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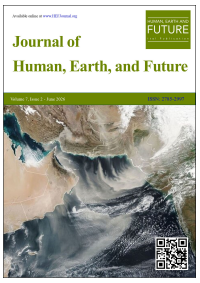

Eel Oil as a Nutritional Intervention: Enhancing Growth and Biochemical Profiles in Stunted Rat Models

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Abstract

Stunting is a major chronic nutritional disorder that requires effective nutritional interventions to improve growth and metabolic outcomes. This study aimed to analyze the potential of eel (*Anguilla bicolor*) oil supplementation as a lipid-based nutritional intervention to enhance growth and biochemical profiles in stunted male Wistar rats. A completely randomized design experiment was conducted using 35 male rats, which were divided into seven treatment groups based on different supplementation doses. The observed variables included body weight, body length, hemoglobin concentration, liver function (SGOT–Serum Glutamic Oxaloacetic Transaminase and SGPT–Serum Glutamic Pyruvic Transaminase), kidney function (urea and creatinine), and insulin growth factor-1 (IGF-1) level. The data were analyzed using One-Way Analysis of Variance followed by Duncan's post hoc-test with a significance level of 0.05 The results showed that eel oil supplementation significantly improved body weight, body length, and hemoglobin concentration ($p<0.05$), whereas liver enzyme activity and creatinine levels showed no significant differences among groups ($p>0.05$), indicating maintained organ function in rats. Elevated urea levels observed in stunted rats decreased following supplementation. These findings indicate that eel oil supplementation improves growth-related indicators without adversely affecting liver and kidney function, suggesting its potential as a promising lipid-based nutritional intervention for stunting prevention.

Keywords: Eel Oil; Body Weight; Hemoglobin; Nutritional Intervention; Male Rats.

1. Introduction

Stunting is a form of chronic malnutrition caused by prolonged inadequate nutrient intake, leading to impaired growth and development in children [1]. Indonesian nutritional status survey reported that the percentage of stunted children decreased from 21.5% in 2023 to 19.8% in 2024, although further reduction was expected to meet the national goal of 18.8% by 2025. Compared to acute diseases, stunting does not cause immediate mortality but leads to short and long-term detrimental effects, including cognitive impairment, diminished physical capacity [2], and decreased productivity in adulthood [3]. This shows the need for adequate, diverse, and balanced nutrition as a preventive method. Animal-source foods such as fish are recognized to provide a broader range of essential nutrients compared to plant-based foods, thereby supporting optimal growth and development during early life [4].

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Fish is one of nutritious foods from animal sources, providing high-quality protein, fatty acids, vitamins, and minerals that contribute significantly to children growth [5]. However, conventional cooking with high temperatures leads to the degradation of nutrients in fish. This issue can be overcome through the extraction of fish into oil, a practical alternative to preserve nutrients, particularly the omega-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [6]. Fish oil consumption is associated with several health benefits, including improved growth and development [7], cognitive performance [8], and maternal-child health outcomes [9]. Several studies have shown that fish oil supplementation with omega-3 and omega-6 fatty acids improves human anthropometric outcomes and hemoglobin levels without compromising organ function [10, 11]. The results show that fish oil supplementation represents a promising strategy for the prevention of stunting. However, most of the studies in this field focused on marine-derived fish oil, whereas the potential of freshwater fish oil as a lipid-based nutritional intervention to improve growth performance and biochemical profiles remains underexplored.

Eel (*Anguilla bicolor*) is a freshwater fish widely distributed in Indonesia, which contains high amount of monounsaturated fatty acids (MUFAs), balanced omega-6 fatty acids, and moderate omega-3 fatty acids [12, 13]**.** Unlike marine fish oil, eel oil provides a distinct fatty acid composition that may offer complementary metabolic effects. MUFAs have been associated with enhanced mitochondrial efficiency and improved nutrient partitioning toward lean tissue accretion which is essential during recovery from growth restriction. Omega-6 fatty acids play a central role in membrane biogenesis and growth-related signaling pathways necessary for tissue development. The coexistence of MUFAs and polyunsaturated fatty acids (PUFAs) suggests that eel oil may facilitate anabolic recovery through integrated lipid-mediated mechanisms rather than through omega-3 activity alone [13-15].

Eel oil has been explored for various health benefits, including as a functional supplement for cardiovascular disease [9] and as an anti-inflammatory agent in experimental models in a rat model of rheumatoid arthritis induced by complete Freund's adjuvant [11, 13]. Studies have also been conducted on eel processing into flour for high-protein biscuits as a nutritional intervention for stunted children [14]. Eel oil has been investigated for many cases in experimental rat models; its specific role in modulating growth performance and growth-related biochemical markers under stunting conditions in rats remains insufficiently explored.

In nutritional and biological studies, Wistar rats are extensively used due to their well-defined physiology, repeatability, and accessibility. Therefore, this study selects male Wistar rats aged 3 weeks, a critical developmental stage for a strong analogy to early childhood in humans of about 2 years. The age range correlates with the post-weaning period and is recognized as a highly vulnerable phase for the onset of growth faltering [15]. The selection is focused on mitigating the impact of hormonal fluctuations associated with the estrous cycle in females, which can obscure the growth phase and biochemical results. Furthermore, food restriction is applied to establish stunting in Wistar rats, offering a dependable experimental method for investigating growth impairment and associated nutritional modulation [16]. In the context of this established stunted Wistar rats model, eel oil intervention is comprehensively evaluated. The investigation focuses on key anthropometric indicators such as body weight and length as direct measures of catch-up growth [17, 18].

Beyond the growth-related parameters, this study incorporates a multifaceted biochemical analysis to show a holistic picture of the intervention's efficacy and safety. The analysis includes assessing the hematological profile through hemoglobin levels, as a major indicator of nutritional anemia often associated with stunting [19]. Serum Insulin-like Growth Factor-1 (IGF-1) is also measured, a primary endocrine mediator of growth hormone action that provides a molecular insight into the growth-promoting mechanisms [20]. This is followed by monitoring the safety of prolonged eel oil supplementation by evaluating established clinical toxicity markers of liver functions (SGOT (serum glutamic oxaloacetic transaminase)/AST and SGPT (serum glutamic pyruvic transaminase)/ALT and kidney functions (urea and creatinine), particularly when considering novel lipid-based nutritional sources. Therefore, this study introduces novelty by examining eel oil derived from freshwater sources as an alternative for lipid-based nutritional intervention that simultaneously evaluates growth performance, growth-related biochemical markers, and organ safety parameters in stunted rat models. The findings are expected to contribute to the development of lipid-based nutritional strategies for stunting prevention and to provide new insight into the therapeutic potential of eel oil.

2. Materials and Methods

2.1. Eel Oil Extraction Process

The main material used was eel collected from a cultivation pond in Bogor Regency, West Java, Indonesia. Fresh eel samples were collected and cleaned by removing the head, skin, gills, stomach, and bones to obtain fillets, then washed with distilled water before processing. Fillets were cut into small cubes (approximately 2 × 2 cm), washed several times, drained, and steamed for 5 min. Subsequently, steamed fillets were dried at 40°C for 16 h, and minced using a blender. A sample of 10 g grounded fillet was packed in a plastic clip and stored in a desiccator for the extraction process. Eel oil was extracted from dried fillets using the reflux method [21]. Approximately 10 g of dried eel fillet was placed in a three-neck flask, and n-hexane was added at a solvent-to-solid ratio of 12.21:1 mL/g. The

extraction was conducted at 70°C for 70.56 min with continuous temperature monitoring in the process. The mixture obtained was filtered through Whatman No. 41 filter paper, and the solvent was evaporated using a rotary evaporator at 60°C for 7 min. The resulting crude eel oil was collected in 10 mL amber glass bottles and stored at 4°C until further use. This study used the optimized conditions, which included temperature, extraction time, and solvent-to-solid ratio.

