

Effects of dietary organic selenium supplementation on growth, nutrient retention, and health indicators of climbing perch (*Anabas testudineus*)

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ABSTRACT

Selenium is an essential mineral that, although needed in small amounts, plays a major role in supporting the body's antioxidant system and immune function of fish. This study aims to evaluate the effect of adding organic selenium to feed on growth performance, protein retention, fat retention, blood profile, and antioxidants of climbing perch. This study used a completely randomized design with 4 treatments and 3 replications. The treatments were four doses of organic selenium added to the feed, namely 1, 2, 3, and 4 g Se/kg feed. The climbing perch used has a weight ranging from 3.2–4.4 g and a length ranging from 4.2–5.8 cm. The fish were kept in an aquarium measuring 40×30×30 cm³ with a density of 10 fish/aquarium. The fish were kept for 45 days with a feeding frequency of 2 times a day as much as 5% of the total weight of the fish. The results of this study indicate that climbing perch fed with feed containing 1 g Se/kg feed showed growth performance, and could improve hematological parameters and strengthen the antioxidant system of climbing perch.

Keywords: climbing perch, organic selenium, growth, antioxidant, hematology.

INTRODUCTION

Freshwater fish such as climbing perch are widely favored across Asia, including Thailand, Malaysia, Vietnam, India, Pakistan, the Philippines, and Indonesia, particularly on the islands of Sumatra and Kalimantan. In South Kalimantan, climbing perch aquaculture has recently gained popularity, ranging from small household operations to large-scale enterprises. This species is considered a valuable aquaculture commodity due to its high market price (up to IDR 80,000/kg), resilience to suboptimal water conditions, and ease of cultivation, making it highly attractive for farmers and investors (Akbar, 2018; Akbar, 2021; Sofia et al., 2023; Hanafie et al., 2024).

Despite these advantages, one of the major challenges in aquaculture is ensuring the availability of nutritionally balanced feed to support

optimal growth and health performance. The nutritional requirements of fish vary according to species, age, physiological state, and environmental conditions (Akbar, 2021). Like other cultured species, climbing perch requires not only macronutrients such as protein, fat, and carbohydrates but also essential micronutrients, including vitamins and minerals, which serve as metabolic cofactors. Among these, selenium has been identified as a critical element for fish growth, physiological balance, and overall health (Han et al., 2011; Pattipeilohy et al., 2020).

Previous studies have demonstrated that selenium supplementation reduces oxidative stress, supports the immune system, and enhances growth performance in fish (Prabhu et al., 2014). Selenium plays a key role in pancreatic insulin release, which can accelerate metabolism and protein utilization efficiency (Garg, 2007; Hamzah

et al., 2012a; Hamzah et al., 2012b; Handique et al., 2020). Selenium exists in both inorganic and organic forms, with the latter, including selenomethionine and selenocysteine, offering superior bioavailability, lower toxicity, and improved assimilation due to their ability to bind with body proteins (Kucukbay et al., 2009; Hasrah et al., 2016; Zhou et al., 2018; Suprayudi et al., 2023).

Although selenium is essential, its supplementation must be carefully optimized, as both deficiency and excess can negatively impact fish physiology. Deficiency has been associated with impaired peroxide activity, weakened immunity, poor growth, and reduced feed efficiency, whereas toxicity can lead to growth abnormalities and mortality (Hamzah et al., 2012b). A number of studies have reported beneficial effects of selenium in various fish species, including catfish (*Clarias gariepinus*) (Hasrah et al., 2016; Suprayudi et al., 2023), common carp (*Cyprinus carpio* L.) (Gaber, 2008; Saffari et al., 2018; Luo et al., 2020), tilapia (*Oreochromis niloticus*) (Iqbal et al., 2020; Pattipeilohy et al., 2020; Pattipeiluhu et al., 2023), and striped catfish (*Pangasianodon hypophthalmus*) (Kumar et al., 2020; Kumar et al., 2021; El-Sharawy et al., 2021; Yuniati and Setiawati, 2024).

The specific requirement and physiological role of organic selenium in climbing perch remain largely unexplored. While studies on tilapia and catfish have shown that dietary supplementation with organic selenium at levels around 4 g/kg feed improves growth, nutrient retention, and hematological parameters (Suprayudi et al., 2013; Hasrah et al., 2016), no comparable data are available for climbing perch. This gap in knowledge limits the ability to optimize feed formulations for this high-value species.

It was hypothesized that organic selenium, due to its superior bioavailability, would improve protein and fat retention while positively influencing hematological profiles and antioxidant capacity. The findings of this research are expected to provide new insights into the role of organic selenium in freshwater aquaculture and contribute to the development of more efficient feeding strategies for climbing perch.

In this study, the effects of dietary organic selenium supplementation on climbing perch to determine how it influences growth performance, nutrient retention, and key health indicators, including hematological profiles and antioxidant capacity. Specifically, adding organic selenium to

the diet can enhance protein and fat assimilation, improve metabolic efficiency, reduce oxidative stress, and support overall physiological health. The essence of the study lies in evaluating the functional impact of dietary selenium particularly its organic form on both the growth and biochemical status of climbing perch, aiming to provide practical insights for optimizing feed formulations and improving aquaculture productivity for this high-value freshwater species.

The present study aimed to evaluate the effectiveness of dietary organic selenium supplementation in enhancing nutrient retention, blood parameters, and antioxidant responses in climbing perch.

MATERIAL AND METHODS

Research site and duration

This research was conducted for 45 days, from September 8 to October 23, 2024, at the Wet Laboratory, Faculty of Fisheries and Marine Sciences, Lambung Mangkurat University (ULM).

Experimental design

The research used a completely randomized design (CRD) with 4 treatments and 3 replications. Each aquarium served as one experimental unit containing 10 fish. The treatments were as follows:

- Treatment S1 – addition of organic selenium 1 g/kg feed,
- Treatment S2 – addition of organic selenium 2 g/kg feed,
- Treatment S3 – addition of organic selenium 3 g/kg feed,
- Treatment S4 – addition of organic selenium 4 g/kg feed (Figure 1).

Feed formulation and selenium levels

Feed composition

Artificial feed was formulated with the following composition (Table 1, 2):

Feed processing

Feed ingredients were ground into flour, mixed evenly with organic selenium according to treatment, and combined with warm water



Figure 1. Layout of experimental design (12 aquariums arranged randomly)

Table 1. Artificial feed formulation for climbing perch

Raw material	Composition (%)
Fish meal	57
Bran flour	40
Eco enzyme	2
Molasses	1
Total	100

until forming a smooth dough. The dough was pelletized using a grinder, dried, and blended to obtain uniform particle size suited to fish mouth opening (Figure 2).

