

Dietary organic selenium supplementation improves growth performance and immune response in hybrid groupers (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*)

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Abstract. Fish that have unmet nutritional needs or are unable to use the ingredients and energy in their feed may grow slowly and have a low survival rate (SR). Selenium (SE) is an essential micronutrient that can be added to feed ingredients, directly or indirectly supporting the growth and health of the organism. The purpose of this study was to assess how adding organic SE to hybrid grouper feed could enhance the fry's development, SR, and resistance to *Vibrio alginolyticus* infection. The treatments used in this study were organic SE doses: A: 0.4 g kg⁻¹ feed, B: 0.7 g kg⁻¹ feed, C: 1.0 g kg⁻¹ feed, and D: 0 g kg⁻¹ feed. The results demonstrated that hybrid grouper seeds supplemented with organic Se at a dose of 1.0 g kg⁻¹ feed exhibited the best growth performance (absolute weight, absolute length, and survival rate) and enhanced the fish's immune response to *V. alginolyticus* infection, allowing the fish to survive infection. The study's results suggest that adding 1.0 g kg⁻¹ of organic SE to feed can boost the productivity of hybrid grouper fish farming.

Keywords: selenium, hybrid grouper, seeds, growth, immunity.

Introduction. Selenium (SE) is one of the immunostimulant substances that belongs to the group of essential trace minerals and is known as an important trace mineral needed by all types of animals, including humans (Clyburn 2002), plays a crucial role in the normal functioning of the immune system, stimulating the normal response of immune cells and helping the host body resist viral attacks (Smith et al 2003; Sang 2010; Sritunyalucksanaa et al 2011). SE can also be added to fish and shrimp feed as an immunostimulant agent (Chiu et al 2010). Supplementation at a dose of 0.84 mg kg⁻¹ of feed can improve the survival and growth of whiteleg shrimp (*Litopenaeus vannamei* Boone, 1931) reared in low salinity, as well as enhance the antioxidant capacity of the hepatopancreas (Yu et al 2023). SE is also an essential element that plays a role in various physiological processes and is crucial for maintaining a strong endogenous antioxidant system (McLaughlin & Gunderson 2022). This statement is supported by Li et al (2023), who found that it helps increase the body's antioxidant capacity, reduce oxidative stress, and strengthen the immune system in fish.

Araujo et al (2021) report that Se-Nano is an efficient source for providing SE in the feed of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fry. These selenite chitosan nanoparticles can have a wide range of applications when used in various commercial fish production. Atencio et al (2009) stated that the optimum SE requirement for *O. niloticus* reaches 0.6 mg kg⁻¹ of feed. Furthermore, Lee et al (2016) showed that the optimal dosage content in feed is between 1.06-2.06 mg Se kg⁻¹ feed for juvenile *O. niloticus*. Meanwhile, the toxicity level in feed ranges from 6.31-14.7 mg SE kg⁻¹ feed for juvenile *O. niloticus*, which is characterized by a decrease in growth performance and non-specific enzymatic activity using Se-Methionine as the SE source. SE is beneficial for the growth of *O. niloticus*

at low levels and toxic at high levels. Its immunostimulation may become very weak after long-term feeding of SE-enriched diets (Chen et al 2020).

Halver & Hardy (2003) stated that the SE requirement for Channel catfish is 0.2 mg kg⁻¹ feed. Research by Thangarani et al (2025) showed that SE supplementation in feed can reduce oxidative stress by neutralizing free radicals and reducing lipid oxidation, increasing liver gene expression, and significantly increasing muscle protein content. All of these data support the growth and defense of the catfish *P. pangasius*. Supplementation of organic yeast enriched with SE 1.00-4.00 mg kg⁻¹ feed improves growth performance, increases muscle deposition, increases hepatopancreatic digestive enzyme activity, and strengthens antioxidant and immune capacity in freshwater crayfish, *Cherax quadricarinatus* (Han et al 2025). Administration of selenium-nanoparticles (SNPs) at 1.5 mg/kg feed to freshwater fish *Catla catla* showed superior nutrient digestibility (CP, 76%; CF, 79% and GE, 74%), in addition it also showed increased mineral absorption (K, P, Ca, Na, Zn, Cu and Fe) compared to other doses (Ahmad et al 2024).

