

# Black Soldier Fly (*Hermetia illucens*) larvae oil in diet: Effects of growth, feed utilization; whole-body fatty acid and amino acid profile; immunology responses and Body Coloration of Goldfish Goldfish (*Carassius auratus*)

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## Research Article

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**Black Soldier Fly (*Hermetia illucens*) larvae oil in diet: Effects of growth, feed utilization; whole-body fatty acid and amino acid profile; immunology responses and Body Coloration of Goldfish Goldfish (*Carassius auratus*)**

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**Abstract**

This study evaluated the efficacy of Black Soldier Fly larvae oil (BSFO) as a sustainable substitute for traditional fish oil in the diets of goldfish (*Carassius auratus*). To determine the ideal inclusion rate, a comprehensive 49-day feeding trial was conducted, evaluating six distinct BSFO replacement levels ranging from 0% to 30%. The results demonstrated that incorporating dietary BSFO significantly improved overall growth performance and feed efficiency metrics. Specifically, the 30% BSFO replacement level emerged as the optimal formulation. It successfully maximized the specific growth rate (reaching 1.14% per day), total weight gain, and feed utilization, while simultaneously minimizing the feed conversion ratio. Furthermore, this 30% inclusion level effectively enriched the whole-body essential amino and fatty acid profiles. It also maintained normal hematological parameters, supported healthy immunological responses, and visibly enhanced integumentary carotenoid pigmentation. Conclusively, a 30% BSFO substitution safely improves the growth, nutritional quality, and overall aesthetics in ornamental aquaculture.

Keywords: goldfish; black soldier fly oil, feed; growth; color

## 1. Introduction

Ornamental fish hold significant potential in both domestic and international markets due to their diverse colors, shapes, and captivating movements, which are highly valued by consumers. Among these, goldfish (*Carassius auratus*) are particularly popular because of their wide range of color variations, including orange and reddish hues, as well as their distinctive body shape (Basoruddin et al. 2022). In addition, goldfish are also highly adaptable to new environments, making them relatively easy to maintain (Nigroho et al. 2024).

Feed plays a critical role in the success of goldfish farming. In feed formulations, lipid, along with protein, are essential nutrient. Lipid has a protein-sparing effect, serving as an alternative energy source and thereby allowing dietary protein to be used more effectively for growth (Muin et al. 2024). Fish oil is commonly used as a primary lipid source because it contains omega-3 fatty acids that improve feed quality and support fish growth (Espe et al. 2023). However, fish oil availability is declining, and its cost is relatively high, creating a need for alternative lipid sources in aquafeeds.

Black soldier fly (BSF) oil represents a promising substitute, containing 38.8% lipids and being particularly rich in lauric, palmitic, and oleic acids (Bakar et al. 2021). In addition, BSF contains approximately 42% protein and 35% fat, with linoleic acid levels ranging from 3.6% to 4.5% and linolenic acid levels between 0.08% and 0.74% (Isdadiyanto, 2015). Despite these advantages, research on substituting fish oil with BSF oil in goldfish diets remains limited. Therefore, this study aimed to evaluate the effects of BSF oil substitution in artificial feed on the growth performance and survival rate of goldfish (*C. Auratus*).

## 2. Materials and methods

### 2.1. Test Animals

The test animals used in this study were oranda goldfish with an average weight of  $8.7 \pm 1.2$  g per fish. The stocking density was 10 fish per treatment aquarium. The fish were obtained directly from ornamental fish cultivators in Tulungagung Regency, East java Province, Indonesia.

### 2.2. Goldfish Rearing Containers

The experiment was conducted in 18 aquariums, each with a volume of 125 L, equipped with aeration systems. Each aquarium was filled with water to 70% of its total volume. Before use, the aquariums were thoroughly washed and rinsed to ensure no soap residues or contaminants remained.

### 2.3. Maintenance Media

Freshwater was used as the rearing medium. The water was collected, allowed to settle, and confirmed to be free of contaminants before use.

### 2.4. Research Methods

The research method used in this study was an experimental approach. An experimental method involves manipulating independent variables, controlling external factors, and measuring their effects on dependent variables.

In this study, the method was applied to determine the optimal level of fish oil substitution with black soldier fly (BSF) oil in artificial feed for improving the growth performance and survival of goldfish.

The experimental design followed a Completely Randomized Design (CRD) with six treatments and three replicates each. The treatment levels were as follows:

Treatment A: 0% black soldier fly oil

Treatment B: 10% black soldier fly oil

Treatment C: 15% black soldier fly oil

Treatment D: 20% black soldier fly oil

Treatment E: 25% black soldier fly oil

Treatment F: 30% black soldier fly oil

The experimental diets were formulated using fish meal, shrimp head meal, soybean meal, corn flour, bran meal, fish oil, BSF oil, corn oil, vitamin–mineral mix, and CMC. The nutritional composition of the ingredients and the formulated diets are presented in Tables 1 and 2.

According to Haryono et al. (2015), feed preparation begins by mixing the raw materials in order, from the smallest to the largest proportion. Warm water (50–60°C) was gradually added to the mixture, which was then pelletized using a coconut filter. The pellets were dried in an oven at approximately 30°C until fully dried.

Fish in each treatment group were fed one of the six experimental diets at 3% of their biomass per day, divided into three feedings (08:00, 12:00, and 16:00 Western Indonesian Time), for a period of 49 consecutive days.

#### **2.4.1. Proximate Analysis**

The chemical composition of the sample was determined using standard procedures outlined by AOAC (2005). Crude protein content was calculated by multiplying the total nitrogen content by a conversion factor (typically 6.25), which accounts for differences in carbohydrate content estimation.

#### **2.4.2. Amino Acid Profile**

To analyze amino acid composition, High-Performance Liquid Chromatography (HPLC) (Waters Corporation, USA) was used with Acetonitrile 60%-AccqTag Eluent as the mobile phase. The system operated at a flow rate of 1.0 mL min<sup>-1</sup> and was equipped with a fluorescence detector. Each sample was injected with a volume of 5 µL (AOAC, 2005). Based on the amino acid profile of the diets over the 49-day feeding trial, the treatment with 20% black soldier fly (BSF) larvae meal substitution yielded the highest levels of lysine and methionine, recorded at 3.56% and 3.40%, respectively.

#### **2.4.3. Fatty Acids Profile**

Gas chromatography (GC) was used to test fatty acids. A Shimadzu GC-14B (Shimadzu Corporation, Japan) equipped with a flame ionization detector and a capillary column was used for the GC analysis (AOAC, 2005).

#### **2.4.4. Total Feed Consumption (TFC)**

According to Weatherly (Renitasari et al. 2021), total feed consumption can be calculated using the following formula:

$$F = C - S$$

Where:

F : Feed consumption (g)

C : Feed given (g)

S : Remaining feed (g)

#### 2.4.5. Feed Utilization Efficiency (FUE)

The feed utilization efficiency value was calculated using the formula, as follows:

$$FUE = \frac{W_t - W_0}{F} \times 100\%$$

Where:

FUE : Feed Utilization Efficiency

$W_t$  : Final fish weight at the end of the study (g)

$W_0$  : Initial fish weight at the start of the study (g)

F : Total amount of feed consumed.