Experimental fish and rearing procedure

The fish used were climbing perch (*Anabas testudineus*) with an average length of 4.2–5.8 cm and weight of 3.2–4.4 g, obtained from a fish farmer in Mentaos, Banjar Regency, South Kalimantan. Twelve aquariums (40×30× 0 cm³) were filled with 10 L of water, stocked with 10 fish (1 fish/L). Fish were acclimatized before treatment and fed twice daily (08:00 and 17:00 WITA) at 5% of body weight for 45 days (Figure 3).

Table 2. Proximate feed for climbing perch

Treatment	Analysis parameters				
	Water content (%)	Ash content (%)	Protein content (%)	Fat content (%)	Fiber content (%)
S1	10.03	12.01	29.36	6.89	2.53
S2	11.07	11.20	28.47	7.06	2.22
S3	10.97	11.65	28.78	7.22	2.40
S4	8.83	11.90	28.42	7.34	2.65

Note: Animal Nutrition and Feed Science Laboratory, Faculty of Agriculture, ULM.

Sampling and analysis

Measured parameters and calculations:

- Growth performance,
- Protein and fat retention,
- Survival rate (SR),
- Water quality.

Proximate and chemical analysis

Proximate analyses (protein, fat, ash, crude fiber, moisture) were conducted at the Animal Nutrition and Feed Science Laboratory (ULM) using standard methods:

- Protein – Kjeldahl method,
- Fat (dry) – Soxhlet method,
- Fat (wet) – Folch method,
- Ash – furnace at 600 °C,
- Crude fiber – acid–base digestion,
- Moisture – oven at 105–110 °C.

Selenium levels were analyzed using atomic absorption spectrometry (AAS) at the Banjarbaru Industrial Standardization and Service Center.

Antioxidant enzyme analysis

Antioxidant activities in the meat and liver were measured at the Department of Biochemistry and Biomolecular, Faculty of Medicine, ULM, using:

- Glutathione peroxidase (GSH-Px) – Noguchi et al. (1973),
- Catalase (CAT) – Abei (1984),
- Superoxide dismutase (SOD) – McCord and Fridovich (1969),
- Malondialdehyde (MDA) – Buege and Aust (1978).

Hematological analysis

Blood samples were taken using a syringe inserted into the body midline until reaching the vertebral column. Blood was collected into EDTA

(10%) microtubes to prevent coagulation. The parameters analyzed included erythrocyte count, leukocyte count, hemoglobin, and hematocrit (Lestari et al., 2019).

Statistical analysis and experimental workflow

Data were analyzed using Analysis of Variance (ANOVA) and continued with Tukey's test ($\alpha = 0.05$) to determine significant differences among treatments. Data processing was performed using SPSS 17.0 software. Experimental Workflow is shown in Figure 4.

RESULTS AND DISCUSSION

Results

Absolute length growth

Based on Figure 5, the average value of the highest absolute length growth in treatment S1 is 0.54 cm, followed by treatment S3 of 0.47 cm, while treatments S2 and S4 are 0.34 cm and 0.32 cm respectively. The results of the Liliefors normality test for the absolute length growth of climbing perch obtained a Li max value of 0.216 < Li table 5% 0.242, so it can be concluded that the data is normally distributed. The results of the Barlet variance homogeneity test obtained a calculated X^2 value of $1.034 < X^2$ table 5.991 which means homogeneous. The results of the Anova test showed a calculated F value of $1.007 < F$ table 5% 4.066 which means there was no significant difference between treatments.

Absolute weight growth

Based on Figure 6, treatment S1 produced the highest average absolute weight growth value of 2.13 g, followed by treatment S3 of 1.22 g, and treatment S4 of 1.19 g. Meanwhile, S2 recorded the lowest absolute weight gain of 0.88 g. The results of the Liliefors normality test for the absolute weight growth of climbing perch obtained a Li max value of $0.196 < Li$ table 5% 0.242, so it can be concluded that the data is normally distributed. The results of the Barlet variance homogeneity test obtained a calculated X^2 value of $0.713 < X^2$ table 5.991, which means homogeneous. The results of the Anova test showed a calculated



Mixing



Pelleting



Drying

Figure 2. Flowchart or photos of feed preparation steps



Figure 3. Photo or schematic of aquarium

F value of $8.570 < F$ table 5% 4.066, which means a significant difference between treatments.

The results of the Anova analysis of absolute weight growth showed that treatment S1 had a very significant difference with treatment S2, with an average difference of 1.250 which exceeded the 5% DMRT value (0.767) and 1% DMRT

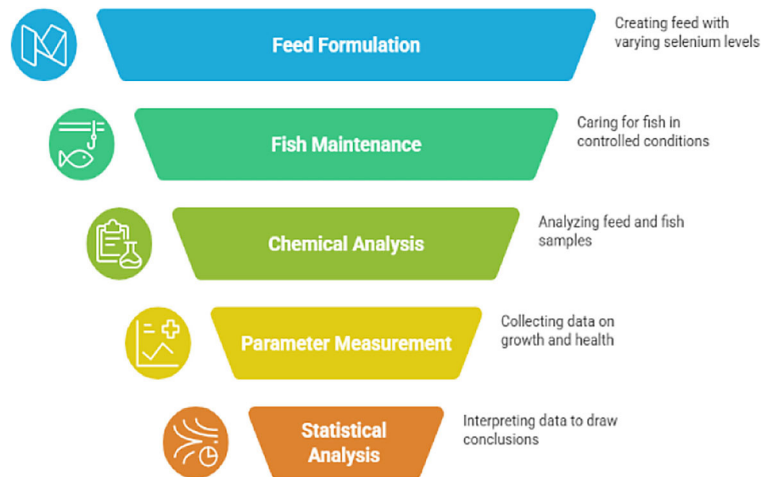


Figure 4. Experimental workflow of organic selenium supplementation in climbing perch

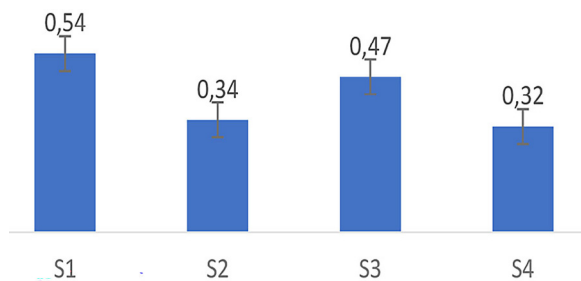


Figure 5. Graph of the average absolute length growth of climbing perch

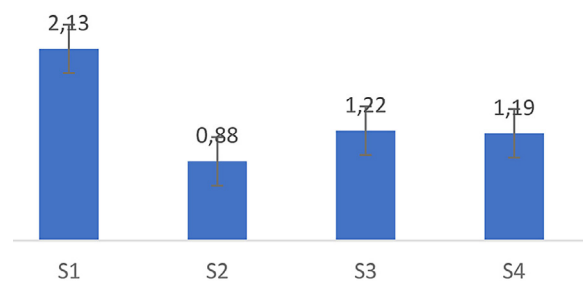


Figure 6. Graph of the average absolute weight growth of climbing perch

(1.171). Treatment S1 also showed a significant difference with treatments S3 and S4, each with an average difference of 0.910 and 0.937, which exceeded the 5% DMRT value (0.787 and 0.755) and 1% DMRT (1.144 and 1.099). There was no significant difference between treatments S2 and S3 or S4, with average differences of 0.340 and 0.313, respectively, which were below the 5% DMRT and 1% DMRT values. Treatments S3 and S4 did not show any significant difference, with an average difference of only 0.027. Treatment S1 gave significantly higher absolute weight growth results compared to other treatments.