One intriguing finding in this regard is that organic SE seems to have a greater capacity to enhance immunological status than inorganic SE. Following nine weeks of feeding sodium selenite, Se-Met, and Se-yeast (0.02, 0.06, 0.20, and 0.40 mg SE kg⁻¹) to channel catfish, the antibody titer levels were Se-Yeast > Se-Met > Na₂SeO₃ following *Edwardsiella ictaluri* administration, and the antibody production levels rose in tandem with the SE levels in the feed (Wang et al 1997). Research on adding it to grouper fish diet has been done, and the results indicate that the optimal SE requirement for juvenile Malabar grouper *Epinephelus malabaricus* is 0.7 mg. kg⁻¹ of feed (Lin & Shiau 2005). High Cu consumption causes oxidative stress in Malabar grouper and reduces their immune response; supplementation of high SE in the feed (2× the optimal dose) reduces oxidative stress and enhances the immune response of the fish (Lin & Shiau 2007).

Organic and inorganic sources of SE are used differently. In addition to metallic and organic forms like selenomethionine, selenocysteine, and selenocystine in nature, it can exist in organic forms like selenite, selenate, and selenide. Compared to inorganic SE forms, which are likewise more promising for creating Se-enriched fish for human consumption, organic SE forms have higher bioavailability, are better at enhancing fish immunological status, and are comparatively less hazardous. Inorganic SE primarily affects hepatic GPx function and is stored in the liver for subsequent selenoprotein production. With greater bioavailability yet less toxicity than Se-Met, selenite, and selenate, nano-SE, as a novel SE source, exhibits a bright future in fish. However, further research is needed on its toxicity, distribution pattern, and metabolic processes. However, when dietary SE levels exceed what some fish species require, both organic and inorganic SE sources are harmful to fish (Wang et al 2022). Compared to organic sources, inorganic SE is maintained in muscle tissue at a significantly lower concentration (Mahan & Parrett 1996). According to reports, organic SE is more bioavailable than inorganic for Channel catfish (Wang & Lovell 1997) and Atlantic salmon (Bell & Cowey 1989). There is a published study on how stocking density and SE source interact in Rainbow trout. One of the best and most practical ways to lessen the impact of environmental stressors on fish growth is through dietary modifications, which have been used in the past. The distribution of SE in various fish tissues is also significantly influenced by the source of the supplement (organic or inorganic). While inorganic SE must be used for selenoprotein synthesis in the liver from a body pool and then transported to various organs and tissues, organic SE is more quickly stored in skeletal muscle through nonspecific pathways as methionine (Prabhu et al 2020; Berntssen et al 2018; Wischhusen et al 2019).

The purpose of this study was to investigate the impact of adding organic SE to hybrid grouper feed on the development, survival, and resistance of grouper fry to *Vibrio alginolyticus* infection during the nursery phase of hybrid grouper fry, based on prior research data and reports. In order to enhance the growth performance, survival rate, and resistance of grouper fry to *V. alginolyticus* infection, this study sought to discover the optimal recommended dosage of organic SE added to the hybrid grouper diet.

Material and Method

Feed preparation. The organic SE used is technical organic SE (Sel-Plex®), which contains 1,000 ppm of organic Se. Therefore, the organic SE dosage is in g kg⁻¹ of feed. Organic SE is added to commercial feed using a coating technique, utilizing one chicken egg white and adding PBS solution to a total of 100 mL for every kg of feed. The manufactured feed is then air-dried at room temperature for 3 hours, then placed in plastic feed bags. The feed can be given immediately or stored at a room temperature of around 16°C.

Feed treatment testing. The fish used in this study were hybrid grouper fry with an average total length of (3.510±0.155) cm, an average weight of (0.566±0.759) g, and a stocking density of 200 fish/tank. The hybrid grouper fry were reared in a 1 x 1 x 1 m tank and equipped with aeration as an oxygen source.

