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Organic Selenium Improves Growth Performance and Metabolic Biomarkers in African Catfish (*Clarias gariepinus* Burchell, 1822) Fed Fishmeal-Free Diets

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ABSTRACT

Raw material cost including protein sources account for at least 70% of total aquafeed production cost. Protein is among the top three most expensive nutrients to supply in aquaculture diets, after energy and phosphorus. It is imperative to identify effective and practical strategies to ensure that African catfish (*Clarias gariepinus;* Burchell, 1822) utilizes amino acid intake for optimum growth. The ever-increasing cost-price squeeze between aquaculture input costs and output prices have forced aquafeed to reduce dietary nutrient specifications particularly amino acids. This occurs against a backdrop of sustainability concerns regarding fish meal as a protein source. This work studied 450 *C. gariepinus* in 15 experimental aquariums for a 62-day culture period. Implementing an experimental design with five fish meal-free dietary treatments and three replicates, differing in organic selenium content, Se0.5 (0.5 ppm), Se2 (2.0 ppm), Se4 (4.0 ppm), Se6 (6.0 ppm) and Se8 (8.0 ppm). Se4 achieved the highest final biomass at 2092.97g ($P>0.05$) and ADG at 34.79 g/day/aquarium ($P<0.05$). The superior performance was supported by favorable metabolic biomarkers, showing highest expression of the *igf1* gene at 2.75 ($P<0.05$), avoided hyperglycemia with blood glucose maintained at 33.67mg/ dL ($P<0.05$), hepatosomatic index of 1.85 ($P<0.05$) and highest body selenium retention at 18.53% ($P<0.05$). Selenium supplementation exerted a positive effect on *C. gariepinus* energy metabolism. In conclusion, practical African catfish diets can implement the 4.0mg/ kg organic selenium supplementation regime to achieve optimum growth and enable cost-saving lower protein diets.

INTRODUCTION

Freshwater aquaculture constitutes the primary source of aquatic protein in Asia **(Buchmann, 2022)**. Indonesia, along with China and India, ranks among the leading global producers **(Rimmer *et al.*, 2013; Kristanto *et al.*, 2019)**. The African catfish (*Clarias gariepinus*) was introduced to Indonesia in 1985 and has since become a fundamental component of freshwater aquaculture due to its rapid growth, resilience, favorable meat quality, and potential for genetic enhancement through crossbreeding **(Sunarma *et al.*, 2016; Adesuyi *et al.*, 2023)**. *C. gariepinus*

belongs to the family Clariidae, which exhibits an omnivorous diet with carnivorous tendencies **(Oluyemi, 2019)** and has relatively high protein requirements compared to other freshwater species **(Onyia *et al.*, 2021)**.

Meeting these protein demands presents a significant challenge, particularly in light of increasing feed costs, as protein constitutes one of the costliest elements of aquaculture diets **(Zebua *et al.*, 2025)**. Consequently, it is imperative to identify effective strategies to optimize amino acid utilization for optimal growth. Microminerals, notably selenium (Se), may contribute to enhancing protein utilization in diets with reduced protein content. Selenium is vital for fish, with requirements ranging from 0.2 to 12mg/ kg depending on species **(Prabhu *et al.*, 2014)**. It is a crucial component of antioxidative enzymes such as glutathione peroxidase, selenoprotein P, and thioredoxin reductase **(Khan *et al.*, 2017)**, which safeguard cells from oxidative damage caused by reactive oxygen species (ROS) generated during metabolic processes **(Saito, 2022)**.

Under aquaculture conditions, fish are frequently exposed to environmental stressors such as pollutants, crowding, hypoxia, and handling **(Dong *et al.*, 2002; Segner *et al.*, 2011; Lyorah *et al.*, 2023)**. Consequently, oxidative stress occurs when reactive oxygen species (ROS) production surpasses the antioxidant capacity, resulting in DNA damage, protein denaturation, and lipid peroxidation **(Ji & Yeo, 2021; Li *et al.*, 2023)**. Managing oxidative stress diverts energy and amino acids away from growth toward maintenance, thereby increasing production costs. Adequate selenium supplementation may mitigate ROS-induced damage, conserve energy, and improve growth efficiency.

In addition to its antioxidative function, selenium plays a substantial role in thyroid hormone metabolism through the iodothyronine deiodinases (DIO1–3), which necessitate selenocysteine for the conversion of thyroxine (T4) to the biologically active triiodothyronine (T3) **(Kohrle, 2009)**. Triiodothyronine (T3), in conjunction with insulin-like growth factor 1 (IGF1), facilitates enhanced glucose uptake via GLUT4 and GLUT3 transporters, thereby supporting ATP synthesis **(Kido *et al.*, 2016; Peng *et al.*, 2020)**. Selenium also indirectly promotes IGF1 signaling by mitigating oxidative stress **(Yeap *et al.*, 2010)** and governs energy metabolism through AMP-activated protein kinase (AMPK), which promotes glucose uptake and fatty acid oxidation **(Hardie *et al.*, 2012; Oka *et al.*, 2021)**.

In aquafeeds, selenium is commonly supplemented as inorganic sodium selenite or as organic forms such as selenium yeast, selenomethionine, and hydroxy-selenomethionine **(Kianersi *et al.*, 2022; Wu *et al.*, 2022)**. Although sodium selenite may induce toxicity at levels exceeding 1mg/ kg, organic variants are generally better tolerated and demonstrate greater efficacy in promoting growth when included at higher concentrations **(Zhu *et al.*, 2016)**. Given the substantial oxidative and metabolic demands present in commercial aquaculture, reliance solely on sodium selenite may be insufficient to optimize fish performance.

This study evaluated the effects of graded dietary organic selenium supplementation (0.5, 2.0, 4.0, 6.0, and 8.0mg/ kg) on African catfish over a 62-day trial under the zero-fish meal feed formulation. Zootechnical performance, metabolic biomarkers, and transcriptomic responses were

assessed. The findings offer insights into the effective use of organic selenium in practical African catfish diets, highlighting its potential role in reducing protein requirements and thereby improving both the economic efficiency and sustainability of catfish aquaculture.

MATERIALS AND METHODS

1. Ethical use of experimental fish

The procedure for handling and managing catfish in the experiment conformed to the SNI national accreditation (number SNI 7306:2009) of the Republic of Indonesia, pertaining to catfish culture and utilization.