Protein retention and fat retention

Protein retention

The results of protein retention and fat retention of climbing perch can be seen in Figure 7. The highest protein retention of climbing perch was in treatment S1 (5.04%), followed by treatment S2 (2.00%), treatment S3 (1.32%), and the lowest in treatment S4 (0.35%). The results of the

Liliefors normality test for protein retention of climbing perch obtained a Li max value of $0.158 < \text{Li table } 5\% \ 0.242$, so it can be concluded that the data is normally distributed. The results of the Barlet variance homogeneity test obtained a calculated X_2 value of $-6.401 < X_2 \text{ table } 5.991$ which means homogeneous. The results of the Anova test showed a calculated F value of $0.998 < F \text{ table } 5\% \ 4.066$ which means there was no significant difference between treatments.

Fat retention

Based on Figure 8, treatments S1 and S2 showed the highest fat retention of 28.16% and 28.19%, while treatments S3 and S4 had lower values of 22.30% and 19.62%. The results of the Liliefors normality test for catfish fat retention obtained a Li max value of $0.231 < \text{Li table } 5\% \ 0.242$, so it can be concluded that the data is normally distributed. The results of the Barlet variance homogeneity test obtained a calculated X_2 value of $-6.453 < X_2 \text{ table } 5.991$ which means homogeneous. The results of the Anova test showed

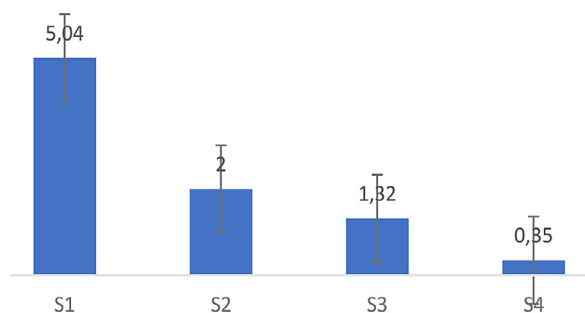


Figure 7. Graph of average protein retention of climbing perch

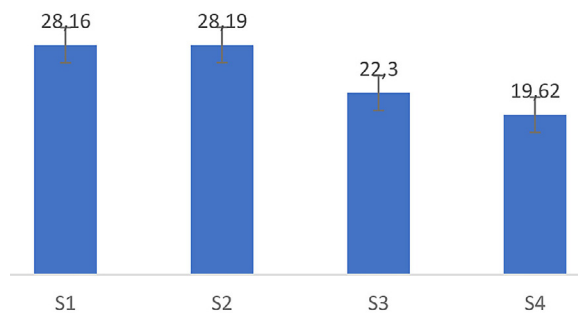


Figure 8. Graph of average fat retention of climbing perch

a calculated F value of $0.586 < F_{\text{table } 5\%} 4.066$ which means there was no significant difference between treatments.

Blood profile

Leukocytes

The average leukocytes of climbing perch after maintenance can be seen in Figure 9. The parameters of leukocyte of climbing perch range from $250\text{--}1.850 \times 10^3 \mu\text{L}$. The highest leukocyte parameter is in treatment S2 with an average value of $1.850 \times 10^3 \mu\text{L}$, followed by treatment S4 with an average value of $1.450 \times 10^3 \mu\text{L}$, then treatment S3 with an average value of $550 \times 10^3 \mu\text{L}$ and the lowest average leukocyte value in treatment S1, which is $250 \times 10^3 \mu\text{L}$. The results of the Liliefors normality test of climbing perch leukocytes obtained a Li max value of $-0.524 < Li_{\text{table } 5\%} 0.242$, so it can be concluded that the data is normally distributed. The results of the Barlett variance homogeneity test obtained a calculated X^2_2 value of $-1.208 < X^2_{\text{table } 5.991}$ which means homogeneous. The results of the Anova test showed a calculated F value of $3.167 < F_{\text{table } 5\%} 4.066$, which means there was no significant difference between treatments.

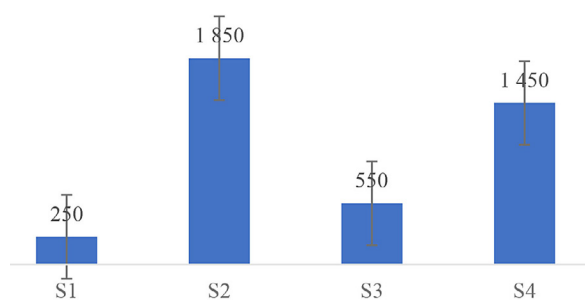


Figure 9. Leukocytes of climbing perch

Erythrocytes

Based on the data in Figure 10, the parameters of erythrocytes of climbing perch range from 1.08 to $2.75 \times 10^6 \mu\text{L}$. The highest average value of erythrocytes of climbing perch was in treatment S1 ($2.75 \times 10^6 \mu\text{L}$), followed by treatment S2 ($1.63 \times 10^6 \mu\text{L}$), treatment S3 ($1.44 \times 10^6 \mu\text{L}$), and the lowest value of erythrocytes was in treatment S4 ($1.08 \times 10^6 \mu\text{L}$). The results of the Liliefors normality test of climbing perch erythrocytes obtained a Li max value of $-0.544 < Li_{\text{table } 5\%} 0.242$, so it can be concluded that the data is normally distributed. The results of the Barlett variance homogeneity test obtained a calculated X^2_2 value of $1.048 < X^2_{\text{table } 5.991}$, which means homogeneous. The results of the Anova test showed a calculated F value of $2.150 < F_{\text{table } 5\%} 4.066$, which means there was no significant difference between treatments.