The treatments given were organic SE doses, namely A: 0.4 g kg⁻¹ feed; B: 0.7 g kg⁻¹ feed; C: 1.0 g kg⁻¹ feed; and D: 0 g kg⁻¹ feed, with 3 replications for each treatment. The artificial feed enriched with organic SE was given to the hybrid grouper fry *ad libitum* (until satiation), with a frequency of 4 times a day (at 07:00, 11:00, 13:00, and 16:00 h). The maintenance of hybrid grouper fry was carried out for 40 days. To maintain the suitability of the maintenance media, siphoning of feces and 100% water changes were carried out twice a day (morning and evening).

The observations conducted include: growth (absolute length and absolute weight) and survival rate (SR) at the end of the study, which are each calculated using the formula by Effendie (2002):

Absolute length growth using the formula, as follows:

$$L = L_t - L_0$$

Where:

L = Absolute length (cm)

L_t = Fish length at the end of the observation (cm)

L₀ = Fish length at the beginning of the observation (cm)

Absolute weight growth using the formula, as follows:

$$\Delta W = W_t - W_0$$

Where:

ΔW = Absolute weight (g)

W_t = The weight at the end of the observation (g)

W₀ = The weight at the beginning of the observation (g)

Survival rate (SR) is calculated using the formula, as follows:

$$SR = \frac{N_t}{N_0} \times 100 \%$$

Where:

SR = Survival rate (%)

N_t = Number of live fish at the beginning of the observation

N₀ = Number of live fish at the end of the observation

Meanwhile, the water quality of the rearing media is observed periodically every 3 days.

Challenge test. After administering organic SE for 40 days through feed, the hybrid grouper fish were challenged with *V. alginolyticus* at a concentration of 10⁷ CFU mL⁻¹, 0.1 mL fish⁻¹, by injection, to determine the fish's immune response. The fish were transferred to the Fish Health and Environmental Laboratory with two replications for each treatment. Each experimental container (100L plastic container) was filled with 10 fish. During maintenance, the fish were fed according to the treatment *at satiation* with a frequency of 3 times a day (morning, afternoon, and evening). For 5 days, the fish were acclimatized to allow them to adapt to their new environment. Then, on the 6th day, the fish were infected

intramuscularly (IM). Furthermore, clinical symptoms and mortality were observed for 14 days. The observations conducted included challenge test survival, relative percent survival (RPS), and hematocrit.

RPS is a method for measuring the effectiveness of a treatment or intervention on fish, especially in the context of aquaculture, or the percentage survival rate, which is a value indicating the effectiveness of a treatment against the tested sample. RPS is calculated based on the comparison between the SR of treated fish and the SR of control fish (without treatment), using the formula (Amend 1981):

$$\text{RPS} = [1 - (\% \text{ Mortality in vaccinated group} / \% \text{ mortality in control group})] \times 100$$

Blood was drawn from the fish's tail area for the hematocrit calculation after the syringe had been soaked with 10% EDTA. The collected blood was then placed in a 1.5 mL microtube. The hematocrit capillaries are filled with blood to the volume limit and sealed with the available covers. They are then centrifuged using a hematocrit centrifuge (fixed at 12,000 rpm) for 5 minutes. The length of the erythrocyte sediment in the hematocrit capillaries is measured using a hematocrit reader.

Statistical analysis. Each treatment was replicated three times, and the average of all data across all parameters was used for statistical evaluation. Analysis of Variance (one-way ANOVA) was conducted to assess the parameters with a 95% confidence interval. Duncan's test was performed to determine the significance of growth (weight and length), survival rate, and other parameters using SPSS® software version 7.0. The results are presented as mean ± standard deviation of the mean.

Results. Growth is the result of strong regulation between factors present in the environment, such as feed availability, temperature, and photoperiod, which affect the regulation or metabolism in the bodies of hybrid grouper fry after feeding with organic SE, as shown in Table 1.