#### 2.4.6. Absolute Weight Gain

The weight growth value was calculated based on the (Srisuksai et al. 2024) namely:

$$W = W_t - W_0$$

Where:

W : Weight growth (g)

$W_t$  : Biomass weight of the test fish at the end of the study (g)

$W_0$  : Biomass weight of the test fish at the beginning of the study (g)

#### 2.4.7. Specific Growth Rate (SGR)

According to (Usman et al. 2022) the specific growth rate during the maintenance period is calculated using the following formula:

$$SGR = \frac{\ln W_t - \ln W_0}{t} \times 100 \%$$

Where:

SGR : Specific growth rate (%/day)

$W_t$  : Total weight of fish at the end of the study (g/tail)

$W_0$  : Total weight of fish at the beginning of the study (g/tail)

t : Time (duration of the maintenance period, in days)

#### 2.4.8. Food Conversion Ratio (FCR)

The food conversion ratio represents the amount of feed required to produce 1 kg of meat. According to (Effendie, 1997) the food conversion ratio can be calculated using the following formula:

$$FCR = \frac{F}{(W_t - W_0 - D)}$$

Where:

FCR: Food conversion ratio

$W_t$ : Fish biomass weight at the end of the study (g)

$W_0$ : Fish biomass weight at the beginning of the study (g)

F: Amount of feed consumed (g)

D: Fish biomass weight that died during maintenance (g)

#### 2.4.9. Survival Rate (SR)

According to (Effendie, 1997), survival rate is the percentage of cultured fish that survive, which can be calculated as follows:

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

Where:

$N_t$ : Number of fish at the end of the study

$N_0$ : Number of fish at the beginning of the study

### 2.5. Blood profile

#### 2.5.1. Erythrocytes

Erythrocyte counts were determined using a Neubauer hemocytometer (Mahardika et al. 2020):

$$\text{Total erythrocytes (cells/mm}^3\text{)} = \Sigma E \times 10^4$$

Information:

$\Sigma E$  = Number of erythrocytes counted

#### 2.5.2. Leukocytes

Leukocytes were counted using the same hemocytometer method. Counting was performed systematically, starting from the upper left corner, then moving right, down, and left in sequence (Lestari et al., 2019). The formula used was:

$$\text{Total leukocytes (cells/mm}^3\text{)} = n \times 50$$

Information :

n = Number of leukocytes counted in 64 squares

### 2.5.3. Leukocyte Differential

The leukocyte differential is calculated as follows according to (Hartika et al. 2014):

$$\% \text{ Lymphocytes} = \frac{L}{100} \times 100\%$$

$$\% \text{ Monocytes} = \frac{M}{100} \times 100\%$$

$$\% \text{ Neutrophils} = \frac{N}{100} \times 100\%$$

### 2.5.4. Hemoglobin

Hemoglobin levels were measured using the Sahli method (Yusuf et al. 2023). Blood was drawn with a Sahli pipette up to the 20 mm<sup>3</sup> mark, then transferred into a hemoglobinometer tube containing 0.1 N HCl up to the 10 (red) mark. The mixture was stirred for 3–5 minutes. Distilled water was added dropwise until the red color matched the standard solution in the hemoglobinometer. Hemoglobin levels were expressed in g/dL or g/%.

### 2.5.5. Hematocrit

Hematocrit levels were determined by comparing the volume of packed red blood cells with the total blood volume on a hematocrit scale (Dahlifa et al. 2016). The formula, adapted from Anderson et al. (1993), is:

$$\text{Hematocrit (\%)} = \frac{\text{red blood cell volume}}{\text{total blood volume}} \times 100\%$$

## 2.6. Color level

The coloration of goldfish was quantitatively evaluated using the Hue, Saturation, and Value (HSV) color model, a widely adopted method for assessing pigmentation in aquaculture and image-based studies (Sural et al. 2002; Ho et al. 2013). The HSV model separates chromatic content (hue) from intensity (value) and vividness (saturation), making it suitable for analyzing fish coloration.

## 2.7. Chromatophore cell

Chromatophore observations in the dermal layer were conducted at the end of the study using histological techniques. Skin samples were fixed, processed, and sectioned at a thickness of 3 µm, then stained with hematoxylin and eosin (H&E) to visualize tissue structure and chromatophore distribution. Following [56], histological slides were prepared for each treatment group and examined under a light microscope at 400× magnification. Chromatophore cells were identified based on location and pigmentation, and their numbers were quantified to assess pigmentation intensity. An increased chromatophore count was associated with enhanced color intensity in goldfish.

## 2.8. Water Quality

Water quality parameters observed in this study included temperature, acidity (pH), and dissolved oxygen (DO). Measurements were taken once per week, either in the morning or evening, before feeding.

## 2.9. Data Analysis

Data analysis was conducted after the study was completed statistically using IBM SPSS Statistics 25 software. Statistical analysis was performed on crime scene data, TFC, FUE, FCR, absolute weight growth, SGR and SR. The statistical analysis used is the Complete Randomized Design (RAL) test by conducting normality tests, homogeneity tests and additivity tests. The results obtained were then analyzed for variance (ANOVA). The data were analyzed variously (F test) at a 95% confidence level. If the results of analysis variance (ANOVA) had significant, Duncan's double region test is carried out to determine the difference in the middle value between treatments. Analysis of water quality data is carried out descriptively to be further compared with the feasibility value in existing references.

## 3. Results

The growth performance of goldfish (*Carassius auratus*) after 49 days of rearing is presented in Table 3. Among all treatments, treatment F (30% BSF oil per 100 g feed) showed the best results for Total Feed Consumption (TFC), Feed Utilization Efficiency (FUE), Feed Conversion Ratio (FCR), Average Body Weight (ABW), Specific Growth Rate (SGR), and Survival Rate (SR), with values of 141.66 g, 43.37%, 2.31, 61.45 g, 1.14%/day, and 100%, respectively. Treatment A (0% BSF oil) contained the lowest levels of lysine ( $3.05 \pm 0.06\%$ ) and methionine ( $3.08 \pm 0.09\%$ ), whereas feed F contained the highest levels of lysine ( $4.70 \pm 0.02\%$ ) and methionine ( $4.88 \pm 0.08\%$ ). Similarly, the amino acid profile analysis showed that treatment A had the lowest lysine ( $3.05 \pm 0.06\%$ ) and methionine ( $3.08 \pm 0.09\%$ ) deposits, while treatment F recorded the highest lysine ( $4.70 \pm 0.02\%$ ) and methionine ( $4.88 \pm 0.08\%$ ) deposits.

The fatty acid profile of the experimental feeds showed that treatment A contained the lowest levels of linoleic acid ( $2.89 \pm 0.04\%$ ), linolenic acid ( $2.66 \pm 0.02\%$ ), eicosapentaenoic acid ( $1.03 \pm 0.07\%$ ), and docosahexaenoic acid ( $0.65 \pm 0.08\%$ ), whereas treatment F contained the highest levels of linoleic acid ( $4.55 \pm 0.03\%$ ), linolenic acid ( $5.79 \pm 0.05\%$ ), eicosapentaenoic acid ( $3.27 \pm 0.09\%$ ), and docosahexaenoic acid ( $3.38 \pm 0.08\%$ ).