2. Experimental diets

A completely randomized design consisting of five dietary treatments was organized with three replicates each. Experimental feeds were prepared by supplementing a commercial fishmeal-free catfish pellet (SL2-2mm, PT. Wonokoyo Jaya Kusuma, Indonesia) with graded levels of commercial organic selenium (SeOxG®, PT. Aquacell Indo Pasifik, Indonesia, registration number KKP RI D 2312767 PBS): 0.5 (Se0.5), 2.0 (Se2), 4.0 (Se4), 6.0 (Se6), and 8.0 mg/kg (Se8). All diets were identical in formulation, differing only in selenium inclusion. Proximate composition was determined following AOAC standard procedures, and selenium concentrations were quantified by inductively coupled plasma–mass spectrometry (ICP-MS). Ingredient inclusion and nutrient profiles are presented in Tables (1, 2).

Table 1. Five dietary treatments with increasing levels of selenium

Ingredient (%inclusion as-fed basis)	Se 0.5	Se 2.0	Se 4.0	Se 6.0	Se 8.0
Soybean meal	31.14	31.14	31.14	31.14	31.14
Wheat pollard	15.15	15.15	15.15	15.15	15.15
Wheat flour	9.65	9.65	9.65	9.65	9.65
Poultry meat meal	9.63	9.63	9.63	9.63	9.63
Meat and bone meal	8.00	8.00	8.00	8.00	8.00
Wheat bran	7.62	7.62	7.62	7.62	7.62
Copra meal	4.86	4.86	4.86	4.86	4.86
Tapioca flour	4.00	4.00	4.00	4.00	4.00
Industrial flour	4.00	4.00	4.00	4.00	4.00
Crude palm oil	2.26	2.26	2.26	2.26	2.26
Fish oil	0.70	0.70	0.70	0.70	0.70
L-Lysine HCI	0.79	0.79	0.79	0.79	0.79
DL-Methionine	0.88	0.88	0.88	0.88	0.88
Monocalcium phosphate	0.50	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25	0.25
Vitamin E	0.02	0.02	0.02	0.02	0.02
Vitamin C	0.10	0.10	0.10	0.10	0.10
Mineral mix	0.23	0.23	0.23	0.23	0.23
Vitamin mix	0.15	0.15	0.15	0.15	0.15

Ingredient (%inclusion as-fed basis)	Se 0.5	Se 2.0	Se 4.0	Se 6.0	Se 8.0
Feed antioxidant	0.01	0.01	0.01	0.01	0.01
Feed antimould	0.05	0.05	0.05	0.05	0.05
Organic selenium	0.01	0.02	0.04	0.09	0.17

All ingredient inclusion rates are consistent across all treatments, except for the dosage of organic selenium supplementation. None of the diets included fish meal.

Table 2. Proximate composition (%), gross energy (kcal kg^{-1}) and selenium (mg kg^{-1}) in the experimental feed

Nutrient	**Se 0.5**	**Se 2.0**	**Se 4.0**	**Se 6.0**	**Se 8.0**
Moisture	7.52	8.67	9.38	9.64	9.53
Protein	32.26	32.04	31.42	31.31	31.75
Fat	6.96	7.07	6.91	7.19	7.67
Fiber	5.16	4.84	4.78	4.94	4.78
Ash	9.35	9.42	9.12	9.18	9.05
Nitrogen-free extract	38.75	37.96	38.39	37.74	37.22
Gross energy	4069.11	4034.62	4002.22	3995.73	4044.49
Selenium (Se)	0.8	1.04	1.51	3.36	5.17

3. Water quality monitoring

Water temperature and pH were simultaneously measured using a handheld pH meter with an integrated temperature sensor (Mediatech ATC9908, Mediatech, Indonesia). Prior to daily use, the pH electrode was three-point checked using standard buffer solutions (pH 4.01, 6.86 and 9.18) to ensure accuracy. Measurements were taken directly at the center of all experimental aquarium, approximately 15cm below the water surface, ensuring the probe tip was positioned away from aeration stones or water inlets/outlets. The pH value was recorded once the reading stabilized (~30 seconds).

Dissolved oxygen (DO) concentration (mg/L) was determined using a portable optical DO meter (HI 9142, Hanna Instruments, USA). Measurements were taken immediately following the pH and temperature readings, in the same location within the tank, after allowing sufficient time for the sensor to stabilize (~60 seconds) before recording the final DO value.

4. Fish acclimation and rearing

Two IBC (Intermediate Bulk Container) designed as fish adaptation tanks were aerated for 48h using a central pump connected to six aeration lines (three per IBC). Water temperature was maintained at 29– 31°C using an electrical heater, monitored twice daily. After 48h, 500mg sodium

nifurstyrenate (Elbayou®, Ueno Food Techno Industry Ltd., Japan) and 300g non-iodized salt (98% NaCl) were applied per tank for the disease prevention.

A total of 1050 African catfish fingerlings, measuring 7– 8cm, were obtained from a commercial farm located in Parakan Village, Serang, West Java, Indonesia. The fish were transported in 5-liter plastic bags containing equal proportions of water and air. Upon arrival, the bags were floated in an Intermediate Bulk Container (IBC) for 15 minutes to allow for water temperature equilibration. After this period, the bags were gently tilted toward the water surface to gradually release half of the catfish into the water within the first IBC. Subsequently, the remaining fish were transferred in a similar manner into a second IBC. The fish were fasting for 24 hours post-stocking and were then fed *ad libitum* for a period of 11 days prior to transfer into the experimental aquaria.

During the 11-day fish acclimatization process, thirty 130L aquaria were prepared. Sterile water was employed, continuously aerated, and complemented with a top filter. The aquaria were conditioned with 3g L^{-1} non-iodized salt and 5mg L^{-1} nifurstyrenate. Prior to stocking, the fish were graded and introduced at a biomass of 123– 127g (124.35 ± 1.78 g, mean ± SD) per aquarium. Additional fish were retained in IBCs as replacements. The remaining catfish were returned to the IBCs for an additional 7-day rearing period, serving as substitute fish in case of disease, mortality, or growth abnormalities. Each aquarium was uniquely labelled and assigned specific feed treatments. Every feed container was equipped with a QR code linked to the corresponding aquarium ID, facilitating scan-based verification during feeding. Throughout the experiment, the fish were fed *ad libitum*. On designated days, the catfish received feed until no further feeding activity was observed for 10 minutes. The quantity of feed administered was recorded. When a certain amount of feed was completely consumed (no residual pellets) over two consecutive feeding sessions, the feeding amount was increased, and this process was continued until the rearing period was concluded.