Table 1

Growth of hybrid grouper fish with organic selenium at different doses

Treatment	Growth parameters			
	Final weight (g)	Final length (cm)	Absolute weight (g)	Absolute length (cm)
A	20.418±2.716	10.54±0.505	19.852±1.957	7.03±0.350
B	19.266±3.233	10.27±0.542	18.700±2.474	6.76±0.387
C	24.113±3.545	10.92±0.590	23.547±2.786	7.41±0.435
D	17.485±1.679	10.04±0.565	16.919±0.920	6.53±0.410

Note: A: 0.4 g kg⁻¹ feed, B: 0.7 g kg⁻¹ feed, C: 1.0 g kg⁻¹ feed, D: 0 g kg⁻¹ feed.

Based on Table 1, the hybrid grouper seeds treated with organic SE at a dosage of 1.0 g kg⁻¹ of feed (treatment C) showed the highest absolute weight value (23.547±2.786 g) and was significantly different (95% confidence level) from treatment A (19.852±1.957 g), B (18.700±2.474 g), and control D (16.919±0.920 g). Likewise, the highest absolute length growth results were obtained by administering organic SE at a dose of 1.0 g kg⁻¹ feed (7.41±0.435 cm), significantly different (95% confidence level) from treatment A (7.03±0.350 cm), B (6.76±0.387 cm), and D / control (6.53±0.410 cm). This indicates that the use of organic SE through feed affects the growth of hybrid grouper seeds maintained for 40 days.

The daily weight growth rate of hybrid grouper fish during 40 days of maintenance, fed with organic SE supplementation at different doses, is presented in Figure 1. In contrast to treatments A, B, and D, which had nearly identical values, treatment C demonstrated the best growth rate.

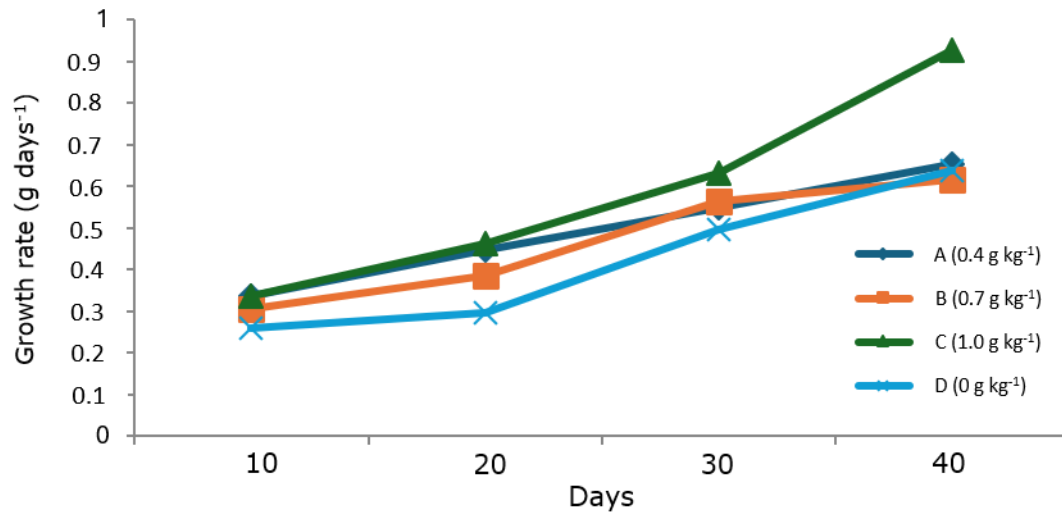


Figure 1. Daily weight growth rate of hybrid grouper.

Survival rate (SR). Comparison of the SR of hybrid grouper seeds with organic SE supplementation and the control is shown in Figure 2. The SR of hybrid grouper seeds with organic SE supplementation showed a higher SR compared to the control (87.33%).