Hemoglobin

Hemoglobin (Hb) has a function in the catabolism process, binding oxygen which will later produce energy. The average hemoglobin levels of climbing perch during maintenance can be seen in Figure 11. The erythrocyte parameters of climbing perch range from $4.2\text{--}6 \text{ g/dL}$. The

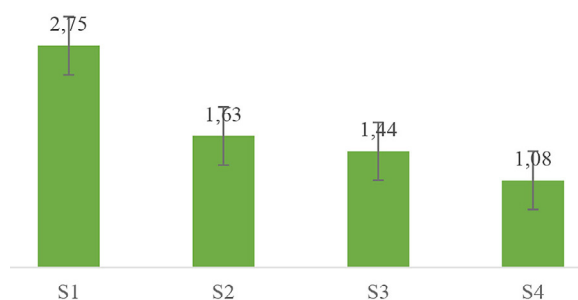


Figure 10. Erythrocytes of climbing perch

highest average hemoglobin value was in treatment S1 (6.0 g/dL), followed by treatment S2 (4.8 g/dL), then treatment S4 (4.5 g/dL) and the lowest hemoglobin value was in treatment S3 (4.2 g/dL). The results of the Liliefors normality test of climbing perch hemoglobin obtained a Li max value of $-0.520 < \text{Li table } 5\% \text{ } 0.242$, so it can be concluded that the data is normally distributed. The results of the Barlet variance homogeneity test obtained a calculated X^2 value of $-1.130 < X^2 \text{ table } 5.991$ which means homogeneous. The results of the Anova test showed a calculated F value of $0.698 < F \text{ table } 5\% \text{ } 4.066$ which means there was no significant difference between treatments.

Hematocrit

Hematocrit reflects the percentage of erythrocyte volume in total blood, showing the same trend as erythrocytes and hemoglobin. The average hematocrit levels of climbing perch during maintenance can be seen in Figure 12. The hematocrit parameters of the climbing perch range from 8.57 to 22.3%. The highest average hematocrit level was in treatment S1 (22.3%), followed by treatment S2 (10.71%), then treatment S3 (10%) and the lowest hematocrit value was in

treatment S4 (8.57%). The results of the Liliefors normality test of the climbing perch hematocrit obtained a Li max value of $0.217 < \text{Li table } 5\% \text{ } 0.242$, so it can be concluded that the data is normally distributed. The results of the Barlet variance homogeneity test obtained a calculated X^2 value of $-2.064 < X^2 \text{ table } 5.991$ which means homogeneous. The results of the Anova test showed a calculated F value of $3.191 < F \text{ table } 5\% \text{ } 4.066$ which means there was no significant difference between treatments.

Meat and liver antioxidants of climbing perch

Glutathione peroxidase

Glutathione peroxidase (GSH-Px) levels in the meat and liver of betok fish during rearing can be seen in Figures 13, 14. Based on the data, the increase in GPx activity in the meat and liver of climbing perch was more prominent with the S3 treatment (3 g/kg selenium), which achieved the highest GPx levels compared to other treatments. In fish meat, GPx increased from 1.911 U/L (control) to 2.405 U/L, while in fish liver, GPx activity increased from 1.363 U/L to 1.494 U/L. Higher GPx activity reflects the efficiency of the

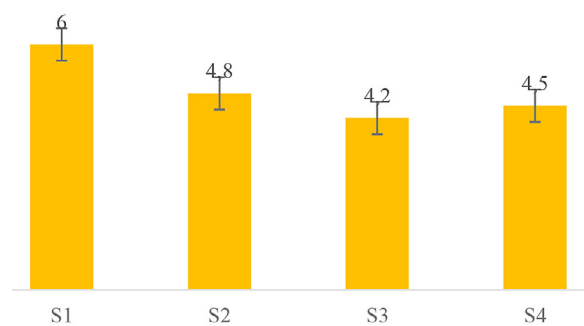


Figure 11. Hemoglobin of the climbing perch

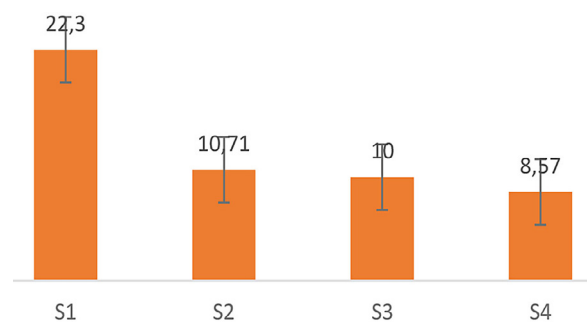


Figure 12. Hematocrit of climbing perch

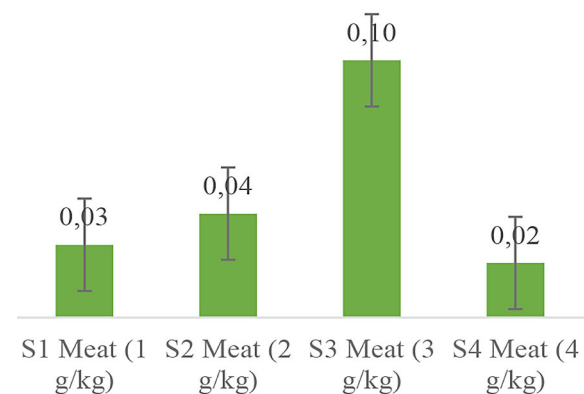


Figure 13. GSH-Px meat of climbing perch

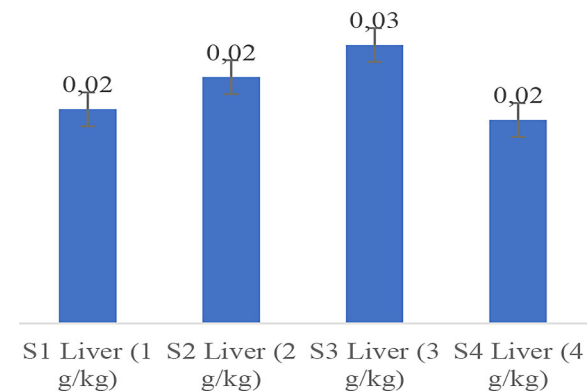


Figure 14. GSH-Px liver of climbing perch

antioxidant system in neutralizing peroxides and protecting biomolecules from oxidative stress. The results of the Liliefors GPx normality test of climbing perch meat obtained a Li max value of $-5.505 < \text{Li table } 5\% \text{ } 0.242$, so it can be concluded that the data is normally distributed. The results of the Barlet variance homogeneity test obtained a value of $X_2 \text{ count } 1.374 < X_2 \text{ table } 5.991$ which means homogeneous. The results of the Anova test showed a value of $F \text{ count } 3.018 < F \text{ table } 5\% \text{ } 4.066$ which means there was no significant difference between treatments.

Malondialdehyd

Malondialdehyde (MDA) levels in the meat and liver of climbing perch during maintenance can be seen in Figures 15, 16. The results of the Liliefors MDA normality test of climbing perch meat obtained a Li max value of $-0.563 < \text{Li table } 5\% \text{ } 0.242$, so it can be concluded that the data is normally distributed. The results of the Barlet variance homogeneity test obtained a X_2 count value of $-1.107 < X_2 \text{ table } 5.991$ which means homogeneous. The results of the Anova test showed

a calculated F value of $1.466 < F \text{ table } 5\% \text{ } 4.066$ which means there was no significant difference between treatments.

