-Met and Se-S, respectively. Based on SGR and whole-body Se content, the biological utilization of Se-Met was 1.20 and 2.90 times that of Se-S, respectively.

Keywords: juvenile cobia (*Rachycentron canadum*); selenium; growth performance; antioxidant enzyme; selenium accumulation

Introduction

Selenium exists in nature as inorganic or organic forms and was long considered a toxic substance. In 1957, Schwartz et al. confirmed that Se could prevent liver necrosis in rats caused by Se and/or vitamin E deficiency [1]. Subsequent research revealed that Se is an essential trace element component of glutathione peroxidase (GSH-Px), with selenocysteine at its active center. This enzyme converts hydrogen peroxide and lipid peroxidation products into water and alcohols, protecting polyunsaturated phospholipids in cell and subcellular membranes from oxidative damage [2]. Additionally, Se promotes animal growth, improves reproductive performance, and enhances immunity.

Different Se sources exhibit varying bioavailability, utilization efficiency, and stress resistance in fish. Lorentzen et al. [3] found that Atlantic salmon (*Salmo salar*) fed diets supplemented with selenomethionine had higher Se concentrations in muscle and whole body than those fed selenite. Wang et al. [4] reported that organic Se sources (selenium yeast and selenomethionine) required lower dietary levels and showed higher relative bioavailability than inorganic sodium selenite in channel catfish (*Ictalurus punctatus*), with higher GSH-Px activity and Se deposition. Dietary Se supplementation increased GSH-Px activity in rainbow trout (*Oncorhynchus mykiss*), with organic Se showing superior antistress effects compared to selenite [5]. Jaramillo et al. [6] demonstrated that selenomethionine had 3.3 times higher bioavailability than sodium selenite in hybrid striped bass (*Morone chrysops* × *M. saxatilis*) using slope-ratio methodology.

Cobia (*Rachycentron canadum*), belonging to the order Perciformes, family

Rachycentridae, and genus *Rachycentron*, is also known as black kingfish or sergeant fish. As a large carnivorous species, cobia is characterized by rapid growth, easy adaptation to artificial feeds, and excellent flesh quality, making it an important aquaculture species in offshore cage culture along southern China coasts.

Currently, only the Se requirement from selenomethionine has been reported for cobia, with Liu et al. [7] indicating a requirement of 0.788–0.811 mg/kg for juvenile cobia. No studies have examined the effects of different Se sources and levels on the nutritional physiology of cobia. Therefore, this experiment was designed to investigate the nutritional effects of varying Se sources and levels in juvenile cobia diets, aiming to provide fundamental data for improving nutritional requirement databases and developing efficient formulated feeds for this species.

1.1 Experimental Design and Diets

A basal diet was formulated using vitamin-free casein as the primary protein source, corn starch as the carbohydrate source, and fish oil and corn oil as the main lipid sources. The composition and nutrient levels of the basal diet are presented in Table 1 . The basal diet was supplemented with Se from either sodium selenite (purchased from Sinopharm Chemical Reagent Co., Ltd.) or selenomethionine (purchased from Changsha Xingjia Bioengineering Co., Ltd.) at levels of 0 (control), 0.3, 0.6, 0.9, and 1.2 mg/kg, resulting in nine experimental diets (with a shared control). The analyzed Se content in the control diet was 0.35 mg/kg (designated C-0.35). The four sodium selenite-supplemented diets had analyzed Se levels of 0.68, 1.09, 1.26, and 1.65 mg/kg (designated Se-S-0.68, Se-S-1.09, Se-S-1.26, and Se-S-1.65, respectively). The four selenomethionine-supplemented diets had analyzed Se contents of 0.67, 1.02, 1.33, and 1.69 mg/kg (designated Se-Met-0.67, Se-Met-1.02, Se-Met-1.33, and Se-Met-1.69, respectively).

Table 1 Composition and nutrient levels of the basal diet (dry matter basis) %

1.2 Experimental Fish and Culture Management

The feeding trial was conducted at a floating sea cage farm in Nansan Island, Zhanjiang, Guangdong Province. Juvenile cobia were purchased from a local fish farmer in Yingli Town, Zhanjiang. Fish were acclimated for two weeks and fed commercial diets during this period. Prior to stocking, fish were fasted for 24 h. Healthy fish with uniform size and an initial body weight of (22.18$±$0.35) g were randomly selected and distributed into nine treatment groups, each with three replicates. Each replicate consisted of one 2.5 m × 1.2 m × 1.4 m cage, totaling 27 cages with 30 fish per cage. To minimize experimental error, all cages were arranged randomly. Fish were hand-fed to satiation twice daily (07:00 and 18:00) at 6%–9% of body weight. During the trial, water temperature ranged from 28 to 33 °C, pH was 7.6–7.8, salinity was 29–31, and dissolved

oxygen concentration was >6.0 mg/L. The experimental period lasted 10 weeks. Selenium was not detected in the culture seawater.