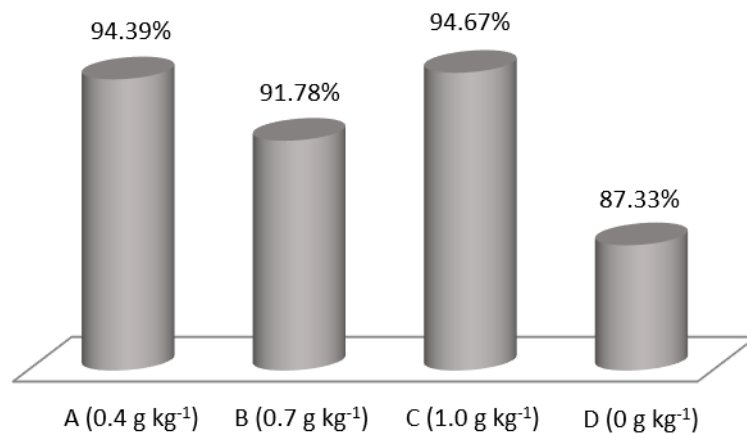


Figure 2. Survival rate of hybrid grouper seeds with organic selenium supplementation and control.



Figure 3. Clinical symptoms observed in the dead fish (hemorrhage at the anus and wounds on the abdominal fin).

Challenge test. On the 7th day post-infection, clinical symptoms such as decreased appetite, bleeding/inflammation in the anus, and wounds on the abdominal fins (Figure 3) began to appear. Hemorrhage or bleeding from the anus indicates that the fish is infected with *V. alginolyticus*, which signifies inflammation/bleeding in the fish's digestive organs.

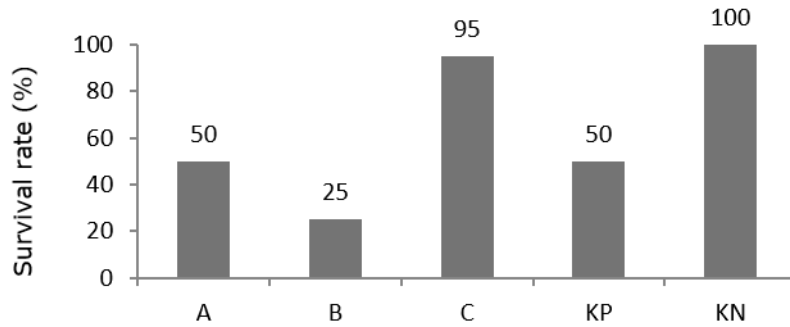


Figure 4. Survival Rate of hybrid grouper after challenge test (Note: A: 0,4 g kg⁻¹, B: 0,7 g kg⁻¹, C: 1,0 g kg⁻¹, KP: positive control, KN: negative control).

Figure 4 showed that the addition of organic SE at a dose of 1.0 g kg⁻¹ feed (C) can enhance the immune response of hybrid grouper to *V. alginolyticus* infection, allowing the fish to survive post-infection (on day 14) with a SR of 95±5% and significantly different (95% confidence level) compared to other treatments, namely treatment A (50±10%) and treatment B (25±5%), as well as the positive control (KP) with an SR of (50±10%) and the negative control (KN) with an SR of (100±0%) due to very favorable environmental conditions.