Similarly, the fatty acid composition of goldfish muscle reflected the feed treatments. Treatment A recorded the lowest levels of linoleic acid ( $1.30 \pm 0.07\%$ ), linolenic acid ( $4.47 \pm 0.05\%$ ), eicosapentaenoic acid ( $2.60 \pm 0.05\%$ ), and docosahexaenoic acid ( $4.25 \pm 0.03\%$ ), while treatment F showed the highest levels, with values of  $6.37 \pm 0.02\%$ ,  $7.75 \pm 0.08\%$ ,  $5.02 \pm 0.05\%$ , and  $5.15 \pm 0.04\%$ , respectively. The color quality of goldfish fed BSF oil for 49 days is shown in Figure 8, with HSV analysis results presented in Table 7. Goldfish in treatment F displayed the most vibrant and aesthetically desirable coloration, described as "Tiger Orange" with "Daffodil Yellow" highlights.

Based on erythrocyte count data for goldfish (*C. auratus*), presented in Figure 1, the average erythrocyte count ranged from  $1.23$  to  $2.39 \times 10^6$  cells/mm<sup>3</sup>. The highest erythrocyte count was observed in treatment F (30% BSF oil) at  $2.39 \pm 0.05 \times 10^6$  cells/mm<sup>3</sup>, while the lowest was in treatment A (0% BSF oil) at  $1.23 \pm 0.08 \times 10^6$  cells/mm<sup>3</sup>. Leukocyte count data, presented in Figure 2, showed that average leukocyte counts ranged from  $0.70$  to  $1.90 \times 10^5$  cells/mm<sup>3</sup>. Treatment F recorded the highest leukocyte count at  $1.90 \pm 0.14 \times 10^5$  cells/mm<sup>3</sup>, and treatment A had the lowest at  $0.70 \pm 0.10 \times 10^5$  cells/mm<sup>3</sup>.

Lymphocyte counts in goldfish, presented in Figure 3, ranged from 63% to 88%, with treatment A showing the lowest count ( $63.00 \pm 3.00\%$ ) and treatment F the highest ( $88.30 \pm 2.07\%$ ). Hemoglobin counts in goldfish (*C.*

*auratus*) during the study are presented in Figure 6, ranging from 8.2 to 11.2 g/dL. The lowest hemoglobin level was observed in treatment A (0% BSF oil) at  $8.02 \pm 0.39$  g/dL, while the highest was in treatment F (30% BSF oil) at  $11.2 \pm 0.54$  g/dL. Hematocrit values, shown in Figure 7, ranged from 30% to 39%, with treatment F exhibiting the highest hematocrit at  $39.14 \pm 1.77\%$ .

#### 4. Discussion

The substitution with black soldier fly oil (*Hermetia illucens*) in goldfish (*Carassius auratus*) feed resulted in improved growth performance. Growth was evaluated using total feed consumption (TFC), feed utilization efficiency (FUE), feed conversion ratio (FCR), absolute body weight (ABW), and specific growth rate (SGR). Analysis of variance (ANOVA) showed that replacing fish oil with BSF oil significantly affected all growth parameters. The best performance was observed in treatment F (30% BSF oil per 100 g feed), with mean values of TFC ( $127.17 \pm 1.46$  g), FUE ( $44.01 \pm 0.40\%$ ), FCR ( $2.27 \pm 0.02$ ), ABW ( $56.15 \pm 1.19$  g), and SGR ( $1.24 \pm 0.02\%/day$ ). The improved growth in treatment F is likely associated with the nutritional composition of BSF oil, which contains approximately 42% protein and 28% lipid (dry weight) (Xu et al., 2020).

The TFC value in treatment F was higher than in treatments A (0% black soldier fly oil), B (10%), C (15%), D (20%), and E (25%), likely due to the fatty acid content of BSF oil. In particular, *H. illucens* oil contains 40.1% lauric acid (C12:0), 13.1% palmitic acid (C16:0), 9.88% myristic acid (C14:0), 3.6–4.5% linoleic acid (18:2n-6), and 0.08–0.74% linolenic acid (18:3n-3) (Fawole et al. 2021), (Li et al. 2016). Feed intake is generally proportional to fish size; larger fish require more feed, which leads to higher total feed consumption (Karimah et al. 2018). Additionally, feeding behavior is influenced by feed aroma and palatability. Treatment F (30% BSF oil) appeared to have an aroma and taste preferred by goldfish, resulting in the highest feed intake. This is consistent with (islama et al., 2020), who noted that feed with a distinctive aroma that is well accepted by cultured fish is considered high quality. Similarly, Filawati et al. (2018) reported that feed palatability is influenced by smell, taste, and texture, which are closely linked to attractants present in the diet. Black soldier fly oil, with its distinctive odor, may act as such an attractant. At the appropriate inclusion level, it can enhance feed palatability and promote efficient feed utilization.

The highest feed utilization efficiency (FUE) was observed in treatment F (30% BSF oil), with a value of  $43.37 \pm 0.94\%$ . This value was higher than in treatments A (0%), B (10%), C (15%), D (20%), and E (25%), likely due to the fatty acid composition of BSF oil. In particular, the lauric acid content, combined with 43% protein in treatment F, promoted optimal energy absorption and supported efficient growth, indicating that the feed met the nutritional requirements of goldfish (*C. auratus*). This aligns with Usman et al. (2022), who reported that omnivorous fish generally require 20–60% protein, with an optimum range of 30–37%. High feed utilization efficiency reflects the digestibility and effective use of the feed for growth (Iskandar, 2015). Proximate analysis of treatment F showed a fat content of 14.28%, and black soldier fly oil provides saturated fatty acids, primarily lauric acid, which can act as antioxidants and increase HDL (62. Sandhya et al. 2016). The combination of lauric acid and high protein content in treatment F likely contributed to improved energy absorption and more efficient growth.

In several previous studies, (Bakar et al. 2021); (Fawole et al. 2021) stated that the effect of the digestibility level of BSF oil on the growth of carp (*Cyprinus carpio*) was very positive and significant. The mechanism of BSF oil

digestibility and its impact on growth is because BSF oil has a medium chain fatty acid profile (MCFA) which is very digestible, especially lauric acid which can reach a range of 21-49%. In contrast to long chain fatty acids, MCFA molecules are much smaller so they can be hydrolyzed quickly by lipase enzymes in the digestive tract of carp and absorbed efficiently by the intestines. The presence of BSF oil can trigger a Protein-Sparing Effect because MCFA from maggot oil is very easily absorbed so that the fat content in it will be directly transported to the liver through the portal vein to be oxidized into instant energy (beta-oxidation). Through this digestive mechanism, goldfish do not need to break down amino acids (protein) from their feed to meet their basic energy needs (Gatlin et al. 2024). As a result, the feed's protein content can be allocated optimally and purely for the formation of flesh tissue and somatic growth. The high level of lipid digestibility in BSF oil plays a central role in optimizing nutrient metabolism, which directly impacts fish growth performance. This numerical difference in fish growth results occurs due to the high lipid content in BSF oil, commonly known as chitin. The higher chitin content in BSF oil has also been identified as a cause of decreased digestibility in some cases. Chitin is thought to reduce access to chitinases and proteinases, which can affect protein and lipid absorption in the intestine, thus preventing natural protein absorption by the intestine.