The results of the Liliefors malondialdehyde (MDA) normality test of climbing perch liver obtained a Li max value of $0.241 < \text{Li table } 5\% \text{ } 0.242$, so it can be concluded that the data is normally distributed. The results of the Barlet variance homogeneity test obtained a X_2 count value of $-2.407 < X_2 \text{ table } 5.991$ which means homogeneous. The results of the Anova test showed a F count value of $1.985 < F \text{ table } 5\% \text{ } 4.066$ which means there was no significant difference between treatments.

Catalase

Catalase (CAT) levels in the meat and liver of climbing perch during maintenance can be seen in Figures 17, 18. The results of the Liliefors CAT) normality test of climbing perch meat obtained a Li max value of $0.151 < \text{Li table } 5\% \text{ } 0.242$, so it can be concluded that the data is normally distributed. The results of the Barlet variance homogeneity test obtained a X_2 count value of $1.415 <$

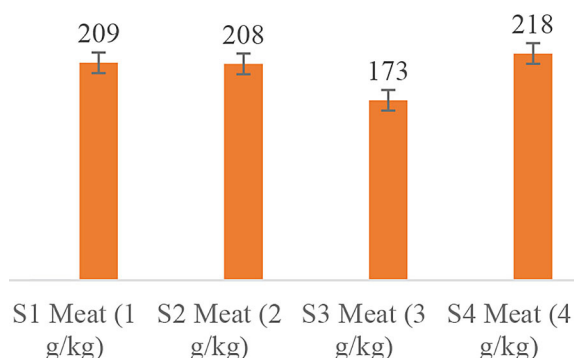


Figure 15. MDA meat of climbing perch

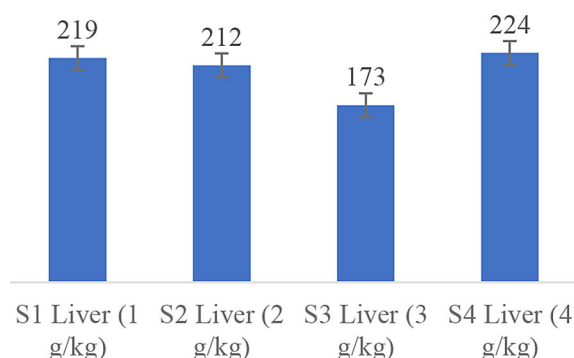


Figure 16. MDA liver of climbing perch

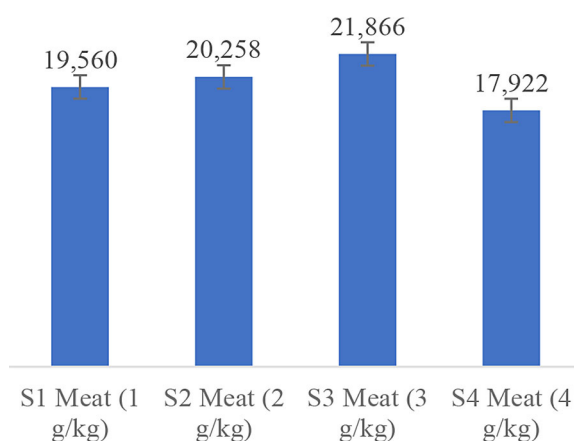


Figure 17. CAT meat of climbing perch

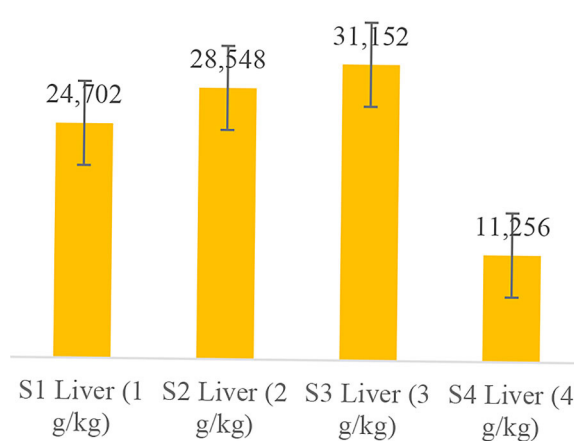


Figure 18. CAT liver of climbing perch

X_2 table 5.991 which means homogeneous. The results of the Anova test showed a F count value of $0.010 < F$ table 5% 4.066 which means there was no significant difference between treatments.

The results of Liliefors normality test of CAT activity of catfish liver obtained a value of Li max $0.203 < Li$ table 5% 0.242, so it can be concluded that the data is normally distributed. The results of Barlet's homogeneity test of variance obtained a value of X_2 count $1.431 < X_2$ table 5.991 which means homogeneous. The results of the Anova test showed a value of F count $0.293 < F$ table 5% 4.066 which means there is no significant difference between treatments.

Superoxide dismutase

Superoxide dismutase (SOD) levels in the meat and liver of climbing perch during maintenance can be seen in Figures 19, 20. The results of the normality test of Liliefors superoxide dismutase of climbing perch meat obtained a Li max value of $0.190 < Li$ table 5% 0.242, so it can be concluded that the data is normally distributed. The results of the Barlet variance homogeneity test obtained a value of X_2 count $1.746 < X_2$ table 5.991 which means homogeneous. The results of the Anova test showed a value of F count $0.004 < F$ table 5% 4.066 which means there was no significant difference between treatments.

The results of Liliefors normality test of SOD activity of catfish liver obtained a Li max value of $0.181 < Li$ table 5% 0.242, so it can be concluded that the data is normally distributed. The results of Barlet's homogeneity test of variance obtained a value of X_2 count $1.795 < X_2$ table 5.991 which means homogeneous. The results of the Anova test showed a value of F count $0.009 < F$ table 5% 4.066 which means there was no significant difference between treatments.

Survival rate

Based on Figure 21, the survival rate of climbing perch during the study ranged from 73.3 to 90%. The highest survival rate was obtained in treatments S1, S2, and S4 at 90%, while the lowest survival rate was in treatment S3 at 73.3%. The presence of death in each treatment was related to the feed made. The results of Liliefors normality test of survival rate activity of climbing perch obtained a value of Li max $0.209 < Li$ table 5% 0.242, so it can be concluded that the data is normally distributed. The results of

Barlet's homogeneity test of variance obtained a value of X_2 count $-7.342 < X_2$ table 5.991 which means homogeneous. The results of the Anova test showed a value of F count $0.260 < F$ table 5% 4.066 which means there was no significant difference between treatments.

Water quality

Water quality is one of the factors that affect the growth and survival of climbing perch that are kept during the study. Water quality parameters observed in this research were temperature, DO, pH, and ammonia level as presented in Table 3.