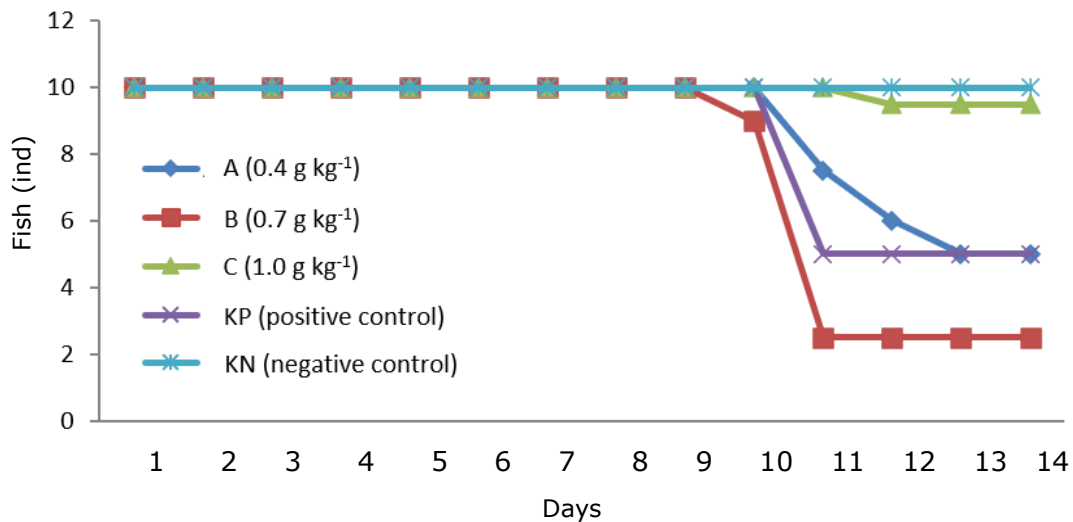


Figure 5. The average number of fish that survived after the challenge test.

Based on Figure 5, it can be seen that fish mortality began to appear on the 10th day after being infected with *V. alginolyticus*, specifically in treatment B. Mortality occurred between the 10th and 13th days post-challenge.

Table 2
Results of hematocrit measurement in the challenge test of organic SE addition in hybrid grouper

Treatments	Before challenge test (%)	Day 7 th after challenge test (%)	Day 14 th after challenge test (%)
A	23.5±2.12	23.5±2.12	30±1.41
B	25±1.41	28±11.31	26±5.66
C	25.5±12.02	22.25±6.72	27±0.00
KP	30±5.66	16.5±0.71	24±0.00
KN	28.5±0.71	15.5±3.54	22.5±3.54

Note: A: 0.4 g kg⁻¹, B: 0.7 g kg⁻¹, C: 1.0 g kg⁻¹, KP: positive control, KN: negative control.

On the 7th day after infection with *V. alginolyticus*, fish treated with organic SE showed higher hematocrit values compared to the control groups. Specifically, hematocrit values were A: $23.5 \pm 2.12\%$, B: $28.0 \pm 11.31\%$, and C: $22.25 \pm 6.72\%$, while the KP and KN recorded $16.5 \pm 0.71\%$ and $15.5 \pm 3.54\%$, respectively (Table 2). By day 14 after the challenge, hematocrit values remained higher in the SE-treated groups (A: $30.00 \pm 1.41\%$, B: $26.00 \pm 5.66\%$, C: $27.00 \pm 0.00\%$) compared to the controls (KP: $24.00 \pm 0.00\%$, KN: $22.50 \pm 3.54\%$). These results indicate that the addition of organic SE can effectively increase red blood cell levels in hybrid grouper following bacterial infection.

Mortality rate and RPS was calculated to assess the treatment's efficacy; the results are shown in Table 3. Specifically, mortality rate were A: 50%, B: 75%, C: 5%, and D: 50%; and RPS values were A: 0% (ineffective), B: -50% (ineffective), C: 90% (effective), and D: -. It shows that treatment C is effective for application in the rearing of hybrid grouper fish.

Table 3

RPS calculation for organic SE supplementation in the feed of hybrid grouper

Treatment	Mortality rate (%)	RPS (%)	Information
A	50	0	Ineffective
B	75	-50	Ineffective
C	5	90	Effective
D	50	-	-

Note: A: 0.4 g kg⁻¹ feed, B: 0.7 g kg⁻¹ feed, C: 1.0 g kg⁻¹ feed, D: 0 g kg⁻¹ feed, RPS: Relative percent survival.

Water quality of media. During the maintenance of hybrid grouper seeds, water quality measurements are taken every 10 days (Table 4). The water quality parameters in this research on the maintenance of hybrid grouper seeds using organic SE are still within normal limits and continue to support the life of the maintained hybrid grouper seeds.