The digestive potential of chitin can vary among fish. In certain species, chitin can be considered to have immunostimulatory properties that are beneficial in preventing fish from contracting diseases and bacterial infections (Rangkuti et al. 2026). In goldfish, this is also affected based on previous research by (Muin et al. 2024) which found that the chitin content in BSF oil can increase the innate immune response and disease resistance in goldfish. BSF oil is well accepted by tilapia with higher nutrient digestibility than fish meal. Furthermore, these results are supported by increased growth of fish fed BSF oil, indicating that the addition of insect oil to goldfish feed can provide efficient energy and high digestibility, the overall rate of nutrient digestion, including facilitating the absorption of fat-soluble vitamins, to be very optimal. This can directly boost the increase in Feed Utilization Efficiency (FUE); Specific Growth Rate (SGR) which is comparable to the decrease in the Feed Conversion Ratio (FCR) value (Fisher et al. 2020).

The best feed conversion ratio (FCR) was observed in treatment F (30% BSF oil), with a value of  $2.31 \pm 0.05$ . This value was higher in BSF oil content compared to treatments A (0%), B (10%), C (15%), D (20%), and E (25%). FCR values below 3 are generally considered efficient for fish (Mubarak et al. 2021). The lowest performance was seen in treatment A (0% BSF oil), with an FCR of  $3.37 \pm 0.21$ , exceeding the optimal threshold. This is likely due to the lower protein and fat content in treatment A, which may have limited the ability of goldfish to meet their nutritional requirements. Lauric acid in BSF oil has been reported to increase high-density lipoprotein (HDL), reducing blood vessel blockage and supporting metabolism [(Sandhya et al. 2016); (Isdadiyanto, 2015)]. Feed conversion ratio is closely linked to feed utilization efficiency; a lower FCR indicates higher efficiency, meaning that fish are able to convert consumed feed into growth more effectively (Rararsati et al., 2021)

The highest absolute weight gain was observed in treatment F (30% BSF oil), with a value of  $37.40 \pm 2.1$  g. The combination of lauric acid and 43% protein in treatment F likely promoted optimal energy absorption, supporting high-quality feed that meets the nutritional needs of goldfish (*C. auratus*). Goldfish growth is influenced by total feed consumption, feed utilization efficiency, feeding frequency, and the content of macro- and micronutrients in the diet. Treatment F provided essential nutrients, including 11.10% fat, 37.13% protein, and 21.56% carbohydrates, yielding

approximately 400% energy at 0.59 kcal/g. In general, goldfish require 30–37% protein and 4–10% fat (Usman et al. 2020).

Black soldier fly oil contains 3.6–4.5% linoleic acid and 0.08–0.74% linolenic acid (Li et al. 2020) and is also rich in lauric acid (40.1%), palmitic acid (13.1%), and myristic acid (9.88%) (Fawole et al. 2021). Lauric acid acts as an antioxidant, antimicrobial agent, and supports HDL production, helping reduce blood vessel blockage and facilitating metabolism (Nugroho et al. 2024). Palmitic acid contributes to energy storage, linoleic acid is a precursor for eicosapentaenoic acid (EPA), and linolenic acid is a precursor for docosahexaenoic acid (DHA) (Herawati et al. 2020).

The highest specific growth rate (SGR) was observed in treatment F, in which 30% of fish oil was replaced with BSF oil, with a value of  $1.14 \pm 0.04\%$ /day. This SGR was higher than in treatments A (0%), B (10%), C (15%), D (20%), and E (25%). One of the key factors influencing SGR is feed quality, which includes nutrient content, water stability, digestibility, aroma, and taste (Takeuchi et al., 2002). Higher feed quality ensures that the maximum energy from the diet is available for goldfish (*C. auratus*) growth (Zainuddin et al. 2017). Fish growth is also influenced by the balance of nutrients in the feed; a well-balanced diet promotes optimal growth (Muin et al., 2024).

SGR is closely linked to feed utilization efficiency; the more efficiently feed is converted into biomass, the higher the growth rate. Black soldier fly oil contributes to improved SGR due to its high lauric acid content (44.9%), which is absent in conventional fish oil. Lauric acid functions as an antioxidant, protecting and repairing fish cells (Sandhya et al. 2016); (Mukhlis et al. 2020). By reducing cellular damage, lauric acid allows absorbed protein to be effectively used for growth. Additionally, energy stored from dietary fat and protein supports metabolism, activity, and growth (Riyanti et al. 2020). When dietary energy exceeds immediate needs, it can be allocated to growth, further enhancing SGR (Renitasari et al. 2021).

The survival rate (SR) of goldfish (*C. auratus*) was 100% across all treatments, from A (0% BSF oil) to F (30%). Survival can be influenced by feed and water quality, as noted by [1], who reported that both the quantity and quality of feed, as well as water conditions, affect fish survival. Nutritional content also plays a key role; protein supports growth and helps regenerate damaged cells, while micronutrients such as vitamin C enhance the immune system (Munisa et al. 2015).

In this study, feed containing black soldier fly oil provided essential fatty acids, including EPA and DHA, which are beneficial for growth and disease resistance. Li et al. (2016) reported that EPA and DHA in black soldier fly oil can produce survival rates of 96–100%. Additionally, black soldier fly oil contains 44.9% lauric acid, absent in conventional fish oil (Sukowati et al., 2021). Lauric acid acts as an antioxidant, protecting and repairing fish cells, thereby supporting overall health and survival (Mukhlis et al. 2020).

Water quality is a critical factor supporting the growth and survival of goldfish (*C. auratus*). Monitoring parameters such as temperature, pH, and dissolved oxygen (DO) helps minimize the risk of aquaculture failure. During the study, water temperature ranged from 25 to 26.5 °C, which is within the optimal range for goldfish growth (25–32 °C) (Solihah et al., 2015). The temperature remained relatively stable throughout the maintenance period, supporting appetite and reducing disease risk. pH values ranged from 7.1 to 8.11, and dissolved oxygen ranged from 4.02 to 6.11 mg/L. These values fall within the optimal ranges for goldfish (pH 6.5–8.0 and DO > 3 mg/L), ensuring that water quality did not negatively affect growth or survival (Khairunnisa et al. 2020).

Methionine and lysine are key limiting amino acids for fish growth. Methionine not only promotes growth but also serves as a precursor for other metabolites dependent on its availability (Espe et al. 2023). Lysine plays multiple roles in fish physiology, including preventing fin rot, supporting gastrointestinal and immune system development, enhancing protein deposition and somatic growth, and synthesizing carnitine, which transports fatty acids into mitochondria for subsequent oxidation (Liaqat et al. 2024). As the methionine and lysine content in the feeds increased, their deposition in the fish body also increased. The highest deposition was observed in treatment F (30% BSF oil), with lysine and methionine levels of  $4.70 \pm 0.02\%$  and  $4.88 \pm 0.08\%$ , respectively. Overall, all experimental feeds met the methionine and lysine requirements for goldfish. Additionally, methionine contributes to immunity, antioxidant responses, intestinal enzyme activity, gut microflora, and lipid and amino acid metabolism (Mai et al. 2022).