1.3 Sample Collection and Analysis

At the end of the trial, fish were fasted for 24 h before harvest. All fish in each cage were collected, anesthetized with eugenol (1:10,000), and weighed. Five fish per cage were randomly selected for blood collection via cardiac puncture using a 2.5 mL syringe. Blood samples were placed in 1.5 mL Eppendorf tubes, centrifuged at 4,500 r/min for 10 min at 4 °C, and the serum was stored at -80 °C for subsequent analysis. Another five fish per cage were dissected to obtain liver samples, which were immediately frozen in liquid nitrogen and then stored at -80 °C. Eight additional fish per cage were euthanized, eviscerated, boiled for 3 min, and muscle was removed to isolate vertebrae. Vertebrae were rinsed with ultrapure water to remove attached muscle tissue, dried at 105 °C, ground to pass through an 80-mesh sieve, extracted with ether for 12 h to remove lipids, and redried at 105 °C [8].

Diet composition analysis: Moisture content was determined by oven drying at 105 °C; crude protein content was measured by the Kjeldahl method (total nitrogen × 6.25); crude lipid content was determined by Soxhlet extraction; and crude ash content was measured by combustion in a muffle furnace at 550 °C.

Liver and serum antioxidant indices: Activities of GSH-Px, glutathione reductase (GR), total superoxide dismutase (T-SOD), catalase (CAT), and malondialdehyde (MDA) content were determined using assay kits from Nanjing Jiancheng Bioengineering Institute. Sample preparation, reagent preparation, and analytical procedures were performed strictly according to the manufacturer' s instructions.

Selenium content in diets, whole body, vertebrae, and liver: Samples were digested with nitric acid and hydrogen peroxide, and Se content was determined by inductively coupled plasma mass spectrometry.

1.4 Calculation Formulas

Specific growth rate (SGR, %/d) = 100 × [ln(final mean weight) -ln(initial mean weight)] / experimental days;

Weight gain rate (WGR, %) = 100 × (final mean weight -initial mean weight) / initial mean weight;

Survival rate (SR, %) = 100 × (final number of fish) / (initial number of fish);

Feed conversion ratio (FCR) = total feed intake / (final total weight + total weight of dead fish -initial total weight).

1.5 Statistical Analysis

Results are expressed as means ± standard error. Two-way ANOVA was performed using SPSS 16.0 general linear model (GLM) to analyze the main effects of Se source, Se level, and their interaction. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$.

2.1 Effects of Selenium Source and Level on Growth Performance of Juvenile Cobia

As shown in Table 2 , Se source had no significant effect on SGR, WGR, FCR, or SR ($P>0.05$). Se level extremely significantly affected SGR and WGR ($P<0.01$) but had no significant effect on SR or FCR ($P>0.05$). The interaction between Se source and Se level did not significantly affect any growth parameters ($P>0.05$). Both SGR and WGR increased initially and then decreased with rising Se levels. Quadratic regression analysis indicated that maximal SGR was achieved at dietary Se levels of 1.29 mg/kg and 1.46 mg/kg for selenomethionine and sodium selenite, respectively (Figure 1 [Figure 1: see original paper]). Based on SGR, the biological utilization of selenomethionine was 1.20 times that of sodium selenite (selenomethionine: $y = 0.209x + 2.111$, $R^2 = 0.98$; sodium selenite: $y = 0.175x + 2.218$, $R^2 = 0.99$; where x represents dietary Se level and y represents SGR).

Table 2 Effects of Se source and Se level on growth performance of juvenile cobia