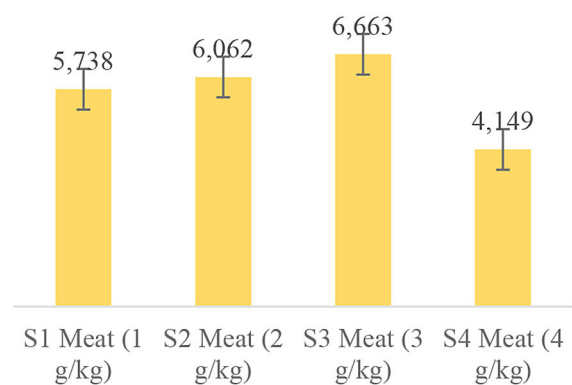


Figure 19. SOD meat of climbing perch

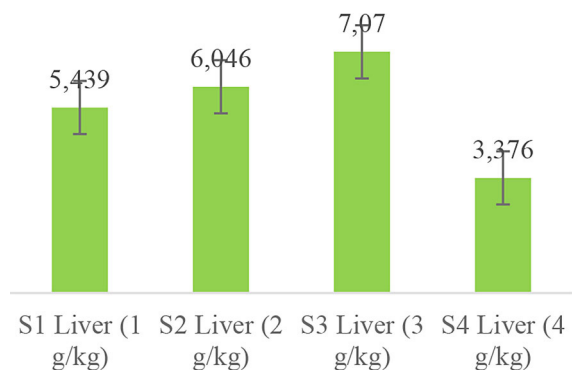


Figure 20. SOD liver of climbing perch

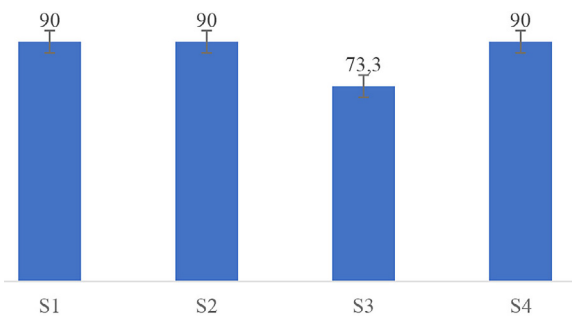


Figure 21. Survival rate of climbing perch

Table 3. Parameters of water quality in this research

Parameters	Outcome		Reference
	Pre	Post	
Temperature (°C)	28.5	26.5–26.6	25–32 °C (Cholik <i>et al.</i> , 1986)
DO (mg/L)	3.75	2.82–2.95	> 2 mg/L (Cholik <i>et al.</i> , 1986)
Ph	6.98	6.86–7.11	6.5–9.0 (Cholik <i>et al.</i> , 1986)
Ammonia (mg/L)	0.5	0.5–1	< 1 mg/L (Wardoyo, 1978)

DISCUSSION

Absolute length and weight growth

Growth in fish can generally be seen by the increase in length and weight of fish in a certain period of time. Fish growth and development are influenced by internal factors such as genes and hormones as well as external factors such as nutrition, water quality, feed availability and disease attacks (Wisnuwati and Nugroho, 2018). According to Mudjiman (2000) growth can occur due to the remaining energy from the process of metabolism, respiration, digestion and other physiological processes.

The results of absolute length growth of catfish ranged from 0.32 to 0.54 cm. Based on the data can be seen that treatment S1 showed the highest absolute length increase of 0.54 cm and the lowest in treatment S4 of 0.32 cm. This shows that a certain dose of selenium (S1) is able to support optimal growth compared to other treatments. Variations in absolute length increase in different treatments are influenced by the level of selenium availability in the feed absorbed by climbing perch. The right dose can increase the efficiency of nutrient use, while doses that are too low or too high can produce suboptimal effects due to metabolic imbalance.

The provision of selenium in climbing perch feed showed a significant effect on increasing fish weight. Selenium is an essential micro-nutrient for normal growth and physiological function of fish, including supporting the activity of antioxidant enzymes such as glutathione peroxidase, which protects cells from oxidative damage and increases the efficiency of nutrient metabolism. Based on the data can be seen that treatment S1 produced the highest absolute weight growth of 2.13 g, followed by S3 of 1.22 g, and S4 of 1.19 g. Meanwhile, S2 recorded the lowest weight increase of 0.88 g. These results indicate that the dose of selenium in S1 provides

an optimal effect on fish metabolism and growth, supporting the efficiency of feed to be converted into energy and body tissue.

Feeding with different treatments did not show any significant effect on the absolute length growth of the climbing perch. This is thought to be because the nutrient content in the feed is sufficient for the needs of the climbing perch for growth. All feeds given to the climbing perch have protein content (28.42–29.36%) within the optimal range for climbing perch. Optimal growth performance of climbing perch depends on the provision of feed with a protein content of around 20–40% (Hossain *et al.*, 2012).

The addition of organic selenium with different doses in the feed between treatments showed a significant effect on the absolute weight growth of the climbing perch. The results of the Anova analysis showed that the S1 treatment gave significantly higher absolute weight growth results compared to other treatments. This is thought to be due to the S1 treatment having the highest protein content in the feed at 29.36% compared to other treatments.

Protein retention and fat retention

External factors that affect fish growth include feed nutrients such as protein and fat. Protein is a source of energy for fish, in addition to carbohydrates and fat. The speed of fish growth is determined by the amount of protein absorbed and utilized by fish for building materials (Vega *et al.*, 2018). Protein in feed that is absorbed and utilized properly by fish can be described through protein retention. Protein retention is protein that is converted from feed protein that is stored in the fish's body (Setiawati *et al.*, 2013). The protein retention value will indicate the deposition index (formation) as body tissue or that utilized for growth (Buwono, 2000).

The highest average protein retention of climbing perch was in treatment S1 (5.04%) and

the lowest was in treatment S4 (0.35%). The results of the Anova test showed that the addition of organic selenium to fish feed with different doses did not significantly affect the protein retention of climbing perch. The addition of organic selenium to fish feed is not directly proportional to protein retention in the body of climbing perch. The more organic selenium is added to fish feed, the lower the protein retention value. This is thought to be because the increasing organic selenium content causes a decrease in nutrient digestibility.

The fat retention of climbing perch in treatments S1 and S2 had a fat retention of 28.16% and 28.19% respectively, which were higher than treatments S3 and S4 of 22.3% and 19.62% respectively. The results of the Anova test showed that the addition of organic selenium to fish feed with different doses had no significant effect on the fat retention of climbing perch.

High protein retention results in high fat retention as well. High fat digestibility will result in high protein digestibility (Iswadi *et al.*, 2016), because the fat used can contribute to fish metabolism so that it can affect the level of protein digestibility. In the increased fat content of feed in treatments S3 (7.22%) and S4 (7.34%), retention actually decreased. This is thought to be due to a decrease in nutrient digestibility due to the high organic selenium content in treatments S3 (3 g/kg feed) and S4 (4 g/kg feed) in fish feed.