Glutamic acid is a non-essential amino acid; however, it can contribute to enhanced growth performance in fish. The glutamic acid content varied across treatments, with the highest level observed in treatment F (30% BSF oil) at  $7.30 \pm 0.01\%$ . Glutamic acid improves feed utilization efficiency by supporting the synthesis of arginine, proline, and glutathione, thereby influencing gut health and nutrient absorption in the intestine (Indrianti et al. 2023). In addition to its role in amino acid synthesis, glutamic acid is involved in various metabolic processes, serving as a metabolic fuel in the intestine, liver, kidneys, and skeletal muscle, and participating in antioxidative (Li et al. 2020).

Linoleic and linolenic acids are polyunsaturated fatty acids (PUFAs) that serve as precursors to longer-chain PUFAs. Linolenic acid is an omega-3 fatty acid, whereas linoleic acid is an omega-6 fatty acid. Several studies have examined the impact of the linolenic/linoleic ratio on fish metabolism. In grass carp, an optimal ratio improved growth performance, altered muscle fatty acid composition, and promoted glucose metabolism (Yao et al. 2023). Gonçalves (2021), also reported that feeds with optimal ALA/LNA ratios increased total n-3 fatty acid content in fish. In this study, all experimental feeds met the required levels of linolenic and linoleic acids, with feed F showing the highest deposition: linolenic acid at  $7.75 \pm 0.08\%$  and linoleic acid at  $6.37 \pm 0.02\%$ .

Dietary inclusion of Black Soldier Fly (*Hermetia illucens*) larvae oil (BSFLO) significantly modulates the whole-body fatty acid profile of goldfish (*Carassius auratus*), characterized by a linear deposition of medium-chain saturated fatty acids (MCFAs), particularly lauric acid (C12:0), which serves as a readily available energy substrate through direct portal transport and rapid  $\beta$ -oxidation (Belghit et al. 2019; Dumas et al. 2024). This efficient lipid-driven energy supply exerts a prominent protein-sparing effect, preventing the catabolism of dietary essential amino acids (EAAs) for gluconeogenesis and consequently maximizing their allocation toward somatic protein synthesis (Li et al. 2020). While the strict homeostatic regulation of structural proteins maintains a highly stable whole-body amino acid profile and preserves essential amino acid indices (EAAI), excessive substitution of conventional oils with BSFLO suppresses the endogenous bioconversion of Alpha linolenic acid (ALA) into long-chain polyunsaturated fatty acids (LC-PUFAs) like EPA and DHA due to substrate saturation of 50-60 desaturases (Kousoulaki et al. 2021; Xu et al. 2023). Therefore, a judicious or partial replacement strategy is imperative to harness the metabolic and protein-sparing benefits of MCFAs without compromising the physiological essential fatty acid requirements and membrane fluidity of the fish (Fawole et al. 2021).

Goldfish in treatment F displayed the most vibrant and aesthetically desirable coloration, described as "Tiger Orange" with "Daffodil Yellow" highlights. This combination is highly favored in ornamental goldfish for its deep

golden hue and even distribution across the body and fins. Treatments E and D followed, classified as "Pumpkin Orange" and "Amber," respectively, both showing good chromatic depth but with either slightly reduced shine (E) or over-saturation and dullness (D). Fish from treatments C and B exhibited more muted or patchy orange tones, while treatment A had the weakest visual appeal, shifting toward a yellowish "Tomato Red–Ivory" combination, considered less ideal according to goldfish color standards.

HSV analysis provided an objective quantitative assessment. Treatment F had the highest brightness value at 72.6%, confirming the strong visual intensity observed. Treatment D (20% BSF oil) showed the highest saturation at 22.7%, indicating deeper pigmentation but with reduced brightness. Treatment A exhibited a hue shift toward yellow ( $36.1^\circ$ ), suggesting a possible pigment imbalance.

The results of chromatophore cell analysis in goldfish fed varying levels of BSF oil for 49 days are shown in Figure 9. Treatment A (0% BSF oil) exhibited a high abundance of chromatophores with dense clusters, indicating strong baseline pigmentation. Treatments B (10%) and C (15%) showed moderate chromatophore numbers, maintaining good pigmentation consistent with brighter and more vibrant coloration. Treatment D (20%) displayed very dense chromatophore clusters but with lower brightness, suggesting that pigmentation effectiveness may be reduced or altered. Treatment E (25%) had the lowest chromatophore abundance, correlating with duller colors and reduced pigmentation. Treatment F (30%) showed a moderate-to-low chromatophore presence, higher than treatment E but lower than the moderate groups, corresponding with somewhat diminished color quality. Overall, moderate BSF oil inclusion (10–15%) supports optimal chromatophore abundance and coloration, while higher inclusion levels appear to reduce pigment cell presence and color vibrancy. This pattern aligns with previous reports on the effects of BSF oil on fish pigmentation and skin cell health. Lymphocyte counts in goldfish from 63% to 88%, with treatment A showing the lowest count ( $63.00 \pm 3.00\%$ ) and treatment F the highest ( $88.30 \pm 2.07\%$ ). Normal lymphocyte counts in goldfish range from 59.6% to 80.2% (Li et al. 2016). Monocyte counts ranged from 12.10% to 22.07%, with treatment A the lowest ( $12.10 \pm 1.73\%$ ) and treatment F the highest ( $22.07 \pm 1.30\%$ ). Neutrophil counts ranged from 5.01% to 17.05%, with treatment A recording the lowest value ( $5.01 \pm 0.28\%$ ) and treatment F the highest ( $17.05 \pm 0.47\%$ ).

Hemoglobin counts in goldfish (*C. auratus*) during the study ranging from 8.2 to 11.2 g/dL. The lowest hemoglobin level was observed in treatment A (0% BSF oil) at  $8.02 \pm 0.39$  g/dL, while the highest was in treatment F (30% BSF oil) at  $11.2 \pm 0.54$  g/dL. Hematocrit values ranged from 30% to 39%, with treatment F exhibiting the highest hematocrit at  $39.14 \pm 1.77\%$ . Normal hematocrit values for koi carp range from 29.7% (Chew and Gibson-Kueh, 2023) to 43.8% (Radu et al. 2009).

The normal total erythrocyte count for goldfish ranges from  $1.05$  to  $2.98 \times 10^6$  cells/mm<sup>3</sup> (Yustiati et al. 2020). In this study, treatments D (20% BSF oil), E (25%), and F (30%) showed increased erythrocyte counts, indicating that adding BSF oil to the feed can enhance erythrocyte levels. According to (Somparn et al. 2024) this increase may result from lauric acid and medium-chain fatty acids in BSF oil, which improve lipid metabolism and thereby affect nutrient absorption and energy availability for erythropoiesis. The elevated erythrocyte count also suggests an enhanced immune response, as red blood cells can attract and engulf pathogens via negatively charged membranes (Chan et al. 2022).