2.2. Refining

The refining process of crude eel oil was performed through four sequential stages, namely degumming, neutralization, bleaching, and deodorization [22-24]. In this study, zeolite was selected as the bleaching adsorbent at a concentration of 9%, based on preliminary experiments that compared acid, peroxide, and saponification. The results showed that refining with 9% zeolite produced eel oil that met the chemical quality standards of the Indonesian National Standard (SNI). Degumming was carried out by mixing crude eel oil with 1% citric acid and heating at 60°C under continuous stirring (500 rpm) for 10 min. Subsequently, 20% distilled water (w/w of oil) was added, followed by heating at 60°C with stirring at 500 rpm for another 10 min. The mixture was centrifuged at 5500 rpm for 15 min to separate the hydrated gums. Neutralization was conducted by adding 5% sodium hydroxide solution (1 N, w/w of oil), followed by heating at 60°C, and stirring at 500 rpm for 30 min. The mixture was then centrifuged at 5500 rpm for 15 min to separate the soap stock. Bleaching was conducted by adding 9% zeolite (w/w of oil) to the neutralized oil and heating at 70°C with continuous stirring (500 rpm) for 60 min. After treatment, the oil was centrifuged at 5500 rpm for 15 min to remove the adsorbent. The refined oil was stored in amber glass bottles at 4°C and analyzed for fatty acid profile according to AOAC 991.39-2005 method using gas chromatography (Shimadzu GC-2030) with a flame ionization detector (FID).

2.3. Experimental Animals and Design

A total of 35 Wistar rats (*Rattus novergicus*) aged 3-4 weeks with an initial body weight of 123–128 g were used as experimental animals. During this study, welfare of animals was supervised by a veterinarian, who monitored the health status of rats twice per week. Before intervention, all rats were acclimatized for 7 days in cages with proper ventilation. The cages (43 × 38 × 17 cm) were made of polycarbonate and bedded with wood shavings, which were replaced twice weekly, with the design shown in Figure 1.

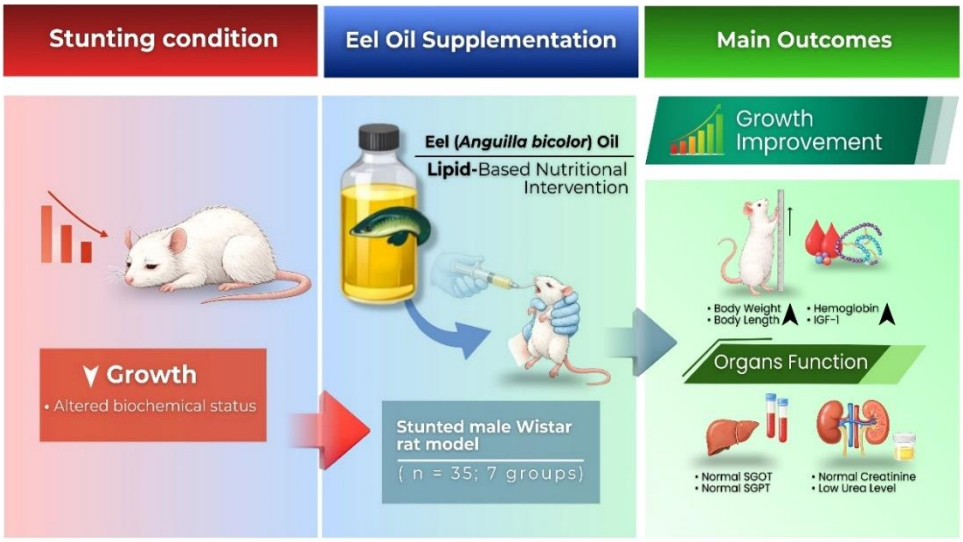


Figure 1. Study design for the experiment with Wistar rats

During the acclimatization phase, rats were given normal feed containing 14% moisture, 8% ash, 19.18% crude protein, 8% crude fat, 7% crude fiber, 1.2% calcium, 1% phosphorus, 0.7% lysine, 0.3% methionine, and 0.5% methionine+cystine. Drinking water was supplied ad libitum using bottles with ball-valve sipper tubes and replenished daily to prevent microbial contamination. Before treatment administration, the initial body weight of individual rats was measured to ensure accurate dosage determination. The sample size was determined using Federer's formula for experimental designs to ensure adequate error degrees of freedom for analysis of variance (ANOVA), expressed as:

$$(t-1)\,(r-1)\;\geq 1 \tag{1}$$

where t represents the number of treatment groups, and n represents the number of experimental units per group. With seven treatment groups (t = 7), the calculation yielded a minimum requirement of four animals per group. To anticipate potential drop-out during the experimental period, one additional animal was included in each group, resulting in five rats per group and a total of 35 rats. The eel oil doses (Table 1) were derived from the recommended human dosage of

the commercial fish oil emulsion (15 mL/day), which served as the positive control reference in this study. Dose translation from human to rat was performed using proportional body weight adjustment and guided by body surface area (BSA) normalization principles commonly applied in interspecies dose conversion [25].

Based on the average body weight of 200 g per rat, the human-equivalent dose was converted to a rat-equivalent range to ensure physiologically relevant intake levels [26]. To systematically evaluate potential dose–response relationships, the converted reference dose was subsequently arranged in a two-fold incremental design (allowing assessment of submaximal, moderate, and higher supplementation levels while remaining within a nutritionally relevant and metabolically safe range). Both normal and stunted rats were provided with the same diet formulation, but differed in the amount administered. Feed restriction (50% of standard intake) was applied only during the stunting induction phase. After the stunted phenotype was established, all rats were returned to the standard feeding.

Table 1. Experimental groups and treatments

Group (K)	Treatment	Description
K1	Normal feed without oil supplementation	Normal group
K2	Without eel oil supplementation	Negative control
K3	Commercial fish oil emulsion supplementation	Positive control
K4	Eel oil supplementation 0.02 mL/200 g/day	Treatment 1
K5	Eel oil supplementation 0.04 mL/200 g/day	Treatment 2
K6	Eel oil supplementation 0.08mL/200 g/day	Treatment 3
K7	Eel oil supplementation 0.16 mL/200 g/day	Treatment 4

During the intervention period, feed restriction was discontinued and all stunted groups (K2–K7) received standard feeding at normal intake levels, while the normal control group (K1) received unrestricted feeding throughout the experiment. Feed was provided 6 h after oil administration to ensure gastric emptying and to optimize nutrient absorption. Eel oil was administered once daily by oral gavage for 30 consecutive days at the designated doses to ensure accurate and consistent delivery of the intervention. To minimize potential confounding effects, the feeding schedule and dietary management were maintained consistently across all experimental groups during the intervention phase. Therefore, any observed differences in growth performance and physiological responses were attributed primarily to the type and dose of oil supplementation administered.