Blood profile

The blood leukocyte value of the climbing perch ranges from 250 to 1.850×10^3 μL . Leukocytes of all treatments of climbing perch have values above the standard. The number of normal fish leukocytes ranges from 42.70 to 88.50×10^3 μL (Muliari *et al.*, 2020), which means that the blood leukocytes of climbing perch have values above normal. The high value of blood leukocytes of climbing perch is thought to be because the fish experienced stress when blood was taken. This is supported by the opinion of Dias *et al.* (2001) after stress, blood glucose, cortisol, plasma, number of leukocytes, number of lymphocytes, and neutrophils increased.

The blood leukocyte levels of climbing perch tend to increase with the addition of organic selenium doses in fish feed. This is thought to occur because the effect of organic selenium doses causes stress, so that leukocyte levels increase as an immune response to the stress. This is

supported by Pratiwi (2019) that an increase in the body's defense against bacterial infections affects the increase in the number of leukocytes. Setiawati *et al.* (2007) stated that fish in poor aquatic environmental conditions (water quality), stress factors and disease infections result in an increase in the number of leukocytes.

The blood erythrocyte value of the climbing perch ranges from 1.08 to 2.75×10^6 μL . The highest erythrocyte value was found in treatment S1 (2.75×10^6 μL) and the lowest erythrocyte value was found in treatment S4 (1.08×10^6 μL). The results of the Anova test of climbing perch erythrocytes showed that the addition of organic selenium in feed with different doses had no significant effect between treatments. The blood erythrocyte value of climbing perch was in normal condition. This is reinforced by the statement of Rocha *et al.* (2018), the erythrocyte value of the pomfret (*Colossoma macropomum*) ranged from $1.08\text{--}5.07 \times 10^6$ μL . Meanwhile, the erythrocyte value in the Siamese jambal fish (*Pangasius hypophthalmus*) ranged from $1.57\text{--}3.41 \times 10^6$ μL (Lukistyowati *et al.*, 2007).

According to Bastiawan *et al.* (1995) abnormal fish appetite, when fish appetite decreases is caused by low hematocrit values and abnormal blood erythrocyte counts. This can be seen in the S4 treatment, where the fish in this treatment have less appetite so that there is a lot of leftover food at the bottom of the aquarium. Increasing the dose of organic selenium in the fish feed causes the fish to respond to this with a low appetite. This is in accordance with the opinion of Yanto *et al.* (2015) that other factors that affect fish erythrocyte values include environment, gender, age, nutrition, and oxygen deficiency conditions. If the leukocyte value is low, the higher the erythrocyte count, this indicates that the leukocyte and erythrocyte values in climbing perch are negatively correlated. According to Johnny *et al.* (2003), leukocytes are different from erythrocytes because they have the ability to move freely and are able to leave the blood vessels to the tissue in carrying out their functions. The number of leukocytes that deviates from normal conditions has important clinical significance in evaluating health disorders.

The hemoglobin value of the blood of the climbing perch ranges from 4.2–6 g/dL. According to Dopongtonung (2008), the normal hemoglobin range in blood ranges from 12–14 g/dL. Hemoglobin is closely related to erythrocytes.

Low hemoglobin levels cause the metabolic rate to decrease and the energy produced to be low (Matofani *et al.*, 2013). This can be seen in the treatments S3 (4.2 g/dL) and S4 (4.5 g/dL) where the fish in these treatments had less appetite so that there was a lot of leftover food at the bottom of the aquarium. The increase in organic selenium in the feed, the fish responded to this with a low appetite in the fish. This can be seen from the tendency for the growth in length and weight of the catfish to be increasingly low.

Low hemoglobin content affects the low amount of oxygen in the blood. Many factors affect low hemoglobin levels. According to Dellman and Brown (1989), hemoglobin levels below the normal range, due to poor water quality, namely high ammonia content in this experiment ranging from 0.5–1.0 mg/L and also indicating low feed protein content, seen in low protein retention in treatment S4 (0.35%). According to Royan *et al.* (2014) hemoglobin levels below the normal range, due to poor water quality, indicate low feed protein content, vitamin deficiency and poor water quality or fish are infected.

The hematocrit value of the blood of the climbing perch ranges from 8.57 to 22.3%. The results of the Anova test of the hematocrit of the blood of the climbing perch show that the addition of organic selenium in feed with different doses has no significant effect between treatments. Lukistyowati *et al.* (2007) stated that the normal hematocrit level of farmed fish is 15–40%. Meanwhile, according to Sarjito and Haditomo (2017) the normal hematocrit value of fish ranges from 28–40%. In treatments S2, S3, and S4, the hematocrit value is below the normal limit of fish hematocrit. The low hematocrit value of the climbing perch is due to the lack of appetite of the fish and it is suspected that the fish are stressed due to changes in environmental conditions and handling when blood is taken. This is in accordance with the statement of Lukistyowati and Kurniasih (2012) that loss of appetite results in a decrease in hematocrit levels. Added by Hardi (2011) stress in fish can be caused by several factors, such as environmental factors, handling when blood is taken (injection) or due to infection.

Overall, the results of the study indicate that the addition of selenium in feed has a positive role in improving hematological parameters of climbing perch, especially in the S1 treatment with a moderate dose. Selenium helps support the production of erythrocytes, hemoglobin, and

hematocrit, and improves the immune response by increasing the number of leukocytes. However, inappropriate doses of selenium, such as in the S3 and S4 treatments, can cause a decrease in hematological parameters due to toxicity or oxidative stress. Therefore, attention is needed in determining the optimal dose of selenium so that its benefits can be maximized without causing negative impacts on the health of climbing perch.

Antioxidants of climbing perch

The addition of selenium in fish feed significantly increases the activity of antioxidant enzymes such as glutathione peroxidase (GPx) which plays an important role in protecting fish body cells from damage caused by free radicals. Based on the data, the increase in GPx activity in the meat and liver of climbing perch fish was more prominent with the S3 treatment (3 g/kg selenium), which achieved the highest GPx levels compared to other treatments. In fish meat, GPx increased from 1.911 U/L (control) to 2.405 U/L, while in fish liver, GPx activity increased from 1.363 U/L to 1.494 U/L. Higher GPx activity reflects the efficiency of the antioxidant system in neutralizing peroxides and protecting biomolecules from oxidative stress (Atencio *et al.*, 2009).

The addition of selenium at doses that are too low (S1, 1 g/kg) or too high (S4, 4 g/kg) indicates less than optimal GPx activity. At low doses, selenium may not be enough to support the formation of antioxidant enzymes, while at high doses, the possibility of toxic effects or inhibition of enzyme activity can reduce the effectiveness of the antioxidant system. In addition, the results of the study showed that selenium was more effective than selenium in improving these antioxidant indicators, because selenium has higher bioavailability and can be directly integrated into enzyme structures such as GPx. Overall, the addition of selenium in fish feed at optimal doses not only increases the activity of antioxidant enzymes, but also supports fish health and performance by minimizing oxidative damage.