5. Growth monitoring

Every 15 days (DOC 15, 30, 45, and 62), fish were fasted for 12 hours and subsequently sedated using 150 ppm tricaine methanesulfonate (MS-222; Finquel®, Argent Laboratories, Philippines). Four 30-liter containers were employed (two designated for sedation and two for recovery). Fish were individually weighed to evaluate growth and uniformity, then rehoused in their aquaria following recovery. Feeding was resumed the following morning.

6. Sample collection

At DOC 62, fish were sedated and sampled for final analysis. Blood was collected from 2–3 fish per aquarium via the caudal vein into 10% EDTA tubes. Two fish per aquarium were sacrificed for tissue collection. Samples included liver, muscle, gonads, and whole fish. Blood was used for glucose, hydrogen peroxide-challenge viable cell count, ALT, and AST analyses. Liver was analyzed for hepatosomatic index, MDA, GPx, SOD, and *IGF-1* expression. Muscle and

whole fish were analyzed for proximate composition and selenium content. Gonads were weighed for hepatosomatic and gonadosomatic index calculations. Liver samples for gene expression were preserved in GENEzol™ (Geneaid, Taiwan) and stored at −80°C. Fish remains were biorecycled as compost fertilizer.

7. Heat challenge exposure

The catfish were subjected to a temperature of 40°C for a duration of five minutes within a plastic rectangular container filled with five liters of freshwater. Following this exposure, the catfish were transferred to an alternate container containing freshwater at a standard temperature of approximately 28- 29°C, which represents the typical water temperature available at the research station. The selection of the challenge temperature and exposure duration was determined through modifications to the fish heat challenge experimental protocol previously conducted by **Metz *et al.* (2025)**.

8. Blood biochemical analysis

Blood glucose was measured using a portable kit (GlucoDr AGM-2100, Allmedicus, Korea) employing the NAD-dependent glucose dehydrogenase (GDH) method **(Price & Spencer, 1979)**. This method is robust against glucose overestimation due to icodextrin and oxygen interference **(Perera *et al.*, 2011; Rajendran & Rayman, 2014)**.

9. Proximate composition and selenium retention

Initial whole-body samples (10 pooled fingerlings) and final carcass samples (one fish per treatment) were analyzed for proximate composition. Moisture analysis was conducted according to **AOAC (1984)**, calculated as weight loss after drying. Crude protein was assessed referring to the method of **Kjeldahl (1883)** and **Saez-Plaza *et al.* (2013)**. Measured nitrogen content was then multiplied by 6.25 as a factor to derive the crude protein content of the specimen. Crude fat was determined referring Soxhlet extraction method **(Jensen, 2007)**. Ash was analyzed by Furnace at 550°C for 3h **(St. John, 1943)**. Crude fiber examination followed the method of **Henneberg and Stohmann (1864)**, using a sequential acid and alkali digestion.

Whole-body selenium was determined by ICP-MS **(Hirtz & Gunther, 2020)** from pooled initial samples and one fish per aquarium at harvest. Selenium retention was calculated as:

$$\text{Se retention (\%)} = \text{total Se intake}[(\text{final biomass} \times \text{final Se \%}) - (\text{initial biomass} \times \text{initial Se \%})] \times 100$$

10. Hepatosomatix index (HSI)

This was measured as the percentage of liver mass compared to total body mass **(Pham & Nguyen, 2019)**. HSI was calculated as: HSI (%)= [liver weight/bodyweight]×100

11. Gene expression

Liver RNA was extracted from liver using GENEzol™ reagent. Purity was assessed by NanoDrop spectrophotometer (Thermo Fisher, USA). Reverse transcription followed **Nasrullah *et al.* (2021)**. qPCR was performed using Verso 1-Step RT-qPCR Kit (Thermo Fisher, USA) on an FQD-48A LineGene K Plus (Bioer, China). Primer sequences of the analyzed genes are listed in Table (3). Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method **(Livak & Schmittgen, 2001)**. All target genes were normalized to the highly conserved beta-actin gene as reference.

Table 3. qPCR primers sequence used in the study

Gene	Type	Sequence	Reference
Beta-actin	Forward	*ACC GGA GTC CAT CAC AAT ACC AGT*	Nasrullah *et al.*, 2021
	Reverse	*GAG CTG CGT GTT GCC CCT GAG*	Nasrullah *et al.*, 2021
Superoxide dismutase (*sod*)	Forward	*TGC TCC CGT AGT GGT TAA AGG G*	Nasrullah *et al.*, 2021
	Reverse	*TTC ATC AAG TGG CCC ACC ATG*	Nasrullah *et al.*, 2021
Insulin-like growth factor 1 (*igf-1*)	Forward	*TAC TTC AGC AAG CCA ACA GG*	Nasrullah *et al.*, 2021
	Reverse	*CTC TGG AAG CAG CAT TCA TCT A*	Nasrullah *et al.*, 2021

12. Zootechnical performance

Final biomass was determined by weighing all fish per aquarium **(Rodríguez-Sánchez *et al.*, 2018)**. Average daily gain (ADG) was calculated as biomass gain ÷ 62 days **(Hassan *et al.*, 2020)**. Feed conversion ratio (FCR) was calculated as feed intake ÷ biomass gain **(Kokkali *et al.*, 2023)**.

14. Statistical analysis

All data generated in this study were stored, organized and summarized in a Google Spreadsheet (Google Inc, USA). Statistical analysis for hypothesis and subsequent post-hoc testing was performed using R language **(R Core Team, 2025)** and the R-Studio IDE software (Posit, USA). Hypothesis testing on both zootechnical and biological parameters implemented the one-way ANOVA procedure, followed by the Tukey HSD post-hoc test. Statistical significance was set at $P < 0.05$.

RESULTS

Throughout the 62-day experimental rearing period, water used as habitat for the catfish was carefully maintained to have an average temperature of 29.03ºC, pH of 8.09, and dissolved oxygen level of 6.40mg/ L. The initial biomass of each aquarium containing 20 catfish fingerlings was 124.6 grams on average. The differences in the initial biomass between the six dietary treatments were not significantly different ($P > 0.05$). Right at the onset of the aquarium experimental period (T0), the whole-body proximate content of the catfish fingerlings is shown in Table (5). Furthermore, the whole-body selenium content at T0 was analyzed to be 46.58mcg/ 100g.