2.2 Effects of Selenium Source and Level on Liver and Serum Antioxidant Indices of Juvenile Cobia

As shown in Table 3 , Se source extremely significantly affected liver GR, T-SOD, and CAT activities and MDA content, as well as serum T-SOD activity ($P<0.01$), but had no significant effect on liver GSH-Px activity or serum GSH-Px, GR, CAT activities, or MDA content ($P>0.05$). Se level extremely significantly affected liver GSH-Px and GR activities and MDA content, and serum GSH-Px activity ($P<0.01$), and significantly affected serum GR activity ($P<0.05$), but had no significant effect on liver T-SOD and CAT activities or serum T-SOD, CAT activities, or MDA content ($P>0.05$). The interaction between Se source and Se level significantly affected liver CAT activity and MDA content and serum T-SOD activity ($P<0.05$), but had no significant effect on other antioxidant indices ($P>0.05$). Liver and serum GSH-Px activities increased initially and then stabilized with rising Se levels, while GR activities decreased initially and then stabilized. Among the two Se sources, the highest liver GSH-Px activities and lowest liver GR activities were observed in the Se-S-1.26 and Se-Met-1.33 groups, both significantly higher and lower, respectively, than the control group (C-0.35) ($P<0.05$). Liver CAT activity increased progressively with Se level. In sodium selenite groups, the Se-S-1.65 group showed

significantly higher liver CAT activity than all other groups except Se-S-1.26. In selenomethionine groups, the Se-Met-1.69 group exhibited significantly higher liver CAT activity than all other groups ($P<0.05$). No significant differences in serum CAT activity were detected among groups ($P>0.05$).

Table 3 Effects of Se source and Se level on antioxidant indices in liver and serum of juvenile cobia

2.3 Effects of Selenium Source and Level on Tissue Selenium Content of Juvenile Cobia

As shown in Table 4 , Se source extremely significantly affected vertebrae Se content ($P<0.01$) but had no significant effect on whole-body or liver Se content ($P>0.05$). Se level extremely significantly affected Se content in vertebrae, whole body, and liver ($P<0.01$), with all tissue Se concentrations increasing as dietary Se level increased. The interaction between Se source and Se level had no significant effect on whole-body Se content ($P>0.05$) but extremely significantly affected liver and vertebrae Se content ($P<0.01$). Based on whole-body Se content, the biological utilization of selenomethionine was 2.90 times that of sodium selenite (selenomethionine: $y = 0.938x + 0.052$, $R^2 = 0.99$; sodium selenite: $y = 0.323x + 2.252$, $R^2 = 0.99$; where x represents dietary Se level and y represents whole-body Se content).

Table 4 Effects of Se source and Se level on Se content in tissues of juvenile cobia (mg/kg)

3.1 Effects of Selenium Source and Level on Growth Performance

In this study, juvenile cobia fed the unsupplemented control diet exhibited poor growth. Dietary Se level extremely significantly affected SGR and WGR but not SR or FCR, with SGR and WGR increasing initially and then decreasing as Se levels rose. These results indicate that both Se deficiency and excess can reduce growth performance in juvenile cobia, consistent with findings in yellowtail kingfish (*Seriola lalandi*) [9]. Han et al. [10] reported that dietary Se supplementation promoted growth in gibel carp (*Carassius auratus gibelio*), with growth plateauing at 1.85 mg/kg Se. Previous research on cobia indicated that optimal dietary Se levels promoted growth, with performance stabilizing at Se levels above 0.85 mg/kg [7]. Both Se sources enhanced WGR in malabar grouper (*Epinephelus malabaricus*), with growth stabilizing at 0.80 mg/kg Se [11]. In large yellow croaker (*Larimichthys croceus*), WGR increased with dietary Se level and stabilized at 0.27 mg/kg [12]. Common carp (*Cyprinus carpio*) showed increased WGR with nano-Se supplementation, reaching maximum growth at 1 mg/kg Se [13]. High dietary Se levels (13 mg/kg) caused chronic toxicity in rainbow trout, manifesting as reduced growth and increased mortality [14]. The margin between nutritional requirement and toxicity is narrow in fish [15], with cold-water species being more sensitive to Se toxicity than warm-water

species [16]. In some cases, dietary Se can cause mortality without inhibiting growth, while in others it inhibits growth without causing mortality [17], likely depending on Se form, dosage, fish species, body weight, trial duration, and culture conditions.