Normal total leukocyte counts in goldfish range from  $15.6$  to  $29.1 \times 10^3$  cells/mm<sup>3</sup> (Baghizadeh et al. 2015); (Li et al., 2016). In this study, the highest leukocyte count was observed in treatment F (30% black soldier fly oil) at  $1.90$

$\pm 0.14 \times 10^5$  cells/mm<sup>3</sup>, while the lowest was in treatment A (0%) at  $0.70 \pm 0.10 \times 10^5$  cells/mm<sup>3</sup>. This increase is likely due to BSF oil enhancing phagocytosis and immune enzyme activity, which stimulates leukocyte numbers and functionality (Jiang et al. 2012). Lymphocyte counts, normally ranging from 59.6% to 80.2% (Li et al., 2016), were highest in treatment F, indicating enhanced antibody production. This effect is attributed to lauric acid in BSF oil, which has immunomodulatory and antimicrobial properties that promote the proliferation and function of lymphocytes (Goda et al. 2024). Lymphocytes are critical for defending against infections, as they produce antibodies that target invading bacteria (Novriadi et al. 2024).

Normal monocyte counts in goldfish range from 2% to 12.4% (Nishiguchi et al. 2024). The lowest monocyte count was observed in treatment A (0% BSF oil) at  $12.10 \pm 1.73\%$ , while the highest was in treatment F (30% BSF oil) at  $22.07 \pm 1.30\%$ . Lauric acid in BSF oil can stimulate immune enzyme activity and cytokine gene expression, which play a role in monocyte proliferation and differentiation. This leads to an increased number of monocytes, enhancing phagocytosis and protection against pathogens (Phongpradist et al. 2023).

Treatments with the addition of BSF oil to the feed showed an increase in neutrophil counts, likely due to enzymes associated with neutrophil function and bacterial defense. This suggests that BSF oil not only increases neutrophil numbers but also enhances their functional capacity in immune defense (Khairunnisa et al. 2020). Neutrophils protect the body from bacterial infections by secreting proteolytic enzymes and lysozymes to destroy bacteria (Srisuksai et al. 2024).

Normal hemoglobin levels for goldfish range from 5.9 to 9.09 g/dL (Li et al. 2015). The lowest hemoglobin level was observed in treatment A (0% BSF oil) at  $8.02 \pm 0.39$  g/dL, while the highest was in treatment F (30% BSF oil) at  $11.2 \pm 0.54$  g/dL. Lauric acid, a fatty acid in maggot oil, stimulates feed absorption and improves intestinal development in fish. Improved intestinal health enhances nutrient absorption, including iron and other nutrients essential for hemoglobin synthesis (Indriati et al. 2023). The increased hemoglobin levels are also influenced by the higher number of erythrocytes in the fish's body.

Normal hematocrit levels for koi range from 29.7% (Espe et al. 2023) to 43.8%. The highest hematocrit level was observed in treatment F (30% BSF oil) at  $39.14 \pm 1.77\%$ . Black soldier fly oil contains high levels of lauric acid, a medium-chain fatty acid that is rapidly metabolized into energy (Indriati et al. 2023). This increased energy availability supports better metabolic processes, including erythropoiesis (red blood cell production), which elevates hematocrit values (Srisuksai et al. 2023). Hematocrit levels are closely related to hemoglobin and red blood cell counts; the lower the number of erythrocytes, the lower the hematocrit level in the blood (Yao et al. 2023).

The color quality of ornamental fish is a primary determinant of their commercial value and is often used as a proxy for overall health and pigmentation efficacy (Khieokhajonkhet et al. 2022). Mean hue values ranged from 30.3° to 36.1°, with the control group (A) producing the highest value. Treatments containing black soldier fly oil showed a slight downward trend in hue, particularly at 10%, 20%, and 30% inclusion rates, suggesting a subtle shift away from the “golden” color axis associated with goldfish, possibly toward a slightly duller or less saturated tone. In ornamental fish, carotenoid-based coloration largely determines orange-red hues, and decreases in hue may relate to lower availability or utilization of carotenoids due to BSF oil inclusion, as has been suggested for black soldier fly larvae meal substitutes for fishmeal in goldfish diets (Kamali et al. 2023). Saturation increased with the addition of BSF oil

up to 20%, peaking at 22.7% in treatment D relative to the control (18.8%), with a slight decrease at the highest dose (F; 18.8%). Higher saturation values typically correspond to more vibrant color intensity, indicating that moderate BSF oil supplementation could enhance color vibrancy compared to unsupplemented diets.

However, excessively high inclusion does not further improve saturation and may even diminish this attribute. The mean brightness increased slightly with BSF oil addition, with treatments B, C, and F exceeding the control. This brighter appearance may indicate improved reflective qualities of the skin. Nevertheless, the 20% inclusion (D) appears to decrease brightness, possibly suggesting a threshold above which additional BSF oil may begin to have negative effects on goldfish skin quality or pigment deposition.

Black soldier fly oil is rich in medium-chain saturated and unsaturated fatty acids, which can alter fish lipid metabolism and potentially affect pigment deposition in the skin (Munisa et al. 2015). While research in other species demonstrates that insect-derived oils can efficiently replace traditional oils without major negative impacts on fillet quality or color (Fawole et al. 2021); (Moutinho et al. 2024) the specific effect on goldfish pigmentation appears nuanced. For instance, studies show that black soldier fly larvae meal in goldfish diets can reduce coloration and total carotenoid levels, likely due to reduced dietary pigments. This aligns with the observed slight downward shift in hue and the limited enhancement of saturation and brightness at higher inclusion rates. Additionally, antioxidant compounds present in BSF oil, such as linoleic acid, may play a role in modulating melanin synthesis and pigment stability (Phongpradist et al. 2023). Minor improvements in brightness and color vibrancy at moderate BSF oil doses could partly stem from these functional properties, though the absence of a dose-dependent increase suggests the effect is limited. Chromatophore abundance generally decreases as BSF oil inclusion increases, most notably at 25%, which correlates with duller coloration. Optimal color and chromatophore presence occur at moderate BSF oil inclusion (10–15%). This suggests that excessive BSF oil may negatively impact pigment cell development or maintenance, which is critical for ornamental fish coloration. These findings are consistent with existing literature, which recognizes that insect-derived oils like BSF oil contain fatty acid profiles (e.g., lauric acid and medium-chain saturated fatty acids) that influence lipid metabolism and pigment synthesis in fish (Khieokhajokhet et al. 2022); (Srisuksai et al. 2024). Lauric acid and antioxidant components in BSF oil may support pigment stability at moderate inclusion, but excessive amounts may disrupt pigment cell development or carotenoid deposition, leading to reduced chromatophore abundance and color vibrancy. Studies in ornamental and aquaculture fish species show similar patterns, where insect-based feed ingredients can affect chromatophore dynamics and coloration depending on inclusion levels (Kamali et al. 2023).