Table 5. Initial and final proximate composition (%) of experimental African catfish

Nutrient	Analyzed T0 whole-body level	Analyzed T62 whole-body level	Absolute difference	Percentage difference
Moisture	80.27	73.10	-7.17	-8.93%
Crude protein	14.29	16.45	2.16	13.13%
Crude fat	0.57	0.74	0.17	22.97%
Crude fiber	0.77	3.44	2.67	77.62%
Ash	3.47	3.24	-0.23	-6.63%

Selenium supplementation did not have a statistically significant effect ($P > 0.05$) on gene expression of the superoxide dismutase enzyme (SOD). Exploratory data analysis performed on the gene expression levels revealed that there is an inverse-parabola pattern as selenium dose supplementation increases from 0.5 to 8.0mg/ kg (Fig. 1A). The 4.0mg/ kg supplementation group had the highest gene expression with mean fold change of 1.49. SOD expression increases from 1.10, corresponding to the 0.5 ppm treatment group up to the highest expression corresponding to the 4.0 ppm treatment group, before decreasing to 1.30 of the 6.0 ppm treatment group, and then ultimately reaching the lowest level of 0.89 with 8.0 ppm Se supplementation.

On the other hand, gene expression of insulin-like growth factor 1 (IGF-1) was significantly affected by the dose of organic selenium supplementation ($P < 0.05$). As observed with SOD expression, the highest level of IGF-1 expression, 2.75 mean fold change, was seen in catfish fed the 4.0 ppm Se diet and the lowest level at 0.24 in the 8.0 ppm Se diet (Fig. 1B). Considering data on all treatment groups, the IGF-1 expression data exhibited an inverse parabola pattern, again bearing resemblance to the trend of SOD gene expression. Different from SOD

expression whose variation was weakly explained by Se supplementation dose (R-squared linear = 0.272), variation in IGF-1 expression was considerably explained by Se supplementation dose (R-squared linear = 0.701).

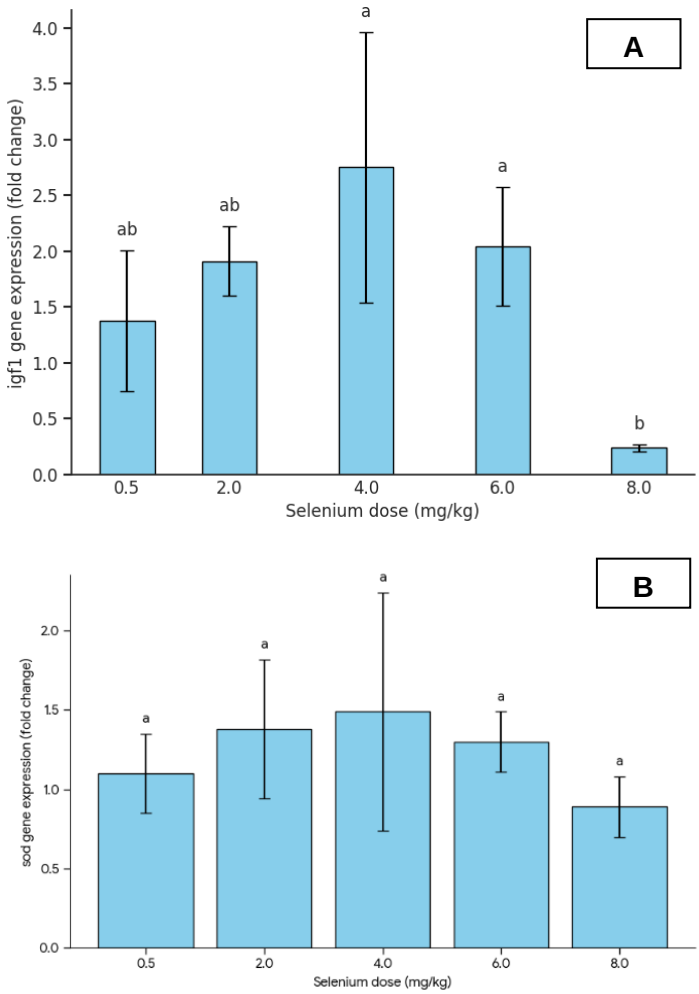

Fig. 1A. Organic selenium supplementation dose effect on the expression of the insulin-like growth factor 1 (*igf1)* gene

Assessing Se supplementation effects at the organ level, Se supplementation dose appeared to have significant effect on liver development ($P < 0.05$), where the 2.0 ppm diet group exhibited the highest hepato-somatic index (HSI) at 2.04%. The proportion of liver compared to whole-body mass of the other dietary treatments ranged from 1.67 - 1.85%. Supplementation dose also explained a considerable amount of variation in HSI (R-squared linear = 0.640).

Post-heat challenge blood glucose was significantly affected by Se supplementation dose ($P< 0.05$). There was a clear pattern in the data, where catfish receiving 2.0 - 8.0 ppm organic selenium and above had lower blood glucose after exposure to 40 degrees for 5 minutes (Fig. 2).

The post-challenge blood glucose levels ranged from 26.00 - 36.00mg/ dL, considerably lower than 94.33mg/ dL of the group supplemented with 0.5 ppm Se.

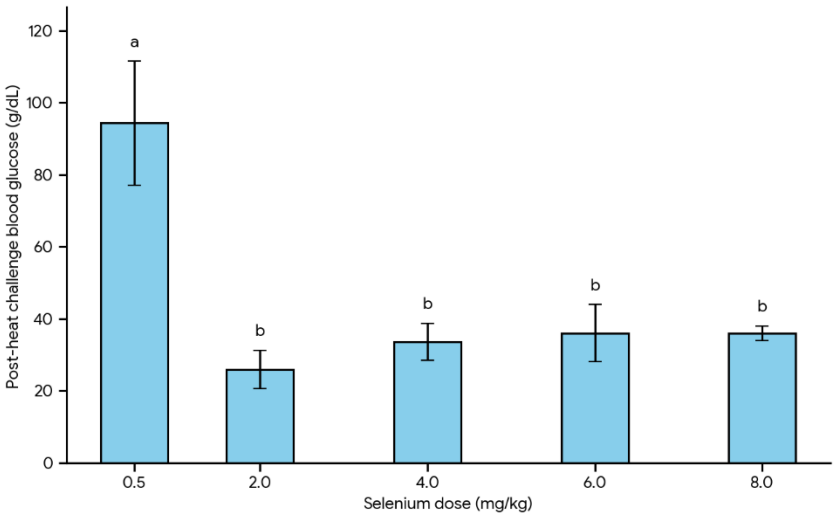

Fig. 2. Differences in blood glucose levels of *C. gariepinus* supplemented with increasing dose of organic selenium (2.0 – 8.0 ppm), in response to exposure to 40 degrees Celcius for 5 minutes as a heat challenge