Table 4

Water quality of hybrid grouper pond

Parameters	Treatments			
	A	B	C	D
pH	7.65-8.22	7.58-8.23	7.61-8.22	7.34-8.23
Salinity (ppt)	32-33	32-33	32-33	32-33
Nitrite (mg L ⁻¹)	< 0.001	< 0.001	< 0.001	< 0.001
TAN (mg L ⁻¹)	0.003-0.046	0.003-0.045	0.003-0.051	0.016-0.042
Temperature (°C)	30-31	30-31	30-31	30-31
DO (mg. L ⁻¹)	5.3-6.1	5.7-6.4	5.7-6.1	5.5-6.3

Note: TAN: Total ammonia nitrate, DO: Dissolved oxygen, A: 0.4 g kg⁻¹ feed, B: 0.7 g kg⁻¹ feed, C: 1.0 g kg⁻¹ feed, D: 0 g kg⁻¹ feed.

Discussion. Growth is a crucial factor in the development of aquatic organisms, as measured by weight gain and length gain. Research shows that supplementing organic SE in feed significantly impacts daily weight gain rates. Energy metabolism in fish relies heavily on Se-driven enzymatic processes. Se, through its role in activating various antioxidant enzymes and regulating thyroid metabolism, can improve the efficiency of energy use in fish, which is crucial in aquaculture to maximize fish growth and health. This is because SE is an essential trace element required in feed for growth and physiological function. SE functions as a component of several protein-containing enzymes, such as the iodothyronine deiodinase (ID) and glutathione peroxidase (GPx) groups (Dhingra & Bansal 2006). ID is a selenoprotein (Se-containing enzyme) that functions as a catalyst for the formation of the hormone triiodothyronine (T3) from the hormone thyroxine (T4) (Brown & Arthur 2001), which plays a role in nutrient metabolism in the body. One of SE's roles is maintaining thyroid gland function. According to McKenzie et al (2002), SE's biological roles and the control of selenoproteins are responsible for its growth-promoting effect. Furthermore, the improved feed utilization characteristics, which are often consistent with the growth performance index, are closely linked to the improvement in fish growth performance

brought about by SE supplementation. The capacity of SE to enhance intestinal antioxidative status from oxidative stress and preserve intestinal integrity, which is advantageous for fish nutritional assimilation, may be linked to its improved feed utilization. Reduced intestinal oxidative stress is associated with maintaining intestinal integrity, which includes improvements in intestinal surface area, brush border enzyme secretion, decrease in enterocyte cell death, and/or enterocyte turnover rates (Jessica et al 2009; Nugroho & Fotedar 2015).

According to Grag (2007), the thyroid gland functions to increase metabolic rate and is very important for normal growth and development. SE also plays a role in the GPx enzyme, which can protect cells from oxidative damage (Tawwab et al 2007). This condition is also supported by the survival rate of hybrid grouper seeds with organic SE supplementation, showing a higher SR (average > 91%) compared to the control (87.33%). Determining the dosage of SE added to feed is crucial. A study by Sumana et al (2023) found that SE is essential for fish development, antibody production, reproduction, and enzymatic function. Therefore, excessive SE intake can cause deformities and other abnormalities, but it is essential for daily biological functions. SE is a nutrient that fish require. For many species, such as grouper (Lin & Shiau 2005) and Yellowtail kingfish (Le & Fotedar 2013), the necessity and nutritional requirements for growth have been proven. Numerous earlier studies and reports that support the results of this study on SE supplementation have involved various fish species, including hybrid striped bass, *Morone chrysops* × *Morone saxatilis* (Cotter et al 2008), *O. mykiss* (Hunt et al 2011), gilthead bream *Sparus aurata* (Saleh et al 2014), and Wuchang bream (*Megalobrama amblycephala*; Long et al 2017).