## 5. Conclusion

The use of BSF oil in artificial feed for goldfish (*C. auratus*) has a significant effect on total feed consumption (TFC), feed utilization efficiency (FUE), feed conversion ratio (FCR), average body weight (ABW), and specific growth rate (SGR), but does not significantly affect survival rate (SR). Based on the research conducted, substituting 30% of BSF oil in feed resulted in the best growth performance, with a TFC of 141.66 g, FUE of 43.37%, FCR of 2.31, absolute weight of 61.45 g, SGR of 1.14%/day, and SR of 100%. Treatment F (30% BSF oil) achieved the highest methionine ( $4.88 \pm 0.08\%$ ) and lysine ( $4.70 \pm 0.02\%$ ) content. Additionally, treatment F also had the highest ALA and LNA deposits, with values of  $7.75 \pm 0.08\%$  and  $6.37 \pm 0.02\%$ , respectively. The hematological parameters of the goldfish were within the following ranges: erythrocytes ( $1.23\text{--}2.39 \times 10^6$  cells/mm<sup>3</sup>), leukocytes ( $0.70\text{--}1.90 \times 10^5$  cells/mm<sup>3</sup>),

lymphocytes (63–88%), monocytes (12.10–22.07%), neutrophils (5.01–17.05%), hemoglobin (8.2–11.2 g/dL), and hematocrit (30–39%)

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### **Author Contribution**

First Author: Conceptualization and methodology for all research; Second and Third Authors: Methodology; Fourth Author: Investigation; Fifth Author: Formal analysis; Sixth Author: Writing, review, and editing.

### **Conflict of Interest**

The authors declare that they have no competing financial, non-financial, professional, or personal interests that could have influenced the work reported in this paper.

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### **Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### **Ethics Statement**

The protocol described and executed in the present study was approved by the Bioethics Committee for Medical / Health Research of The Faculty of Medicine, Sultan Agung Islamic University (Protocol No. 133/III/2026/ Bioethics Committee)

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**Table 1**  
**Preparation of 100 g feed formula (% dry weight).**

| Ingredients                        | Feed Composition (%/100 g Feed) |         |         |         |         |         |
|------------------------------------|---------------------------------|---------|---------|---------|---------|---------|
|                                    | A (0%)                          | B (10%) | C (15%) | D (20%) | E (25%) | F (30%) |
| Fish Meal                          | 24.00                           | 24.00   | 24.00   | 24.00   | 24.00   | 24.00   |
| Shrimp Head Meal                   | 20.00                           | 20.00   | 20.00   | 20.00   | 20.00   | 20.00   |
| Soybean Flour                      | 35.00                           | 35.00   | 35.00   | 35.00   | 35.00   | 35.00   |
| Corn Flour                         | 3.00                            | 3.00    | 3.00    | 3.00    | 3.00    | 3.00    |
| Bran Flour                         | 6.00                            | 6.00    | 6.00    | 6.00    | 6.00    | 6.00    |
| Fish Oil                           | 3.50                            | 3.15    | 2.98    | 2.80    | 2.63    | 2.45    |
| Black soldier fly Oil              | 0.00                            | 0.35    | 0.53    | 0.70    | 0.88    | 1.05    |
| Corn Oil                           | 3.50                            | 3.50    | 3.50    | 3.50    | 3.50    | 3.50    |
| Vitamin-mineral mix                | 3.00                            | 3.00    | 3.00    | 3.00    | 3.00    | 3.00    |
| CMC                                | 2.00                            | 2.00    | 2.00    | 2.00    | 2.00    | 2.00    |
| TOTAL (%)                          | 100.00                          | 100.00  | 100.00  | 100.00  | 100.00  | 100.00  |
| Protein (%)                        | 37.13                           | 37.13   | 37.13   | 37.13   | 37.13   | 37.13   |
| Nitrogen-Free Extract<br>(NFE) (%) | 21.56                           | 21.56   | 21.56   | 21.56   | 21.56   | 21.56   |
| Fat (%)                            | 11.10                           | 11.10   | 11.10   | 11.10   | 11.10   | 11.10   |
| Energy (kcal/g) *                  | 400.59                          | 400.59  | 400.59  | 400.59  | 400.59  | 400.59  |
| E/P ratio **                       | 10.79                           | 10.79   | 10.79   | 10.79   | 10.79   | 10.79   |

Information:

\* Calculated based on digestible energy according to Furuichi (1988): 1 g of protein = 3.0 kcal/g, 1 g of carbohydrates = 2.5 kcal/g, and 1 g of fat = 8.1 kcal/g.

\*\* According to De Silva (1987), the energy-to-protein ratio (E/P) value for optimal fish growth ranges from 8 to 9 kcal/g.

**Table 2.**  
**Proximate Analysis of Feed Ingredients (% dry weight).**

| Ingredients          | Composition (%) |       |      |       |       | Total<br>(%) |
|----------------------|-----------------|-------|------|-------|-------|--------------|
|                      | Protein         | NFE   | Fat  | Fiber | Ash   |              |
| Fish Meal*           | 45.70           | 12.27 | 6.18 | 3.26  | 23.94 | 100.00       |
| Corn Flour**         | 0.40            | 88.18 | 0.61 | 0.03  | 0.24  | 100.00       |
| Shrimp Head Flour*** | 30.45           | 4.69  | 2.87 | 5.26  | 44.75 | 100.00       |
| Soybean Meal Flour** | 46.20           | 31.92 | 2.99 | 3.16  | 6.80  | 100.00       |
| Bran Flour*          | 6.36            | 30.13 | 1.59 | 34.77 | 15.67 | 100.00       |

Description: \* : Hernowo *et al.*, (2020),  
 \*\* : Wibowo *et al.*, (2018)  
 \*\*\* : Sudrajat dan Effendi(2002)

**Table 3.**  
**Growth performance of goldfish (*Carassius auratus*) after 49 days of maintenance.**

| Variable    | Treatment                 |                            |                            |                             |                             |                            |
|-------------|---------------------------|----------------------------|----------------------------|-----------------------------|-----------------------------|----------------------------|
|             | A                         | B                          | C                          | D                           | E                           | F                          |
| TFC (g)     | 125.9 ± 2.22 <sup>a</sup> | 126.41 ± 2.62 <sup>a</sup> | 128.17 ± 1.33 <sup>a</sup> | 136.45 ± 2.87 <sup>bc</sup> | 137.79 ± 1.37 <sup>bc</sup> | 141.66 ± 2.82 <sup>c</sup> |
| FUE (%)     | 29.71 ± 1.81 <sup>a</sup> | 30.32 ± 1.99 <sup>a</sup>  | 32.87 ± 0.12 <sup>b</sup>  | 35.55 ± 0.43 <sup>c</sup>   | 38.80 ± 1.06 <sup>d</sup>   | 43.37 ± 0.94 <sup>c</sup>  |
| FCR         | 3.37 ± 0.21 <sup>a</sup>  | 3.31 ± 0.22 <sup>b</sup>   | 3.04 ± 0.01 <sup>c</sup>   | 2.81 ± 0.03 <sup>c</sup>    | 2.58 ± 0.07 <sup>d</sup>    | 2.31 ± 0.05 <sup>d</sup>   |
| ABW (g)     | 37.40 ± 2.1 <sup>a</sup>  | 38.30 ± 2.03 <sup>a</sup>  | 42.13 ± 0.49 <sup>b</sup>  | 48.50 ± 0.82 <sup>c</sup>   | 53.47 ± 1.46 <sup>d</sup>   | 61.45 ± 2.55 <sup>e</sup>  |
| SGR (%/day) | 0.76 ± 0.04 <sup>a</sup>  | 0.78 ± 0.04 <sup>a</sup>   | 0.84 ± 0.01 <sup>b</sup>   | 0.93 ± 0.01 <sup>c</sup>    | 1.01 ± 0.03 <sup>d</sup>    | 1.14 ± 0.04 <sup>e</sup>   |
| SR (%)      | 100 <sup>a</sup>          | 100 <sup>a</sup>           | 100 <sup>a</sup>           | 100 <sup>a</sup>            | 100 <sup>a</sup>            | 100 <sup>a</sup>           |