Whole-body protein content of all dietary treatments did not exhibit statistically significant differences ($P > 0.05$). The lowest protein content was observed in the carcass of catfish treated with 0.5 ppm Se (76.99 %) and the highest observed in the 6.0 ppm Se catfish group (77.71). Selenium supplementation dose appeared to explain little of the variation in carcass crude protein data (R-squared linear = 0.326). With regards to efficiency of nutrient deposition in the body, there is an inverse parabola relationship in protein retention among the five selenium doses. *Clarias* catfish supplemented with 2.0 ppm Se had the highest protein retention at 42.96%. Examining the whole dataset, an inverse parabola relationship could be seen for this parameter. After reaching the peak of 42.96% retention in the 2.0 ppm Se group, the level continuously goes down up to the lowest level at 37.15% retention in the 8.0 ppm Se group. However, the numerical relationship was not statistically significant ($P > 0.05$). The inverse parabola trend was also apparent in the selenium retention data and for this parameter, statistical significance was observed. The 4.0 ppm supplementation dose resulted in significantly higher selenium retention compared to the level achieved by the 6.0 ppm group, 18.53% and 9.35% respectively ($P < 0.05$). Variation in selenium

retention was moderately explained by selenium supplementation dose, with linear R-squared value of 0.590.

At the conclusion of the 62-day rearing period, the biomass in each aquarium increased by 1842.4g on average. Throughout the dietary trial, each aquarium consumed an average of 2571.2g of extruded feed pellets. Survival rate of all dietary treatments was maintained above 90% throughout the study. There was a trend ($P > 0.05$) in the final biomass of *Clarias* across the five dietary treatments (Fig. 2). The highest final biomass was observed in the catfish group supplemented with 4.0 ppm of organic selenium while the lowest dose treatment of 0.5 ppm resulted in the lowest final biomass. Selenium supplementation dose had a statistically significant effect ($P < 0.05$) on *Clarias* catfish ADG. Supplementation of 4.0 ppm organic selenium resulted in the highest ADG at 34.79 g/day. The ADG of this treatment group is statistically significantly higher than all other groups in this experiment. Feed conversion ratio (FCR) and survival rate (SR) was not significantly affected ($P > 0.05$) by dose of selenium supplementation.

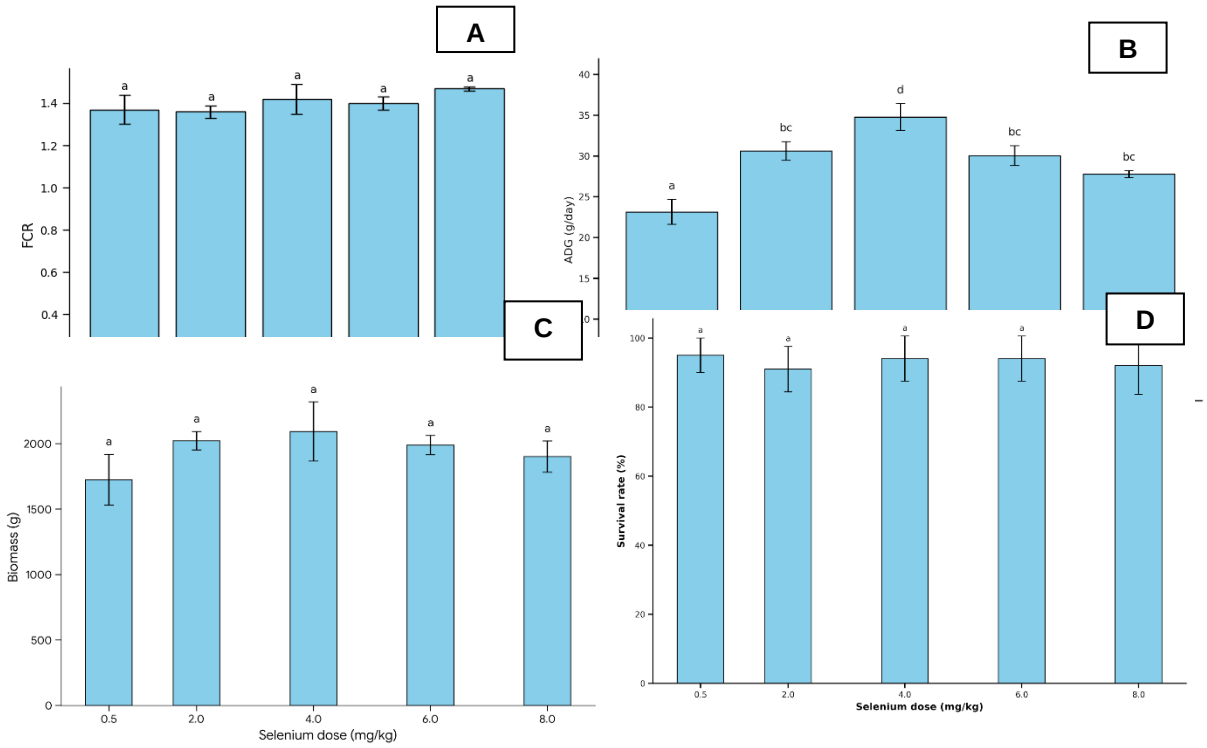

Fig. 3A. Effect of organic selenium supplementation dose on FCR

DISCUSSION

There are two possible approaches to meet the high dietary protein demands of *C. gariepinus*. First is to supply a higher level of amino acids and concurrently crude protein in the feed formulation. Second, as described by **Sadek and Deeb (2021)**, instead of increasing protein intake, the utilization of dietary protein can be enhanced in order to achieve the same level of growth performance with diets of the same or even lower protein specifications. Considering the existing immense economic challenges due to high protein raw material prices, especially fish

meal; the second approach is more commercially feasible and attractive. There is ample literature data on dietary application of exogenous enzymes **(Zheng *et al.*, 2019; Bowyer *et al.*, 2020)** and synthetic amino acids **(Ruben *et al.*, 2023)** with the goal of formulating lower crude protein fish feed to achieve better economic and environmental outcomes.