The addition of SE at a dose of 1.0 g kg⁻¹ of feed increases the immune response of hybrid grouper fish to *V. alginolyticus* infection so that the fish can survive post-infection with SR 95%. SE has been shown to increase fish resistance to *V. alginolyticus* bacterial infection, which is the main cause of hybrid grouper disease in this study. SE supplementation in fish feed can reduce mortality rates and accelerate post-infection recovery, as evidenced by hybrid grouper fish fed organic SE having a higher survival rate than controls. Sumana et al (2023) stated that SE increases the production of pro-inflammatory cytokines and other mediators needed to fight bacterial infections. Under infectious conditions, cytokines such as interleukin (IL) and tumor necrosis factor (TNF) play an important role in initiating and regulating the immune response. SE improves the production of these cytokines, which contribute to the healing process and increase the body's resistance to infection. In fish experiencing stressful conditions, whether due to the environment or infection, there is an increase in the production of free radicals that can damage body cells. SE helps protect fish cells from this oxidative damage, which can disrupt normal metabolism and slow growth (Thangarani et al 2025). According to Lin & Shiau (2005), oxidative stress was decreased by dietary organic SE supplementation. By reducing oxidative stress levels, SE supports fish survival, accelerates recovery from stress, and optimizes disrupted metabolic processes.

RPS is a value that indicates the effectiveness of a treatment against the test being tried. A value of more than 50% indicates that the treatment being tried is quite effective, while a value of less than 50% indicates that the treatment being tried is not effective enough to provide a positive response. In this study, the RPS value given by adding SE in treatment C was 90%, treatment A 0%, and treatment B was 100%. This shows that a dose of SE administration of 1.0 mg kg⁻¹ can be used to increase the immune response of hybrid grouper fish against *Vibrio alginolyticus* infection. While for treatment B, the RPS and SR values were lower than the positive control, this is likely due to the condition of the fish that have not yet adapted to the environment.

The hematocrit test results on the 14th day after being infected with *V. alginolyticus* showed that fish with the addition of organic SE had higher hematocrit values: A (30.00±1.41%), B (26.00±5.66%), and C (27.00±0.00%) compared to fish that did not use organic SE, KP (24.00±0.00%), and KN (22.50±3.54%). The volume of red blood in the blood of fish can describe the health of the fish. Fish that experience anemia have a hematocrit percentage as low as 10%. The color of the blood plasma can also describe the health of the fish. A yellowish color (straw) indicates the presence of bilirubin, an

abnormality of the liver. A whitish color indicates a high fat content, while a reddish color indicates hemolysis. Various types of SE have been demonstrated to enhance hematological indicators in a variety of fish species. For example, African catfish showed improvements in RBC counts, hemoglobin (Hb), and Hct% values (Abdel-Tawwab et al 2007), Nile tilapia showed increased red blood cell (RBC) counts (NeamatAllah et al 2019), and hybrid tilapia showed increased hematocrit percentages (Hct%) (ElHammady et al 2007).

Water quality parameters in this study, such as temperature, salinity, pH, and dissolved oxygen, appeared to be within normal limits and supported the growth of the hybrid grouper seeds. Meanwhile, the total ammonia content in the rearing medium remained within normal limits for all four treatments and supported the growth of the hybrid grouper fry. Ammonia content in the rearing medium is a result of fish metabolism, organic compound decomposition, and bacterial activity. Ammonia is the primary product of protein breakdown and is toxic to fish; therefore, the recommended ammonia content is no more than 1 mg L⁻¹ (Pescod 1974).

Overall, SE plays a crucial role in regulating fish metabolism, particularly through its influence on enzymatic activity involved in energy metabolism, thyroid hormone regulation, and protection against oxidative damage. SE supports the body's energy efficiency, increases basal metabolic rate, and maximizes the conversion of food into energy, which is crucial for optimal fish growth. Therefore, SE supplementation in fish feed can increase feed efficiency, improve fish health, and support fish resistance to stress and infection. By enhancing fish metabolism through SE, we can improve the productivity and sustainability of aquaculture.

Conclusions. This study demonstrated that dietary supplementation with organic selenium improved the growth performance, survival, and immune response of hybrid grouper juveniles. Among the tested treatments, the diet containing 1.0 g kg⁻¹ organic selenium produced the highest growth and survival rates and enhanced resistance to *Vibrio alginolyticus* infection. These findings suggest that supplementation with 1.0 g kg⁻¹ organic selenium may be an effective strategy for improving hybrid grouper production. However, further studies are needed to evaluate the long-term effects and establish the maximum safe and non-toxic dietary level of organic selenium for this species.

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Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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