Fatty acid profiles of eel oil are presented in Table 2. These fatty acids are the main nutritional part that cause biological impacts on growth and metabolism [27]. For comparison, a commercially available fish oil emulsion was used as a positive control. The fish oil emulsion contained approximately 22% fish oil per 15 mL serving, equivalent to 3 g of fish oil in ~13.8 g of total emulsion. Omega-3 fatty acids in fish oil emulsion were approximately 8.5% of the total fish oil, providing a total of 255 mg omega-3 per serving, consisting of 135 mg DHA and 120 mg EPA. This information was obtained from the product packaging label.

Table 2. Fatty acid composition of eel oil (Anguilla bicolor)

Molecular formula	Compound name	Retention time	% area
$C_{12}H_{24}O_2$	Lauric acid	20.282	0.1415
$C_{14}H_{28}O_2$	Myristic acid	25.103	3.9341
$C_{15}H_{30}O_2$	Pentadecanoic acid	27.920	0.7120
$C_{16}H_{32}O_2$	Palmitic acid	30.884	22.8708
$C_{17}H_{34}O_2$	Margaric acid	33.950	0.3379
$C_{18}H_{36}O_2$	Stearic acid	37.812	3.8859
$C_{20}H_{40}O_2$	Arachidic acid	43.280	0.1494
$C_{21}H_{42}O_2$	Heneicosanoic acid	46.631	0.6825
Saturated Fatty Acid (SFA)		**31.5357**	
$C_{16}H_{30}O_2$	Palmitoleic acid	32.242	4.7854
$C_{17}H_{32}O_2$	Heptadecenoic acid	35.524	0.2441
$C_{18}H_{34}O_2$	Oleic acid	37.182	45.2505
$C_{18}H_{34}O_2$	Elaidic acid	38.357	0.1727
$C_{20}H_{38}O_2$	Gondoic acid	44.521	1.3558
$C_{22}H_{42}O_2$	Erucic acid	51.440	0.6825

Monounsaturated fatty acid (MUFA)			**52.491**
$C_{18}H_{32}O_2$**	Linoleic acid	40.378	12.3907
$C_{18}H_{32}O_2$**	Linolelaidic acid	39.032	0.0561
$C_{18}H_{30}O_2$**	Gamma-linolenic acid (GLA)	41.759	0.3853
$C_{18}H_{30}O_2$*	Alpha-linolenic acid (ALA)	42.873	1.3661
$C_{20}H_{34}O_2$*	Eicosatrienoic acid	49.105	0.1127
$C_{20}H_{34}O_2$**	Dihomo-gamma-linolenic acid (DGLA)	47.998	0.5991
$C_{20}H_{34}O_2$**	Arachidonic acid	48.925	0.7363
$C_{20}H_{30}O_2$*	Eicosapentaenoic acid (EPA)	50.486	0.3270
Polyunsaturated fatty acids (PUFAs)			**15.9733**
Omega-6**			**14.1675**
Omega-3*			**1.8058**

2.4. Growth Performances

The evaluation of growth parameters included weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), length gain (L), and specific length growth rate (SLGR). These parameters were determined using the following equations:

W_g = final body weight (g) – initial body weight (g) (2)

L = final body length (cm) – initial body length (cm) (3)

SGR = $(\ln f_w - \ln I_W)/t \times 100\ \%$ (4)

SLGR = $(\ln f_L - \ln I_L)/t \times 100\%$ (5)

FCR = $total\ weight\ of\ feed\ (g)/total\ weight\ gain\ (cm)$ (6)

In the equation, ln f_w is the natural logarithm of rats' weight (g) at the end of the experiment, ln I_w is the natural logarithm of rats' weight (g) at the start of the experiment, and t is the experiment duration (days). Beside ln f_L is the natural logarithm of rats' length (cm) at the end of the experiment and ln I_L is the natural logarithm of rats' length (cm) at the start of the experiment.

2.5. Analysis of Hemoglobin Concentration, Liver, Kidney Function, and Insulin-Like Growth Factor-1 Levels

After completion of the experimental treatment, rats were euthanized using the cervical dislocation method. Immediately after euthanasia, the thoracic cavity was opened using surgical scissors, and blood was collected directly from the left ventricle. A total of 2.5 mL of blood was obtained and divided into two vacutainer tubes, comprising 1.3 mL in a plain tube and 1.2 mL in an EDTA. These tubes were centrifuged for 3 minutes at 2000 rpm, followed by serum and plasma separation, which was into microtubes for storage at –30°C before analysis. Muscle samples were also collected, rinsed with NaCl solution to remove residual blood, and placed into a fixation solution. Blood samples were analyzed for hemoglobin concentration, standard liver function tests (SGOT, SGPT), and kidney function markers (creatinine, urea). Hemoglobin concentration was analyzed using the Hemometer Sahli method, while liver and kidney function were analyzed using a commercial diagnostic kit (YSENMED). IGF-1 concentrations were determined using an ELISA kit (BZ-22186311-EB, Bioenzy). The tissue samples were homogenized in phosphate-buffered saline (PBS), centrifuged to obtain the supernatant, and subjected to sequential incubation with specific antibodies, streptavidin-HRP conjugate, and tetramethylbenzidine substrate, followed by absorbance measurement at 450 nm using an ELISA reader.

2.6. Statistical Analysis

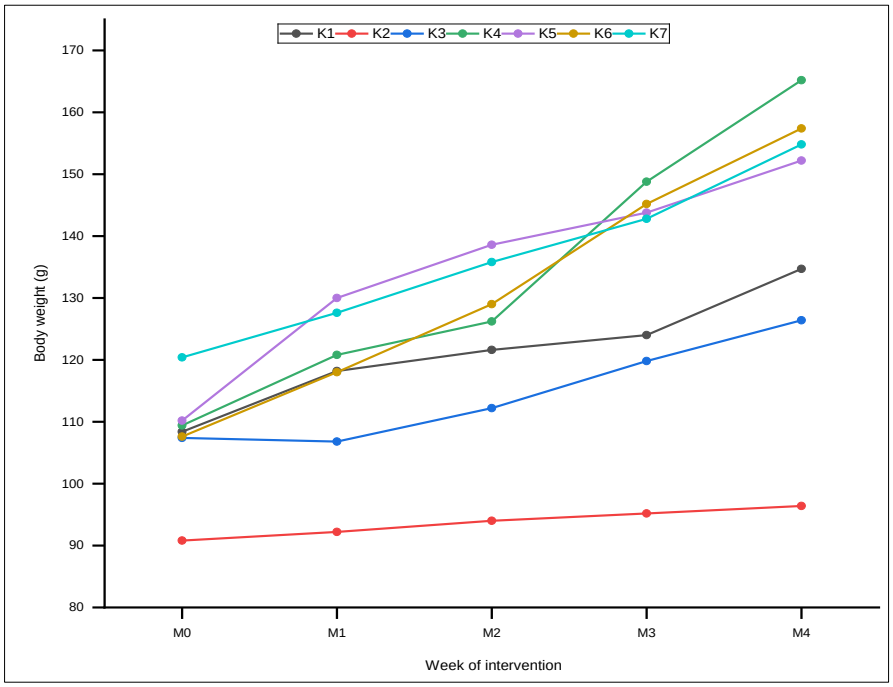
All data were presented as mean ± standard deviation (SD), while statistical analyses were performed using SPSS software. Data distribution was assessed using the Shapiro–Wilk normality test. The association between eel-fish oil supplementation and stunting incidence was examined using the Chi-square test. Subsequently, one-way analysis of variance (Anova) was applied to evaluate the effects of supplementation on growth and biochemical parameters. Duncan's multiple range test was used for post hoc comparisons when ANOVA showed significant differences. Tukey's HSD test was applied to confirm homogeneous subsets for parameters with non-significant ANOVA results. All statistical significance was set at $p < 0.05$, and data analysis was performed using SPSS version 22.