In this study, maximal SGR was achieved at 1.46 mg/kg Se from sodium selenite and 1.29 mg/kg Se from selenomethionine. These values are higher than reported requirements for channel catfish (0.25 [2] and 0.12 mg/kg [4]), cobia (0.85 mg/kg) [7], large yellow croaker (0.18 mg/kg) [12], rainbow trout (0.38 mg/kg) [14], malabar grouper (0.77 mg/kg) [18], Chinese mitten crab (*Eriocheir sinensis*) (0.51 mg/kg) [19], hybrid striped bass (0.40 mg/kg) [20], and Japanese seabass (*Lateolabrax japonicus*) (0.63 mg/kg) [21], but lower than those for yellowtail kingfish (5.56 mg/kg) [9], largemouth bass (*Micropterus salmoide*) (1.60–1.85 mg/kg) [22], cutthroat trout (*Oncorhynchus clarkii*) (9.2 mg/kg) [23], and beluga (*Huso huso*) (11.56 mg/kg) [24]. These values are similar to requirements reported for hybrid striped bass (1.19 mg/kg) [6], gibel carp (1.18 mg/kg) [10], and common carp (1.46 mg/kg) [13]. Variations in Se requirements among fish species may be attributed to differences in fish species, Se supplementation form, waterborne Se concentration, and dietary vitamin E content. Previous studies have demonstrated higher utilization efficiency of organic Se compared to inorganic Se in Atlantic salmon [3], channel catfish [4], grouper [11], and yellowtail kingfish [25]. Methionine-Se was superior to selenium yeast and sodium selenite in improving growth performance and antioxidant capacity of Pacific white shrimp (*Litopenaeus vannamei*) [26]. The interaction between dietary vitamin E and Se can affect Se requirements [18], as these nutrients form an integrated antioxidant defense system; reduced intake of one necessitates higher supplementation of the other [27]. Fish can absorb mineral elements from water via gills and skin. In studies with channel catfish [2] and rainbow trout [14], waterborne Se concentrations were 0.4–2.5 g/L, whereas Se was not detected in the seawater used in this trial, which may partially explain the slightly higher Se requirement observed for juvenile cobia. In this study, the Se requirement from selenomethionine was lower than that from sodium selenite, with selenomethionine showing 1.20 times higher biological utilization based on SGR. Wang et al. [4] reported similar findings in channel catfish, where organic Se sources required lower dietary levels and showed higher bioavailability than inorganic selenite. Comparable results were obtained in hybrid striped bass [6] and yellowtail kingfish [25]. Bell et al. [28] also concluded that Atlantic salmon had higher absorption and utilization of selenomethionine compared to sodium selenite, consistent with our findings.

3.2 Effects of Selenium Source and Level on Liver and Serum Antioxidant Indices

In this study, Se level significantly affected liver and serum GSH-Px and GR activities. GSH-Px activity increased initially and then stabilized with rising Se levels, while GR activity decreased initially and then stabilized. These re-

sults demonstrate that dietary Se supplementation can enhance GSH-Px activity while reducing GR activity in juvenile cobia. Previous studies have reported that Se deficiency decreased GSH-Px activity in channel catfish [4] and yellowtail kingfish [25], whereas GSH-Px activity in cobia [7] and malabar grouper [18] increased with dietary Se level. Zhu et al. [22] observed that liver GSH-Px activity in largemouth bass increased with Se level while GR activity decreased, stabilizing at dietary Se levels above 1.85 mg/kg. Ashouri et al. [13] reported that nano-Se significantly increased GSH-Px activity in common carp. In Chinese mitten crab, serum and hepatopancreas GSH-Px activities and serum GR activity increased initially and then decreased with dietary Se level, while hepatopancreas GR activity increased initially and then stabilized [19]. Organic Se supplementation in diets containing different lupin protein levels increased plasma GSH-Px activity in barramundi (*Lates calcarifer*) [29]. In Japanese seabass, liver and serum GSH-Px activities increased with Se level at 0–0.4 mg/kg but decreased markedly at 0.8–1.0 mg/kg [30], consistent with our results.

Organic Se sources have been shown to more effectively increase GSH-Px activity in channel catfish [4] and common carp [31]. In this study, at 0.9 mg/kg Se supplementation, selenomethionine groups showed higher liver and serum GSH-Px activities than sodium selenite groups. However, Cotter et al. [20] found that in hybrid striped bass, sodium selenite produced higher liver GSH-Px activity than selenium yeast at 0.4 mg/kg Se. Bell et al. [28] reported higher serum GSH-Px activity in Atlantic salmon fed sodium selenite or selenocystine compared to selenomethionine. Other studies found no direct relationship between Se source and GSH-Px activity in erythrocytes of yellowtail kingfish [25] or liver of rainbow trout [32]. These discrepancies may arise from different absorption and utilization pathways of Se sources among fish species, and the metabolic fate of Se in different tissues requires further investigation.