As the living and growth environment of fish, the chemical profile and quality of water is a strong determinant of fish metabolism, health and growth **(Buet *et al.*, 2006; Neeratanaphan *et al.*, 2020)**. This is crucial to ensure that the results of this study are comparable to similar works by other aquaculture researchers. Upon review, there has yet to be data on the whole-body selenium content of *C. gariepinus* catfish in the literature. Review on available literature data on whole-body selenium content of several other freshwater fishes yielded an interesting observation. The selenium content of the catfish in the present study ranged from 46.58 mcg/100 g for T0 fingerlings to 42.35 mcg/100 g for T62 catfish at harvest time. Work done in Thailand **by Singhato *et al.* (2022)** reported the selenium content of the walking catfish *Clarias batrachus* (Linnaeus, 1758), a close relative, at 22.8 mcg/100 g. Their measurements for other freshwater fishes such as the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) and striped snakehead *Channa striata* (Bloch, 1793) were similar at 30.7 and 33.0 mcg/100g, respectively. Assuming that the fish in the Thai study have been raised in aquaculture and consumed feed with typical industry selenium dose supplementation at 0.5 ppm, the resulting whole-body selenium content of catfish supplemented with 0.5 ppm selenium in this study averaged 18.05 mcg/100g (omitting one outlier data point at 130.78 mcg/100 g), which is similar to those reported by **Singhato *et al.* (2022)**. However, if the outlier data point is not excluded from the mean selenium content calculation, interestingly, the analyzed selenium content of *C. gariepinus* in the present study is approximately 52% higher than the levels reported by **Singhato *et al.* (2022)** for freshwater species. The disparity in whole-fish selenium content may be due to the differences in selenium content of the water used to rear the experimental fishes.

The nutritional value of catfish harvested at termination of this experiment can be evaluated from the proximate analysis presented on Table (5). Crude protein content of experimental catfish at harvest analyzed at 16.45% (analyzed moisture content 73.10%) is quite consistent with the level of 17.38% reported by **Aremu *et al.* (2021)**, assuming moisture content of 73.10% to base the comparison on the same dry matter percentage as the current experiment. A study by **Mohammed *et al.* (2023)** documented crude protein content of whole-fresh catfish at 16.24%, in close agreement with the figure found in the present experiment. Similarity of protein content analyzed in this study compared to others in the literature implies that first the catfish observed in this experiment is indeed a typical *C. gariepinus* reared under typical conditions. Secondly, it appears that the dietary protein intake by both the catfish in this study and those in other works in literature is similar, hence the similar whole-body protein content. Another nutrient worth examining is fat. Analyzed using the soxhlet extraction method, fat content of catfish in this study was 0.74%. This is in stark contrast with reported literature values of greater than 3% **(Orire *et al.*, 2013; Sadek & Deep, 2021; Okwakpam *et al.*, 2023)**. This could possibly be due to

differences in the fat contents of diets formulated in this present study compared to those reported in literature**. Orire *et al.* (2013)** had at least 12% crude fat in their experimental diets, compared to the 6-7% fat level of the feed in this experiment. However, this notion is countered by the fact that the diets formulated by **Sadek and Deep (2021)** contained fat levels not more than 5%, yet the fat content of their experimental catfish is still much higher. A possible explanation for this could be differences in the nutrient deposition characteristics during growth of the catfish in this study compared to the one reared by **Sadek and Deep (2021)**. Experimental catfish studied in the present work could have more tendency toward lean muscle growth and deposits less body fat. This may be due to strain or breed differences in the *C. gariepinus* studied. The study by **Okwakpam *et al.* (2023**) observed readily available catfish that were not reared as part of the experiment. As such, there is no information on the dietary nutrient profile received by the catfish in that experiment. The fiber content of catfish in this study at the point of harvest at 62 days of culture (T62) is considerably higher than the level reported by other researchers. For example, **Abeni *et al.* (2015)** took specimens of pond-reared *Clarias* catfish and reported an analyzed whole-body crude fiber content of 0.62%. This is clearly in stark contrast with the 3.44% crude fiber found in the present experiment. Upon observing this phenomenon, we evaluated the feed formula used and analyzed the proximate composition of the feed. The feed fiber content was 3.7%, similar to the fiber content of the experimental catfish. Therefore, the high whole-body fiber content could possibly be explained by residual feed in the GIT of the experimental catfish specimen during sacrifice and sampling. As a result, the analyzed fiber was not only of the fish itself, but also of the remaining feed in the fish digestive tract. This notion is supported by the fact that at the beginning of the experiment, the fiber of the fingerling was at 0.77%, which is a typical figure in *Clarias* literature. It makes logical sense because it is unlikely that contamination of analysis with residual feed happened at two different sampling points. For other proximate parameters, significant differences were not observed between T0 and T62 specimens, indicating that body composition in *Clarias* catfish does not vary much throughout growth progression.

Assessing the effects of organic selenium supplementation on *Clarias* catfish antioxidative capacity, the present study did not reveal any statistically significant effect. This is different from the work of **Kianersi *et al.* (2022)**, in which increases in activities of various antioxidant enzymes were recorded including superoxide dismutase (SOD) in yellowfin seabream *Acanthopagrus latus* (Houttuyn, 1782) as a result of selenium supplementation. In that study, the fish was challenged with mercury exposure to evaluate the potency of selenium in alleviating heavy metal toxicity. It could be the case that considerable elevations in fish antioxidative enzyme activities only occur upon exposure to stressors, as a natural form of metabolic defense. Under normal rearing and physiological conditions such as the one implemented in the present study, the fish body may not be pressured to synthesize SOD in higher amounts than typical baseline levels. This notion is supported by the fact that enzymes are composed of protein as the most expensive nutrient in aquaculture diets definitely would not be supplied in excessive amounts. As such even when selenium as an integral cofactor for these enzymes were supplied, their gene expressions did not increase. In contrast, catfish exposed to heavy metals may require elevated antioxidative defenses

to maintain cellular integrity and in turn physiological homeostasis. Assuming that dietary protein provided to the fish in that study is sufficient, de novo enzyme synthesis is likely directed toward producing SOD. When selenium is supplemented and available in greater amounts, elevated synthesis of the oxidative protector enzymes is enabled, and this potentially explains the increased activities of these enzymes **(Kianersi *et al.*, 2022)**. Indeed, the fish antioxidant defense system has been well documented to have optimal function when dietary selenium intake is sufficient **(Khan *et al.*, 2017)**.