3. Results and Discussion

3.1. Growth Performance

In this study, the body weight and length were monitored weekly to evaluate the effects of eel oil supplementation, with a progressive increase shown in Figures 2 and 3. The results indicated that eel oil supplementation significantly affected the growth performance of stunted rat models. The negative control group (K2), which received 50% feed restriction without oil supplementation, showed the lowest increase in body weight and length in the experimental period. In comparison, groups supplemented with eel oil (K4-K7) showed significant improvements in both body and body length ($p<0.05$). All treatment doses (K4–K7) had statistically similar growth responses, although K4 showed a numerically higher mean growth value than the positive control (K3). The minimal growth observed in the K2 group indicates that the absence of nutritional intervention led to prolonged growth failure.

Weekly body weight assessment showed distinct growth patterns across the groups (Figure 2). In the intervention, all groups gained weight, while K4–K7 had significantly greater gains than both K2 and K3. Specifically, K2 consistently showed minimal growth increase. The limited weight recovery in K2 aligns with contemporary findings in nutritional rehabilitation research, where refeeding without targeted nutrient optimization does not consistently restore growth trajectories following early-life restriction [28]. Recent animal and translational models emphasized that catch-up growth depends not only on caloric adequacy but also on nutrient density and quality, particularly lipid composition [29]. Among eel oil groups, K4 achieved the highest weight gain, reaching approximately 167 g by week 4, followed by K6 and K7, which exceeded K1 and K3. Long-chain polyunsaturated fatty acids, especially omega-3 fractions, are increasingly recognized for their involvement in metabolic regulation, anabolic signaling, and efficient energy partitioning toward tissue synthesis. Experimental studies published within the last five years report that omega-3–containing nutritional interventions improve growth performance and metabolic efficiency during rehabilitation phases [30].



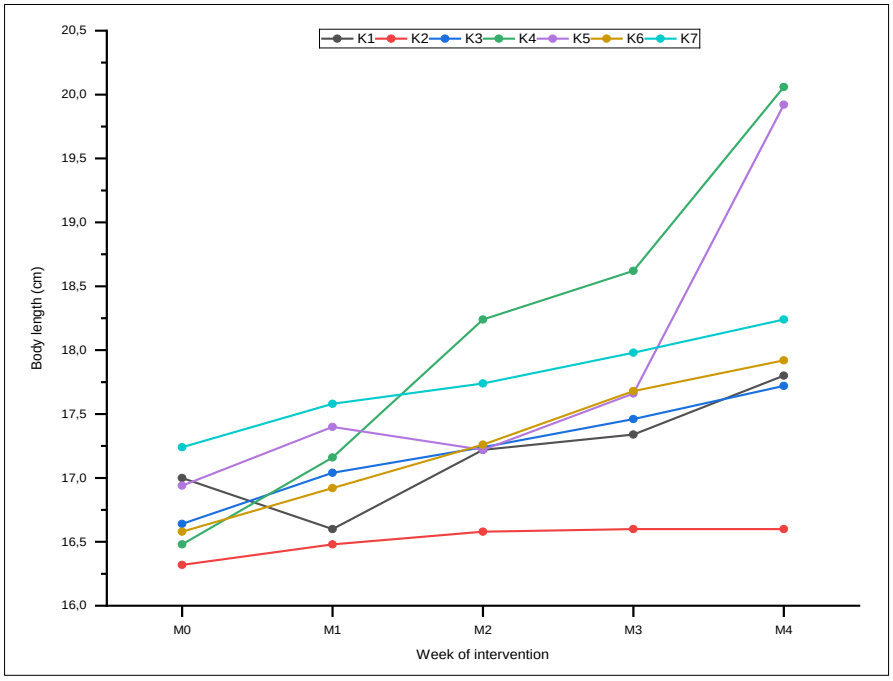
Notes: M0 represents day 0 of treatment initiation, while M1–M4 indicate weeks 1–4 of supplementation. Data are presented as mean ± standard deviation (n=5 per group). Different superscript letters indicate significant differences among treatment groups according to Duncan's multiple range test following one-way ANOVA ($p<0.05$). K1 = normal control group without treatment; K2 = stunted rats without treatment; K3 = stunted rats supplemented with commercial fish oil (Scott's Emulsion); K4–K7 = stunted rats supplemented with eel oil at doses of 0.02, 0.04, 0.06, 0.08, and 0.16 mL/200 g body weight.

Figure 2. Mean body weight gain (g) in stunted male Wistar rats during five weeks of eel oil supplementation

Weekly measurements of body length also demonstrated progressive increases across groups; however, the pattern differed from that observed in body weight. The length-gain profile showed that eel fish oil effectively enhanced linear growth, particularly from weeks 2 to 4. Groups K4 and K5 experienced the greatest linear growth, exceeding the normal and positive controls by week 4. In comparison, K2 showed minimal improvement, confirming impaired

growth under stunting conditions. Significant improvement observed in groups K4–K6 indicated a strong growth-promoting effect of eel fish oil, possibly mediated by enhanced nutrient use and metabolic recovery in previously stunted animals.

Restoration of linear growth is more important than short-term weight gain in stunting, as impaired body length reflects long-term chronic nutritional insufficiency. Contemporary rehabilitation studies indicate that recovery of height or length requires adequate intake of essential nutrients, particularly long-chain fatty acids, which support cell proliferation and skeletal development [31-33]. Optimization of essential fatty acid-based nutritional supplementation may therefore facilitate structural growth recovery during malnutrition treatment [34, 35]. The significant improvement observed in the K4 and K5 groups further highlights the importance of nutrients composition in supporting skeletal recovery. This finding is consistent with recent evidence suggesting that skeletal growth recovery involves both endocrine and metabolic processes [36-38]. Taken together, the present results suggest that eel oil supplementation promotes multidimensional growth recovery following stunting induction, reflected by improvements in both body weight and linear growth.



Notes: M0 represents day 0 of treatment initiation, while M1–M4 indicate weeks 1–4 of supplementation. Data are presented as mean ± standard deviation (n=5 per group). Different superscript letters indicate significant differences among treatment groups according to Duncan's multiple range test following one-way ANOVA ($p<0.05$). K1 = normal control group without treatment; K2 = stunted rats without treatment; K3 = stunted rats supplemented with commercial fish oil (Scott's Emulsion); K4–K7 = stunted rats supplemented with eel oil at doses of 0.02, 0.04, 0.06, 0.08, and 0.16 mL/200 g body weight.