MDA, a lipid peroxidation product, is commonly used as an oxidative stress indicator [33]. Zhu et al. [22] found no significant effect of dietary Se level on liver MDA content in largemouth bass, attributing this to high levels of dietary antioxidants (vitamin E: 400 mg/kg, vitamin C: 1,000 mg/kg) that may have masked Se' s antioxidant effects. In this study, the interaction between Se level and source significantly affected liver MDA content but not serum MDA content. Liver MDA content in both Se source groups decreased initially and then increased with dietary Se level. This may be related to the relatively low antioxidant levels in our experimental diets (vitamin E: 120 mg/kg, vitamin C: 350 mg/kg). At high dietary Se levels, the amount may have exceeded the fish' s requirement and exerted toxic effects, causing oxidative metabolism disorders, decreased T-SOD and GSH-Px activities, increased free radical production, and elevated MDA content. This mirrors the relationship between Se level and biological effects in human nutrition, where appropriate Se levels effectively scavenge reactive free radicals, while excessive levels can catalyze their formation [34]. Similar trends of initial decrease followed by increase in MDA content with dietary Se level have been reported in Chinese mitten crab [19] and Japanese

seabass [21], whereas serum MDA content in Pacific white shrimp decreased progressively with Se level [26].

GSH-Px, T-SOD, and CAT work synergistically in fish to scavenge superoxide anions, hydroxyl radicals, and hydrogen peroxide, reducing lipid peroxidation damage. In this study, liver CAT activity increased progressively with Se level, with the Se-S-1.65 and Se-Met-1.69 groups showing higher activities than other groups within each source. Liver T-SOD activity increased initially and then decreased with Se level, peaking in the Se-S-1.26 and Se-Met-1.33 groups, indicating that dietary Se supplementation can enhance CAT and T-SOD activities in juvenile cobia. Monteiro et al. [35] reported that dietary Se supplementation increased liver CAT and T-SOD activities in matrinxã (*Brycon cephalus*) to resist oxidative stress from methyl parathion exposure, with reduced activities observed in unsupplemented fish. In gibel carp, serum T-SOD activity peaked at 1 mg/kg Se and decreased significantly at 5 mg/kg, while CAT activity showed a declining trend without significant differences [10]. In Japanese seabass, serum and liver SOD activities increased initially and then decreased with dietary Se level [21]. Dietary Se supplementation also increased serum CAT and T-SOD activities in genetically improved farmed tilapia (*Oreochromis niloticus*) [36].

3.3 Effects of Selenium Source and Level on Tissue Selenium Content

Selenium can accumulate in organisms; tissue Se is mobilized when intake is insufficient and accumulates in liver and kidney when intake is excessive, potentially causing toxicity. Han et al. [10] reported that liver and whole-body Se content in gibel carp increased progressively with dietary Se level, with muscle showing stronger Se deposition capacity than liver. Lin et al. [18] found that whole-body Se content in malabar grouper increased with dietary Se level, making it an excellent indicator of Se bioavailability. Previous research on cobia showed that vertebrae and whole-body Se content increased with dietary Se level, stabilizing at 0.793 and 0.811 mg/kg Se, respectively [7]. In this study, vertebrae and whole-body Se content increased with dietary Se level, indicating that Se supplementation promotes Se accumulation in these tissues. Similar patterns of initial increase followed by stabilization have been reported for the peanut worm (*Sipunculus nudus*) [37] and large yellow croaker [12], while continuous increases have been observed in Chinese mitten crab [19] and Japanese seabass [21]. In yellowtail kingfish, liver and muscle Se content increased markedly with dietary Se level, suggesting these tissues as effective indicators of dietary Se status [38].

In this study, Se source and level differentially affected liver Se content in juvenile cobia. Whole-body and vertebrae Se content were higher in selenomethionine groups than in sodium selenite groups, while at 0.6 mg/kg Se supplementation, liver Se content was higher in the sodium selenite group. Lorentzen et al. [3] reported that whole-body, liver, and muscle Se content in Atlantic salmon increased with dietary Se level, with higher whole-body Se in selenomethionine-

fed fish, but higher liver Se in sodium selenite-fed fish at 2 mg/kg Se, suggesting different metabolic pathways for the two sources. Rider et al. [5] found that rainbow trout fed organic Se had lower pyloric ceca Se content but higher whole-body, muscle, and operculum Se content compared to those fed inorganic Se. The higher bioavailability of organic Se may be related to its metabolic route—while sodium selenite follows the conventional Se metabolic pathway, selenium yeast and selenomethionine may follow methionine metabolic pathways [39], allowing selenomethionine to be specifically incorporated into soft tissue proteins and thereby increasing soft tissue Se content [40].

Key findings: 1. Quadratic regression analysis indicated that maximal SGR was achieved at dietary Se levels of 1.46 mg/kg and 1.29 mg/kg for sodium selenite and selenomethionine, respectively. 2. Based on SGR, the biological utilization of selenomethionine was 1.20 times that of sodium selenite; based on whole-body Se content, it was 2.90 times higher.

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