Insulin-like growth factor 1 (IGF1) is a peptide hormone that upregulates anabolic processes involved in growth and physiological development in fish as well as terrestrial organisms **(Xiao *et al.*, 2017)**. The abundance and activity of IGF1 has been shown to be regulated by triiodothyronine (T3). Recently the expression of IGF1 was reported to be upregulated by T3 in the liver of the sapphire devil fish (*Chrysiptera cyanea* Quoy & Gaimard, 1825; **Mahardini *et al.*, 2020)**. This finding validated earlier reports of this phenomenon, for example in zebrafish (*Danio rerio* Hamilton, 1822; **Wang & Zhang, 2011**) and Mozambique tilapia (*Oreochromis mossambicus* Peters, 1852; **Schmid *et al.*, 2003**). In turn, T3 circulating in the fish body is a result of bioconversion from thyroxine (T4), where the iodothyronine deiodinase group of enzymes, specifically iodoythyronine deiodinase (DIO1) and iodothyronine deiodinase (DIO2) catalyze the removal of an iodine atom from the outer ring of T4 **(Farias-Serratos *et al.*, 2021; Suprayudi *et al.*, 2023)**. The iodothyronine deiodinases have selenium as an integral cofactor, because selenocysteine is a key component of their active sites **(Akturk *et al.*, 2013)**. As such, adequate selenium availability is required for optimum energy metabolism and growth. In this experiment, we found that Se supplementation dose exerts a statistically significant effect ($P < 0.05$) on liver IGF1 gene expression in *C. gariepinus*. The 4.0 ppm Se supplementation dose resulted in the highest level of IGF1 gene expression at 2.75 mean fold change. To our knowledge, this study is the first in *C. gariepinus* literature to evaluate and report gene expression effects of organic selenium supplementation, therefore the mentioned optimum dose regarding IGF1 expression is a valuable cornerstone for other aquaculture researchers to validate and elaborate in future experiments. The highest Se supplementation of 8.0 ppm was found to result in the lowest liver IGF1 gene expression. This may possibly be an indication of excessive selenium intake, which explains the inverse parabola relationship in our IGF1 data. This phenomenon is coherent with existing literature on selenium. As a micronutrient, selenium is required for normal body functioning in small amounts. Toxicity of excessive selenium intake in various animals have been extensively documented, in sheep (*Ovis aries* Linnaeus, 1758; **Hosnedlova *et al.*, 2017**), and broiler chicken (*Gallus gallus domesticus* Linnaeus, 1758; **Gautam *et al.*, 2017**). In addition to previously mentioned animals, the Japanese rice fish (*Oryzias latipes* Temminck and Schlegel, 1846**; Li *et al.*, 2008**) experienced high doses of Se. Selenium overdose exerts toxicity by several biological mechanisms. First, excessive selenium can trigger oxidative stress which then leads to mitochondrial dysfunction and (P13K)/Akt pathway disruption, ultimately resulting in cell damage and death **(Hu *et al.*, 2016; Huang *et al.*, 2020)**. It is worth noting that in the present experiment,

the seemingly over supplementation dose of 8.0 ppm results in lower IGF1 expression compared to the lowest supplementation dose of 0.5 ppm. This implies that catfish supplemented with the lowest Se dose did not suffer deficiency, hence it was the highest dose that was potentially excessive that appeared to be detrimental to liver IGF1 expression.

The liver has been well established in the literature as the metabolic powerhouse of fish and other animals **(Dong *et al.*, 2023)**. The role of the liver in lipid, carbohydrate and detoxification metabolic processes makes this organ pivotal in the optimal energy production, growth, health and reproduction of organisms **(Luan *et al.*, 2019; Souza *et al.*, 2023)**. In the present study, the effect of selenium supplementation on the liver was evaluated by measuring catfish liver weights and calculating the hepatosomatic index (HSI). Catfish group supplemented with 2.0 ppm Se had significantly higher HSI compared to the 0.5 ppm treatment group, 2.04 and 1.67 respectively ($P < 0.05$). This is an interesting finding because in pacu (*Piaractus mesopotamicus* Holmberg, 1887) as an example freshwater species, it was reported that Se supplementation ranging from 1-4 ppm did not significantly affect HSI **(Goes *et al.*, 2016)**. However, in that study, the selenium supplied was sodium selenite, an inorganic form of selenium. Probably, the higher bioavailability of organic forms of selenium **(Gawor *et al.*, 2020; Wu *et al.*, 2022)** such as selenomethionine that was used in this study induced a greater biological enhancement of liver tissue growth. This is perhaps due to the higher antioxidative protection activity **(Dalia *et al.*, 2017)** in liver cells that ultimately enabled greater hepatocyte function, maintenance and proliferation. As a result, the liver became more developed and the proportion of its mass relative to total body mass increased.

Catfish that received 0.5 ppm organic selenium supplementation appeared to be hyperglycaemic. Blood glucose level of the lowest supplementation dose group at 94.33mg/ dL was significantly higher than the levels of the 2.0, 4.0, 6.0 and 8.0 ppm supplementation group, which ranged from 26.00- 36.00mg/ dL ($P< 0.05$). In addition to the statistical significance, selenium supplementation dose was found to explain a significant proportion of the variation in catfish blood glucose level (R-squared linear = 0.917). This observation is coherent with literature data, where selenium has been documented to be an integral component of selenoproteins in the iodothyronine deiodinase (DIO) enzymes **(Ventura *et al.*, 2017)**. Iodothyronine deiodinases convert the inactive thyroid prohormone thyroxine (T4) into the active hormone iodothyronine (T3). When DIO abundance and activity are sufficient, circulating T3 levels in the body will be sufficient for optimal IGF1 expression **(Tan *et al.*, 2019)**. Research has shown that IGF1 plays a crucial role in glucose metabolism by upregulating glucose uptake into cells **(LeRoith & Yakar, 2007)**. This mechanism prevents hyperglycemia because circulating blood glucose is reduced and promotes growth as cells have more glucose molecules for metabolism into adenosine triphosphate (ATP) via glycolysis, the citric acid cycle and oxidative phosphorylation **(Keuper *et al.*, 2013; Swerdlow *et al.*, 2013; Nakagawa *et al.*, 2014)**. Subsequently ATP as cellular energy is then utilized intracellularly as a substrate for growth. Data generated in the present study indicate that selenium supplementation dose at 0.5 ppm did not provide sufficient stimulation and upregulation of DIO and subsequent downstream processes in the thyroid-glucose metabolism pathway. As a consequence, too much glucose circulates in blood and not enough absorbed into cells for growth.