Figure 3. Mean body length gain (cm) in stunted male Wistar rats during five weeks of eel oil supplementation

Eel oil supplementation can function as a preventative nutrition against stunting. In this study, each treatment group reported varying degrees of body weight and length gain, leading to obvious disparities in growth performance, as shown in Tables 3 and 4. The increase in body weight was validated by the growth performance metrics, including high weight gain (WG) and specific growth rate (SGR), along with feed conversion ratio (FCR). FCR values in groups K4, K5, K6, and K7 were lower compared to K1, K2, and K3 (Table 3). Previous studies reported that lower FCR values generally indicated better growth efficiency, showing the positive impact of dietary interventions [39, 40].

The standard rats feed used in this study provided a balanced proportion of carbohydrates as the primary energy source to support weight recovery, while dietary protein supplied the essential amino acids required for lean tissue synthesis. The lipid fraction, particularly the essential fatty acids contributed by eel oil supplementation, further enhanced metabolic efficiency by improving lipid oxidation and increasing metabolic sensitivity. A previous report stated that feed with an adequate carbohydrate and protein balance tended to produce lower FCR values due to the supply of optimal substrates for both energy production and nitrogen retention during growth. Additionally, feed enriched with unsaturated fatty acids was shown to improve energy use and reduce protein catabolism, enhancing FCR outcomes in animals receiving nutritional rehabilitation [41-44].

Table 3. Growth performance of stunted rats after eel oil supplementation

Treatment group	Growth variable				
	IW(g)	FW (g)	WG (g)	SGR (%/week)	FCR
K1	113.4±14.80	134.80±14.77^{bc}	21.5±1.12^{ab}	0.58±0.07^{ab}	16.96±2.09^{b}
K2	89.20±1.92	96.40±3.64^{a}	7.20±2.17^{a}	0.26±0.07^{a}	43.55±15.15^{c}
K3	93.00±3.08	126.40±9.74^{b}	33.40±9.63^{c}	1.01±0.25^{c}	10.71±1.92^{ab}
K4	92.20±3.76	165.20±18.48^{d}	73.00±17.94^{d}	1.92±0.39^{d}	5.82 ±1.65^{d}
K5	89.60±1.67	152.20±24.86^{cd}	62.60±25.32^{d}	1.72±0.61^{d}	8.00±4.92^{d}
K6	88.80±7.01	157.40±22.28^{cd}	68.60±23.69^{d}	1.88±0.55^{d}	6.25±2.05^{d}
K7	88.80±4.20	154.80±19.52^{cd}	66.00±20.43^{d}	1.83±0.48^{d}	6.72±2.25^{d}

Note: Data are expressed as mean ± standard deviation (SD). Values in the same column with different superscripts indicate significant differences ($p < 0.05$). Notes: IW=initial weight; FW=final weight; WG=weight gain; SGR=specific growth rate; FCR=feed conversion rate.

The examination of linear growth, determined by the difference between the final and first week, demonstrated a unique dose-dependent variation. Duncan post-hoc test classified K4 and K5 into a separate homogeneous subset, demonstrating that these two groups displayed considerably higher increases in body length compared to K2, K1, K7, K3, and K6 ($p < 0.05$). K4 exhibited the highest increment of mean length (3.80 cm), followed by K5 (3.38 cm), which indicated that moderate eel oil feeding facilitated the highest structural recovery following stunting induction (Table 4). Conversely, the highest supplementation dose (K7) did not demonstrate significant superiority of linear growth compared to other treatments. This pattern suggests that the linear growth response may reach a maximum within an appropriate supplementing range instead of continuously increase with higher doses. These findings correspond with modern rehabilitation models, indicating that catch-up growth in skeletal dimensions relies on sufficient, yet not excessive of nutrient availability, especially for essential fatty acids and overall dietary quality.

Eel oil administered to stunted rats contains essential fatty acids required to support growth and development. Generally, fish oil is considered an important dietary source of polyunsaturated fatty acids (PUFAs). The omega-3 fatty acids present in eel oil are suggested to enhance both body weight and length, thereby contributing to the restoration of impaired growth processes associated with stunting [45]. Eel oil supplementation provides omega-3 and omega-6 essential fatty acids that function synergistically to support growth recovery in stunted rats. Omega-3 fatty acids [32] specifically contribute to improved growth by enhancing metabolic efficiency, promoting lipid oxidation, and stimulating anabolic pathways such as IGF-1 signaling, which is fundamental for tissue accretion and linear growth [46]. Anti-inflammatory and antioxidant effects also help create a physiological environment conducive to catch-up growth during nutritional rehabilitation [47]. Complementarily, omega-6 fatty acids support growth by providing substrates for cell membrane synthesis, which facilitates adipose tissue development, and generate eicosanoid mediators included in muscle protein turnover and lean mass gain [48].

Recent results suggest that an appropriate balance of omega-3 and omega-6 intake enhances metabolic recovery in malnourished animal models by optimizing energy use, improving nitrogen retention, and restoring disrupted anabolic processes [35]. The synergistic actions of omega-3 and omega-6 fatty acids in eel oil play a critical role in reversing growth impairment associated with stunting and promoting effective catch-up growth [49]. The results suggest that eel oil augment the body mass and enhances growth performance. This is in line with previous studies that omega-3 fatty acid plays a role in improving nutritional status and growth performance [50, 51]. The examination of body length gain showed that each treatment group had unique growth responses, causing a significant variation in linear growth rates. Rats in the negative control group showed the significant lower length as compared to the treatment groups at a dose of 0.02 mL/200 g/day and 0.04 mL/200 g/day. Table 4 shows detailed information about the average body length gain by stunted rats in each group.

Table 4. Growth performance of stunted rats based on body length

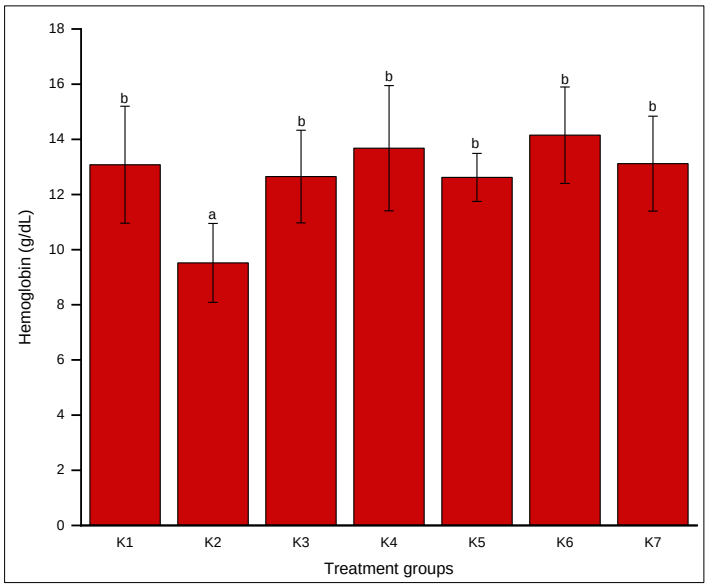
Treatment group	Growth variable			
	IL (cm)	FL (cm)	L (cm)	SLGR (% /week)
K1	16.84±0.49	17.80±0,60^{ab}	0.96±0.11^{a}	0.18±0.01^{a}
K2	16.08±0.73	16.60±0.55^{a}	0.52±0.32^{a}	0.10±0,06^{a}
K3	16.36±1.10	17.72±1.29^{ab}	1.36±0.71^{a}	0.27±0.13^{a}
K4	16.26±0.98	20.06±1.42^{c}	3.80±1.78^{b}	0.69±0.32^{b}
K5	16.54±0.64	19.92±1.27^{c}	3.38±1.51^{b}	0.62±0.27^{b}
K6	16.46±0.95	17.92±1.13^{ab}	1.46±0.85^{a}	0.28±0.17^{a}
K7	16.92±0.43	18.24±1.49^{b}	1.32±0.61^{a}	0.25±0.11^{a}

Note: Data are expressed as mean ± standard deviation (SD). Values in the same column with differing superscripts denote significant differences ($p<0.05$), as established by Duncan's test. Notes: IL=initial length; FL=final length; L=Length; SLGR=specific length growth rate.