The addition of selenium in fish feed showed a significant effect on reducing MDA levels, which is an indicator of oxidative damage to cell membrane lipids. Based on the data, MDA levels in the meat and liver of climbing perch decreased optimally in the S3 treatment (3 g/kg selenium), with MDA levels in the meat of 173 μ M and in the liver of 173 μ M, which are the lowest values

compared to other treatments. This decrease in MDA levels indicates that the addition of selenium can increase protection against oxidative stress through the mechanism of increasing the activity of antioxidant enzymes such as CAT, superoxide SOD, and GPx. MDA levels increased at selenium doses that were too low (S1, 1 g/kg) or too high (S4, 4 g/kg). At low doses, antioxidant protection may not be sufficient to suppress MDA formation, while at high doses, the possibility of selenium toxicity can trigger additional oxidative stress, so that MDA levels increase (218 μ M in meat and 224 μ M in liver for S4).

This study also showed that selenium did not have the same effect, considering its lower bioavailability and effectiveness compared to selenium. Therefore, the addition of selenium at optimal doses has been shown to be effective in improving fish health by reducing oxidative damage and protecting cell membranes against lipid peroxidation. The addition of selenium to fish feed significantly increased catalase (CAT) activity in the meat and liver tissues of climbing perch, especially at doses of 1–3 g/kg. CAT activity in meat increased from S1 (19,560 U/mg protein) to its peak at S3 (21,866 U/mg protein). This increase indicates that selenium supports the function of the antioxidant system by accelerating the decomposition of hydrogen peroxide into water and oxygen, thereby protecting cells from oxidative damage. However, at dose S4 (4 g/kg), CAT activity decreased to 17,922 U/mg protein, which may be due to the toxic effect of selenium at high levels, disrupting the efficiency of antioxidant enzymes.

A similar trend was also seen in fish liver, where CAT activity increased from S1 (24,702 U/mg protein) to S3 (31,152 U/mg protein), showing a more significant response compared to meat. This reflects the role of the liver as the main organ of metabolism and detoxification. However, at dose S4, CAT activity in the liver dropped drastically to 11,256 U/mg protein, indicating that excessive selenium doses can cause oxidative stress, inhibiting catalase activity. These data emphasize the importance of optimal selenium doses in feed to support fish health by increasing antioxidant activity without causing toxic effects on vital tissues.

The addition of selenium to fish feed has a positive effect on superoxide dismutase (SOD) activity in the meat and liver tissues of climbing perch. In meat, SOD activity increased along with

the addition of selenium doses, with the most significant increase at dose S3 (6.663 Units) compared to doses S1 (5.738 Units) and S2 (6.062 Units). This suggests that selenium helps to enhance the antioxidant system's ability to ward off free radicals and reduce oxidative stress in cells. However, the decrease in SOD activity at dose S4 (4.149 Units) suggests that excessive selenium concentrations may cause negative effects, possibly due to excessive toxicity. In the liver, an increase in SOD activity was also seen with the addition of selenium, particularly at dose S3 (7.070 Units). This activity was higher compared to doses S1 (5.439 Units) and S2 (6.046 Units), suggesting that selenium enhances the antioxidant defense mechanism in the liver against oxidative stress. However, as in meat, dose S4 (3.376 Units) showed a decrease in SOD activity, indicating the optimal dose limit for selenium in fish feed. Excessive doses may cause oxidative stress that damages cells, highlighting the importance of determining the right dose to obtain optimal health benefits from selenium in fish feed.

Research shows that the activity of antioxidant enzymes, such as SOD, catalase, and glutathione peroxidase (GSH-Px), in fish is at a low level, accompanied by high levels of malondialdehyde (MDA) as an indicator of oxidative damage. This condition indicates that the body's antioxidant defense system is unable to keep up with the production of free radicals, so that an intake of foods rich in antioxidants is needed to increase the capacity of antioxidant enzymes and reduce the risk of further complications.

Research on climbing perch fish shows that selenium supplementation in feed can increase GSH-Px, CAT and SOD activities in fish meat and liver. The results showed a tendency for significant increase in GSH-Px, CAT, and SOD activities in fish fed 3 g/kg selenium compared to fish fed 4 g/kg selenium feed. Although MDA levels in fish meat and liver did not show significant differences between treatments, the increase in antioxidant enzyme activity reflects the efficiency of the oxidative defense system in protecting biomolecules from peroxide damage (Rotruck *et al.*, 1973).

The relationship between antioxidant enzymes as the main protector against oxidative stress. Low enzyme activity can be an indicator of the need for antioxidant-based nutritional interventions, while in fish, selenium supplementation has been shown to be effective in increasing antioxidant capacity. These findings also provide

insight that nutritional quality improvement strategies for climbing perch can play a significant role in improving antioxidant status and reducing the adverse effects of oxidative stress.

Survival rate

The survival rate of catfish ranges from 73.3–90%. The highest survival rate was obtained in treatments S1, S2, and S4 at 90%, while the lowest survival rate was in treatment S3 at 73.3%. The death in each treatment was related to the feed given. In the first week to the third week, the fish still died. This is thought to be because the fish are still in the adaptation process and have not been able to adapt to the addition of selenium in the feed, causing stress and decreased body resistance and death. According to Linayati *et al.* (2024), the survival rate is categorized as high if the SR value is greater than 70%, SR is categorized as moderate if it is in the range of 50–60%, and low if it is in the range of less than 50%.

Water quality

Water quality is one of the factors that affect the growth and survival of climbing perch that are kept during the study. Water quality measurements during the experiment showed that it was suitable for fish farming. According to (Cholik *et al.*, 1986), water temperature of 25–32 °C is suitable for fish farming. According to (Cholik *et al.*, 1986), dissolved oxygen (DO) > 2 mg/L is good for fish farming. Meanwhile, pH ranges from 6.5–9.0 which is a good pH range for fish farming (Cholik *et al.*, 1986). The ammonia content in this experiment ranged from 0.5–1.0 mg/L. This condition still allows the climbing perch to survive. These water quality parameters are still in reasonable conditions, ideal for the growth of the climbing perch kept during the experiment (Wardoyo, 1978).

CONCLUSION

This study showed that the addition of 1 g/kg organic selenium to catfish increased growth and selenium accumulation in meat and liver tissues, although it did not significantly affect the activities of GPx, MDA, CAT, and SOD. Hematology results showed an increase in fish body resistance, indicating the benefits of organic selenium in

strengthening physiological systems and protecting against oxidative stress. These findings confirm the effectiveness of organic selenium supplementation in supporting the growth and health of catfish in aquaculture.

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