This is in stark contrast with the data of the catfish groups that received supplementation doses above 2.0 ppm, where their blood glucose levels only ranged 26.00 - 36.00 mg/dL. This range is within the normal blood glucose level of *C. gariepinus*, which ranges between 30 and 45 mg/dL **(Adham, 2002)**. This implies in this catfish species, at least 2 ppm organic selenium supplementation is needed to avoid hyperglycaemia and maintain normal blood glucose level. *C. gariepinus* exposed to stress was shown to have significantly elevated blood glucose levels **(Adham, 2002)**. The contrastingly high blood glucose level of catfish supplemented with 0.5 ppm organic selenium in this study could indicate that the fish were experiencing some form of stress during rearing, that was not sufficiently compensated at that supplementation level. The highest glucose level in this study was measured at 94.33 mg/dL. From a biomedical standpoint, actually at this level, the fish was not considered diabetic when referring to the medical definition of diabetes of fasting blood glucose level of 126 mg/dL and above **(Alodhayani *et al.*, 2021)**, despite being relatively the highest. Selenium has been reported to have an insulin-like glycaemic regulation activity **(Hwang *et al.*, 2007; Gurbanov *et al.*, 2016)**. In the rat as a mammalian model, selenium uptake by adipocytes has been shown to induce translocation of glucose transporters that subsequently enhanced cellular glucose transport activity, upregulate cAMP phosphodiesterase activity and stimulate ribosomal S6 protein phosphorylation **(Ezaki, 1990)**. Selenium was demonstrated to support glucose metabolism by enhancing tyrosine kinase activity in the insulin signaling pathway **(McKenzie *et al.*, 2002)**. Furthermore, various proteins involved in signaling activities in the endoplasmic reticulum stress and insulin signaling pathways **(Hwang *et al.*, 2007)**. These literature findings collectively support data from the present study where insufficient dietary selenium intake could potentially be associated with hyperglycemia in *C. gariepinus*.

Optimum biological functioning and zootechnical performance of fish depends on both correct dietary intake and sufficient retention of nutrients. In the present experiment, catfish supplemented with 4.0 ppm selenium appeared to have the best ability to retain selenium. The 4.0 ppm group retained the highest level of selenium, at 18.53%. Looking at the distribution of the retention data of all selenium doses tested in this experiment, it appears that 4.0 ppm is the optimum dose because its corresponding selenium retention is the peak. Both lower and higher supplementation doses had lower retention rates for the micromineral. A study on broiler chickens documented a contrasting observation, where organic selenium retention decreased linearly as supplementation dose increased **(Yoon *et al.*, 2007)**. There is a difference in the method used to calculate selenium retention between the present and the aforementioned study. **Yoon *et al.* (2007)** measured the amount of excreted selenium by analyzing selenium content of their experimental chicken droppings, then calculated retention as the amount of selenium consumed subtracted by amount excreted, multiplied by 100 then dividing by amount of selenium consumed. This method of evaluating nutrient retention is typical of terrestrial animal studies, because excreta collection is relatively straightforward. The present study used a different approach that accommodated the unique challenge to collect excreta and analyze the nutrient contents in aquatic studies, due to leaching to the surrounding water body. In this study, selenium retention was calculated as biomass

at harvest multiplied by whole-body selenium content at harvest, subtracted by biomass at T0 multiplied by whole-body selenium content at T0, divided by total selenium consumed, then multiplied by 100. This approach uses the difference in actual selenium content of experimental catfish before and after selenium supplementation. This removed the need to measure selenium content of excreta with the aforementioned methodological limitation. This difference could explain the different selenium retention patterns between the two studies, apart from species differences in selenium metabolism. Considering the classic plateau effect or law of diminishing return in biological systems **(Ilan, 2019)**, the trend observed in the present study appears to agree, because selenium retention initially has a positive relationship with supplementation dose up to a maximum point, before decreasing as dose increases.

Considering the biomarker data discussed above, it appeared that organic selenium supplementation dose of 4.0 ppm gave the best metabolic and physiological performance in *Clarias* catfish under study. As expected, the highest average daily gain and subsequently final biomass was achieved by the 4.0 ppm supplementation group. The design and execution of this experiment appeared to be sound, as patterns in the metabolic markers were clearly expressed in the zootechnical parameters of the catfish in this study. This highlights the high quality of this study, as numerous other animal nutrition international publications also reported consistency between metabolic and zootechnical performance **(Takahashi *et al.*, 2018; Frota *et al.*, 2022; Sa *et al.*, 2023)**.

Future research in *C. gariepinus* and other freshwater fish species should explore other metabolically significant trace elements such as iodine, elucidate the metabolic pathways involved in their effects on health, growth and reproduction. The optimal supplementation dose of these trace elements in *C. gariepinus* and other aquaculture species would certainly need to be identified to produce industry applicable research data. Furthermore, considering the role of selenium as an antioxidant, successful triggering and occurrence of stress is crucial to evaluate the effectiveness of the micromineral in mitigating stress in aquaculture species. There is an urgent need for future research to establish a formal procedure to systematically induce stress in *C. gariepinus*, so that research on Clarias catfish stress responses and respective mitigation strategies can be more repeatable, reliable, credible and ultimately valuable to aquaculture science and industry.

CONCLUSION

Correct and precise micromineral nutrition, specifically selenium, have direct positive impacts on *C. gariepinus* gene expression, physiology and zootechnical performance. Organic selenium supplementation dose of 4.0 ppm resulted in the highest gene expression of IGF-1 which has an important role in optimal energy metabolism and protein utilization and consistent with this, subsequently manifested in mitigation of hyperglycaemia as dietary supply of glucose that was effectively uptaken by cells. Catfish receiving this dose of supplementation also showed the highest hepatosomatic index. This supplementation was also most effectively retained by the experimental catfish. Collectively, this biomarker data suggests enhanced metabolic balance of *Clarias* catfish receiving the 4.0 ppm selenium diet. Subsequently, the superior biomarker status was translated into higher growth rates and final biomass relative to the other dietary treatments.

To enhance growth efficiency and optimize *Clarias* catfish aquaculture economics, organic selenium supplementation at 4.0 ppm can be practically applied in the feed formulation for this species. The findings from this study sheds light on the vast opportunity for more discoveries in future regarding fish micromineral nutrition, particularly for freshwater aquaculture where literature data are relatively scarce.

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AUTHOR CONTRIBUTIONS

Stevanus Stevanus: Writing original draft, writing and editing revised versions, data collection, data management, exploratory data analysis, statistical data analysis, data visualisation, field operations, methodology
Dedi Jusadi: Conceptualisation, methodology, manuscript review
Agus Oman Sudrajat: Conceptualisation, methodology, manuscript review
Hasan Nasrullah: Conceptualisation, methodology, primer design, manuscript review
Muhammad Agus Suprayudi: Supervision, conceptualisation, methodology, manuscript review, project planning, project resource allocation, experimental fish acquisition

CONFLICT OF INTEREST STATEMENT

We the authors hereby declare that there were no conflicting financial interests or personal relationships that potentially influenced the findings reported in this paper.

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ACCESSIBILITY OF DATA

The data generated from this study are available from the corresponding author upon reasonable request.

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