Although body length does not generate multiple growth indices like body weight, it serves as a critical marker of linear growth and skeletal development, which are essential in stunting [52-54]. The consistent improvement observed in body length, particularly in K4, showed the role of eel oil in enhancing catch-up growth. Furthermore, the parallel increase in body weight and length indicated that the supplementation had a comprehensive effect on somatic and linear growth. The superior performance of K4 compared to higher-dose groups suggested a nutrient threshold effect, where modest supplementation levels produced optimal benefits, while higher doses did not yield proportional improvements due to metabolic saturation or feedback regulation. These results show that eel oil supplementation improves body weight alongside derived growth variables such as WG, SGR, and FCR, including linear growth as measured by increased body length. The dual improvement presents eel oil as a promising nutritional intervention capable of addressing both somatic and linear growth deficits, thereby supporting a potential role in stunting prevention strategies.

3.2. Hemoglobin Concentration

The improvement of nutritional status, assessed using anthropometric measurements, is essential for measuring growth and development. To comprehensively assess nutritional status, there is a need to examine supplementary parameters, including hemoglobin concentration [55]. Therefore, this study evaluated hemoglobin concentration to assess the efficacy of eel fish oil in enhancing oxygen transport capacity, with the results shown in Figure 4.



Note: Values are expressed as mean ± SD (n=5 per group). Different superscript letters above the bars indicate significant differences among treatment groups ($p<0.05$).

Figure 4. Hemoglobin concentration of stunted rats after eel oil supplementation.

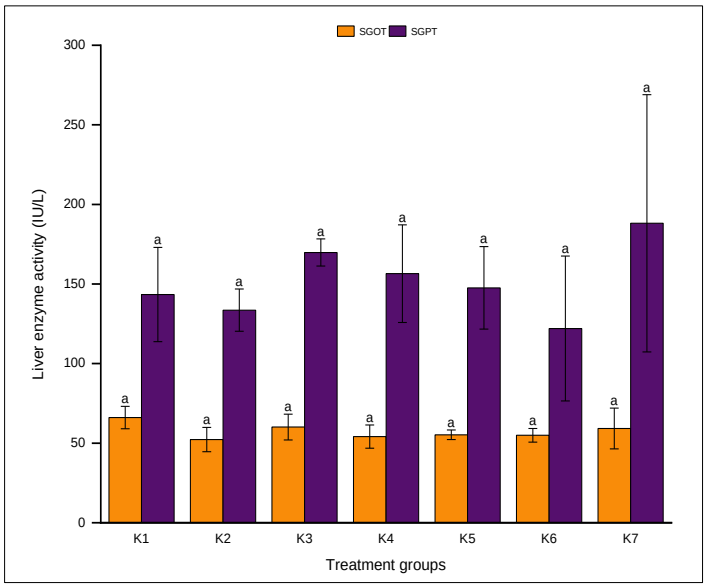
Eel oil supplementation increased hemoglobin concentration in stunted rats compared to K2 ($p<0.05$). Hemoglobin concentrations in K3–K7 were significantly higher than in K2 and were statistically comparable to K1 ($p< 0.05$). These results showed that eel oil supplementation played a role in improving hematological status in stunted rats. The improvements observed in growth performance were accompanied by significant changes in hemoglobin levels. As a major biomarker of nutritional status, hemoglobin shows both protein–energy balance and micronutrient adequacy, particularly iron availability [56]. Recent research on nutritional rehabilitation indicates that lipid-based and nutrient-rich therapies can enhance hemoglobin levels in undernourished groups [57]. In this study, eel oil supplementation significantly increased hemoglobin concentrations in stunted rats compared to the negative control group ($p<0.05$). Among the treatment groups, K4, K5, K6, and K7 showed hemoglobin values that were comparable to the normal and positive control groups. This indicated that eel oil supported the recovery of hematological status in malnourished animals.

These results are consistent with previous studies, showing that omega-3 fatty acids and fish oil supplementation can improve hematological profiles by enhancing erythropoiesis and reducing oxidative stress [19]. In malnourished models, improved hemoglobin is attributed to better nutrient use and protein metabolism, as shown by high growth variables (WG, SGR, FCR). In this study, the protein and PUFAs content of eel oil enhanced iron absorption and hemoglobin synthesis, consistent with reports that nutritional interventions rich in high-quality protein and essential fatty acids can restore hemoglobin levels in growth-retarded rats [58, 59].

3.3. Liver Function

The improvement in hemoglobin levels further shows the ability of eel oil to restore hematological status in stunted rats. However, growth recovery cannot be explained only by hematological outcomes, suggesting the need to evaluate organ function and growth mediators [60]. The improvement in growth performance and hematological status was further complemented by the assessment of liver [61] and kidney functions [62], including IGF-1 as a marker of growth performance [63]. Evaluating these parameters is essential to ensure both the safety and mechanistic plausibility of eel oil supplementation in stunted rats. Liver enzymes are well-established indicators for assessing hepatic integrity and metabolic function in experimental animals. SGOT and SGPT are sensitive markers of hepatocellular injury, with excessive increases potentially signifying hepatic stress or toxicity [64]. The measurement of liver functions is presented in Figure 5.

Serum liver enzyme analysis demonstrated that SGOT (AST) and SGPT (ALT) activities did not differ significantly among experimental groups at the end of the intervention period ($p > 0.05$). All measured values remained within the established physiological reference range for Wistar rats, indicating preserved hepatic integrity across treatments. Given that these enzymes are widely used as indicators of hepatocellular injury, the absence of elevation in supplemented groups suggests that eel oil administration did not induce hepatic stress or toxicity during the intervention period.



Note: Values are expressed as mean ± SD (n = 5 per group). No different superscript letters above the bars indicate significant among treatment groups ($p > 0.05$).

Figure 5. Liver function of stunted rats after eel oil supplementation

The lack of significant variation between groups also indicates that prior nutritional restriction did not produce persistent liver dysfunction once normal feeding was restored. This is consistent with experimental evidence showing that short-term dietary restriction models do not necessarily lead to irreversible hepatic enzyme disruption when adequate nutritional rehabilitation is provided [60, 61]. Moreover, several recent studies report that omega-3–rich lipid supplementation is generally well tolerated in animal models, with no elevation in transaminase levels when administered within physiological dosage ranges [62].

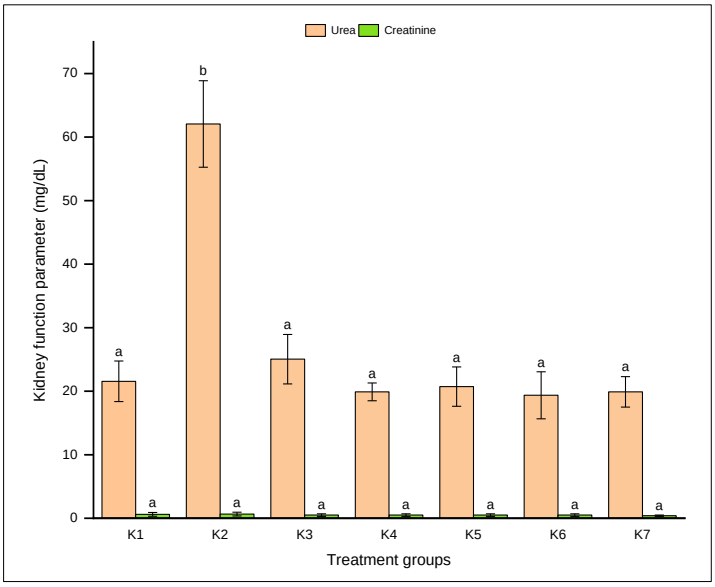
Although not statistically significant on hepatoprotective effect was found, the stability of normal SGOT and SGPT levels in the eel oil supplemented group has clinical relevance. Long-chain PUFAs particularly omega-3 fatty acids, have been reported to modulate oxidative balance and inflammatory signaling in hepatic tissue, thereby supporting liver homeostasis rather than altering enzyme activity under non-pathological conditions [64-67]. The present findings therefore indicate that eel oil supplementation was physiologically safe at the tested doses and did not compromise liver function, supporting its suitability as a nutritional intervention in stunting rehabilitation models.

3.4. Kidney Function

Kidney function is commonly evaluated by measuring serum urea and creatinine, which show protein metabolism and glomerular filtration activity, respectively. High serum urea can indicate impaired protein use or renal dysfunction, while creatinine is considered a reliable marker of renal clearance efficiency [68]. The measurement of kidney function is represented in Figure 6.

Serum urea levels demonstrated a significant overall group effect whereas creatinine concentrations did not differ among groups. Post-hoc analysis clearly separated the negative control group (K2) into an independent subset with markedly elevated urea levels (62.06 mg/dL), while all other groups—including the normal control and eel oil–supplemented rats—clustered within the same homogeneous subset. In contrast, creatinine values remained statistically comparable across all treatments and were maintained within physiological limits, indicating preserved glomerular filtration capacity.

The pronounced elevation of urea in K2 suggests persistent metabolic stress during nutritional restriction. Increased circulating urea is commonly associated with enhanced protein catabolism and negative nitrogen balance, conditions frequently observed in malnutrition states characterized by inadequate nutrient intake and increased tissue breakdown [69]. Contemporary metabolic studies report that undernutrition can elevate urea concentrations through increased amino acid oxidation and hepatic urea cycle activity, even in the absence of structural renal damage [62, 70]. The normalization of urea levels in K4–K7, therefore indicates improved metabolic efficiency and restoration of nitrogen balance following eel oil supplementation.



Note: Values are expressed as mean ± standard deviation (n = 5 per group). Different superscript letters above the bars indicate significant differences among treatment groups ($p > 0.05$).

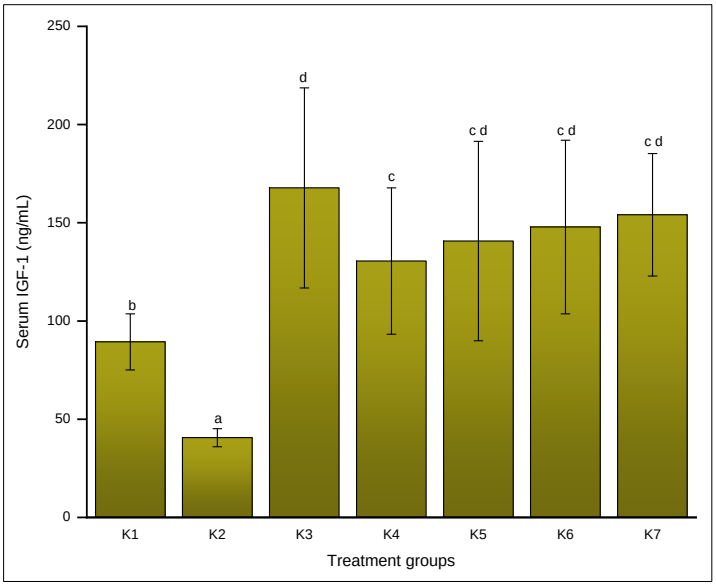
Figure 6. Kidney function of stunted rats after eel oil supplementation

It is important to be underlined that the absence of creatinine elevation across groups confirms that eel oil administration did not impair renal filtration function. PUFAs, particularly omega-3 fatty acids such as DHA and EPA, have been shown to modulate inflammatory signaling, improve mitochondrial efficiency, and support renal hemodynamic stability without inducing nephrotoxicity when administered within physiological ranges [51, 62, 71]. The present findings thus suggest that eel oil supplementation contributed to metabolic stabilization in stunted rats, reflected by reduced urea concentrations, while maintaining normal renal functional integrity

3.5. Insulin-like Growth Factor-1 Levels

IGF-1 is a crucial biomarker for growth and development, showing nutritional status and anabolic activity in animals. Low levels of IGF-1 are often related to growth retardation and stunting, while nutritional therapies that augment protein and energy consumption generally promote IGF-1 production [72]. Eel oil supplementation significantly affected serum IGF-1 levels in stunted rats (Figure 7).

Serum IGF-1 levels varied considerably among groups ($p < 0.001$), indicating that eel oil supplementation influenced endocrine growth signaling in stunted rats. The significantly reduced IGF-1 level in K2 demonstrates ongoing endocrine dysfunction after protein-energy restriction, but the increment of IGF-1 in K4–K7 suggests a restoration of anabolic signaling. Statistically, there is no differences on IGF-1 concentration in K6 and K7 in comparison to the positive control (K3). This indicating that elevated supplementation levels resulted endocrine responses comparable to those of commercial fish oil emulsion.



Note: Values are expressed as mean ± standard deviation (n=5 per group). Different superscript letters above the bars indicate significant differences among treatment groups ($p<0.05$).

Figure 7. Insulin-like growth factor-1 (IGF-1) concentration in stunted rats after eel oil supplementation

The elevation of IGF-1 levels in the supplemented groups provides mechanistic support for the previously observed improvements in linear growth. IGF-1 is a key regulator of longitudinal bone formation, promoting chondrocyte proliferation within the epiphyseal growth plate and enhancing protein synthesis in skeletal tissue [73]. The increase in IGF-1 levels observed at moderate to high eel oil dosages was consistent with the greater body length gain, particularly in the K4 and K5 groups, suggesting coordinated endocrine and structural recovery. Although body weight gain improved across all supplementation levels, the more pronounced response in linear growth indicates that skeletal restoration may be more closely linked to endocrine modulation than to overall mass accretion [74, 75]. These findings further support the interpretation that eel oil supplementation facilitated multidimensional recovery in stunted rats, as reflected by improved anthropometric outcomes, normalized hemoglobin levels, stabilized metabolic markers, and restoration of endocrine signaling through the IGF-1 axis.

The growth-promoting effects observed in eel-oil–treated groups can be attributed to the distinctive fatty acid profile, dominated by MUFAs (52.49%) and enriched with PUFAs fractions, particularly omega-6 (14.16%) and modest omega-3 content (1.80%) (Table 2). MUFAs such as oleic and palmitoleic acids play a key role in enhancing metabolic efficiency, mitochondrial function, and nutrient partitioning toward lean tissue accretion, thereby supporting catch-up growth after nutritional restriction [73]. The substantial omega-6 content, such as linoleic acid, gamma-linolenic acid, DGLA, and arachidonic acid, further contributes to the growth recovery by facilitating membrane biogenesis, modulating eicosanoid-mediated protein synthesis, and supporting adipose expansion required for healthy weight gain [74, 75]. Furthermore, the presence of ALA, EPA, and eicosatrienoic acid is still sufficient to provide anti-inflammatory and IGF-1, enhancing effects that promote linear growth and tissue repair [76].

As structural components of cell membranes, omega-3 enhances cellular signaling and proliferation, which support tissue repair and growth [28]. Mechanistically, this supplementation stimulates anabolic pathways such as IGF-1/mTOR axis, which promotes protein synthesis and muscle accretion. Based on fatty acids profiles, eel oil in this study contained relatively low levels of omega-3 fatty acids but comparatively higher levels of omega-6 fatty acids. Specifically, omega-3 fatty acids are widely recognized for modulating inflammation to support cell membrane integrity and regulate metabolism [28, 30, 77-79]. Omega-6 fatty acids also have important physiological functions as structural components of cell membranes and precursors of signaling molecules that influence growth and development [35, 75]. In humans, linoleic acid (omega-6) can be converted to arachidonic acid, while α-linolenic acid (omega-3) is converted into EPA and DHA through shared desaturation and elongation pathways. This shows the need for a balanced omega-6 to omega-3 ratio and adequate dietary sources of EPA and DHA. A comprehensive review of omega-3 and omega-6 PUFAs shows the nutritional relevance and roles in human physiology, including growth and metabolic health [35, 49, 80]. The result suggests that both classes of PUFAs contribute to overall biological functions rather than acting independently of one another [35]. Despite the relatively low omega-3 content, the combined presence with higher omega-6 levels in eel oil can synergistically support growth recovery and nutritional status in stunted Wistar rats.

The observed growth enhancement cannot be attributed solely to omega-3 fatty acids. Given the predominance of MUFAs and omega-6 PUFAs in the present formulation, the biological effects are likely multifactorial. The substantial MUFA fraction may contribute to improve metabolic efficiency and energy utilization, while the overall

lipid-derived caloric intake may support anabolic recovery following nutritional deprivation [81]. Therefore, eel oil may be better interpreted as a lipid matrix, in which multiple fatty acid classes collectively contribute to growth and metabolic restoration rather than acting through a single dominant pathway

In this study, a controlled experimental model of stunting was established to evaluate the effects of lipid supplementation in previously malnourished rats. Feed restriction in Wistar rats is widely used to model growth faltering, characterized by reduced linear growth, suppressed IGF-1 signaling, and impaired weight gain, thereby reflecting key biological features of pediatric stunting. The 50% feed restriction was applied exclusively during the stunting induction phase and was discontinued once the stunted phenotype had been established [15]. During the intervention period, all groups received standard feeding ad libitum, and differences among groups were therefore attributable solely to the assigned supplementation rather than continued caloric restriction. Although eel oil supplementation contributed additional energy, the administered volumes were modest relative to total daily chow intake and were provided consistently across all supplemented groups.

Thus, the observed improvements are unlikely to be explained solely by caloric compensation, but rather by integrated lipid-matrix effects involving MUFAs and PUFAs that may enhance metabolic efficiency, membrane synthesis, and growth-related endocrine signaling under nutritionally rehabilitated conditions [82]. Previous experimental studies have further validated this feed-restriction model by demonstrating sustained reductions in body length, endocrine alterations, and incomplete spontaneous catch-up growth following nutritional deprivation, supporting its relevance for investigating mechanisms of growth recovery [83, 84]. Nevertheless, extrapolation to human populations should be approached with caution. In addition, formulation differences between the commercial fish oil emulsion used as the positive control and eel oil should be acknowledged. Although oral gavage ensured consistent intake across groups, physicochemical differences between emulsified and non-emulsified lipids may influence dispersion and absorption kinetics. Therefore, comparisons between eel oil and commercial fish oil should be interpreted as functional rather than strictly compositional equivalence.

This study shows that eel oil supplementation provides a comprehensive benefit in stunted rats by improving growth performance, hemoglobin concentration, endocrine regulation through IGF-1, and maintaining normal liver and kidney functions. The results indicate that the supplementation is safe and effective to promote catch-up growth, showing the potential as a novel nutritional intervention for stunting prevention. Moreover, further studies in human populations are recommended to validate the results and explore the translational relevance of eel oil supplementation in addressing children's malnutrition.

4. Conclusion

This study demonstrates that eel oil supplementation represents effective and physiologically safe approach to support recovery from stunting in Wistar rats. Compared with the untreated stunted group, rats receiving eel oil showed improvements in body weight and linear growth, indicating enhanced catch-up growth following dietary deprivation. Although body weight increased consistently with supplementation, linear growth exhibited a more variable response, suggesting that skeletal recovery may depend not only on caloric intake but also on nutrient composition. The restoration of hemoglobin levels further reflects improved nutritional status and systemic recovery. Liver and kidney function parameters remained within normal physiological ranges, indicating that eel oil supplementation did not induce metabolic toxicity during the intervention period.

The increase in circulating IGF-1 provides mechanistic support for the observed growth improvements. IGF-1 plays a central role in longitudinal bone growth and tissue development, reflecting the reactivation of endocrine pathways previously suppressed by undernutrition. The fatty acid profile of eel oil, characterized by high monounsaturated fatty acid content and balanced polyunsaturated components, may have contributed to improved metabolic efficiency and anabolic signaling. These data collectively suggest that eel oil functions not only as an energy source but also as a bioactive nutritional intervention supporting recovery across anthropometric, hematological, metabolic, and endocrine domains. Although extrapolation to human populations requires careful evaluation, the present results highlight the potential of eel oil for further investigation in nutritional rehabilitation and stunting prevention strategies.

5. Declarations

5.1. Author Contributions

Conceptualization, D.F.H., A.S., and S.H.A.; methodology, A.S., S.H.A., and S.; software, D.F.H.; validation, S.H.A., A.S., and S.; formal analysis, D.F.H., S.H.A., and A.S.; investigation, D.F.H., S.H.A., and A.S.; data curation, D.F.H., S.H.A., and A.S.; writing—original draft preparation, D.F.H., A.S., and S.H.A.; writing—review and editing, D.F.H., A.S., and S.H.A.; visualization, D.F.H.; supervision, S.H.A., A.S., and S.; funding acquisition, D.F.H., S.H.A., and A.S. All authors have read and agreed to the published version of the manuscript.

5.2. Data Availability Statement

The data presented in this study are available in the article.

5.3. Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

5.4. Acknowledgments

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5.5. Institutional Review Board Statement

This study was approved by the Health Research Ethics Committee, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia, with the document approval No. 298/KEPH/2024.

5.6. Informed Consent Statement

Not applicable.

5.7. Declaration of Competing Interest

The authors declare that there are no conflicts of interest concerning the publication of this manuscript. Furthermore, all ethical considerations, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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