Note: TFC - total feed consumption; FUE - efficiency of feed utilization; FCR- feed conversion ratio; ABW – average weight gain; SGR - specific growth rate; SR - survival rate; different superscripts indicate significant differences (p<0.05).

**Table 4.**  
**Amino acid profile analysis of goldfish (*C. auratus*) after 49 days of maintenance.**

| <i>Profil asam</i> | <i>Treatments</i>      |                        |                        |                        |                        |                         |
|--------------------|------------------------|------------------------|------------------------|------------------------|------------------------|-------------------------|
| <i>amino (%)</i>   | <i>A</i>               | <i>B</i>               | <i>C</i>               | <i>D</i>               | <i>E</i>               | <i>F</i>                |
| Aspartic acid      | 2.05±0.02 <sup>a</sup> | 2.13±0.04 <sup>a</sup> | 4.63±0.09 <sup>a</sup> | 6.06±0.09 <sup>b</sup> | 5.18±0.06 <sup>b</sup> | 6.31±0.09 <sup>b</sup>  |
| Proline            | 3.10±0.05 <sup>a</sup> | 3.35±0.05 <sup>a</sup> | 5.28±0.06 <sup>b</sup> | 5.75±0.08 <sup>b</sup> | 3.94±0.08 <sup>a</sup> | 4.87±0.07 <sup>b</sup>  |
| Serine             | 4.20±0.02 <sup>a</sup> | 4.56±0.02 <sup>a</sup> | 4.56±0.02 <sup>a</sup> | 4.10±0.04 <sup>a</sup> | 5.46±0.03 <sup>b</sup> | 5.19±0.09 <sup>b</sup>  |
| Glutamic acid      | 5.15±0.01 <sup>a</sup> | 5.26±0.06 <sup>a</sup> | 5.39±0.01 <sup>b</sup> | 5.85±0.07 <sup>a</sup> | 6.16±0.09 <sup>b</sup> | 7.30±0.01 <sup>b</sup>  |
| Glycine            | 4.17±0.04 <sup>a</sup> | 4.53±0.03 <sup>a</sup> | 6.33±0.06 <sup>b</sup> | 6.26±0.05 <sup>b</sup> | 5.91±0.08 <sup>b</sup> | 5.75±0.08 <sup>b</sup>  |
| Histidine          | 4.06±0.08 <sup>a</sup> | 4.05±0.06 <sup>a</sup> | 4.65±0.09 <sup>a</sup> | 5.30±0.08 <sup>b</sup> | 4.80±0.05 <sup>a</sup> | 5.90±0.07 <sup>b</sup>  |
| Arginine           | 4.50±0.07 <sup>a</sup> | 4.70±0.09 <sup>a</sup> | 5.85±0.07 <sup>b</sup> | 6.28±0.06 <sup>b</sup> | 6.30±0.04 <sup>b</sup> | 6.36±0.06 <sup>b</sup>  |
| Threonine          | 4.45±0.03 <sup>a</sup> | 4.75±0.08 <sup>a</sup> | 6.47±0.09 <sup>b</sup> | 5.89±0.06 <sup>b</sup> | 6.75±0.05 <sup>b</sup> | 7.78±0.04 <sup>b</sup>  |
| Alanine            | 5.43±0.04 <sup>b</sup> | 5.67±0.07 <sup>b</sup> | 6.51±0.08 <sup>b</sup> | 4.95±0.05 <sup>a</sup> | 5.17±0.06 <sup>b</sup> | 5.20±0.07 <sup>b</sup>  |
| Valine             | 4.14±0.02 <sup>a</sup> | 4.02±0.05 <sup>a</sup> | 4.72±0.07 <sup>a</sup> | 4.90±0.09 <sup>a</sup> | 6.98±0.07 <sup>b</sup> | 7.10±0.09 <sup>b</sup>  |
| Lysine             | 3.56±0.08 <sup>a</sup> | 3.95±0.02 <sup>a</sup> | 4.70±0.02 <sup>a</sup> | 5.09±0.04 <sup>a</sup> | 5.88±0.09 <sup>b</sup> | 8.33±0.08 <sup>b</sup>  |
| Methionine         | 6.70±0.01 <sup>a</sup> | 6.90±0.05 <sup>a</sup> | 6.89±0.03 <sup>a</sup> | 7.80±0.09 <sup>a</sup> | 8.90±0.07 <sup>b</sup> | 10.74±0.05 <sup>b</sup> |
| Isoleucine         | 4.84±0.03 <sup>a</sup> | 4.97±0.03 <sup>a</sup> | 5.17±0.04 <sup>b</sup> | 4.49±0.08 <sup>a</sup> | 4.95±0.06 <sup>a</sup> | 5.23±0.05 <sup>b</sup>  |
| Phenylalanine      | 4.56±0.02 <sup>a</sup> | 4.61±0.09 <sup>a</sup> | 4.41±0.08 <sup>a</sup> | 4.58±0.07 <sup>a</sup> | 4.80±0.02 <sup>a</sup> | 5.48±0.09 <sup>b</sup>  |
| Leucine            | 4.13±0.03 <sup>a</sup> | 4.17±0.01 <sup>a</sup> | 5.37±0.03 <sup>b</sup> | 3.38±0.04 <sup>a</sup> | 5.07±0.01 <sup>b</sup> | 4.75±0.08 <sup>a</sup>  |
| L-Tryptophan       | 3.12±0.09 <sup>a</sup> | 3.03±0.09 <sup>a</sup> | 6.33±0.05 <sup>b</sup> | 5.93±0.08 <sup>b</sup> | 3.50±0.08 <sup>a</sup> | 4.10±0.04 <sup>a</sup>  |

Note: different superscripts in the same row indicate significant differences (p<0.05).

812 **Table 5.**  
813 **Fatty acid profile analysis of trial feeds for goldfish (*C. auratus*) for 49 days of maintenance.**

| Saturated fatty acid          | Sample |             |             |             |             |             |             |
|-------------------------------|--------|-------------|-------------|-------------|-------------|-------------|-------------|
|                               | Req    | <i>A</i>    | <i>B</i>    | <i>C</i>    | <i>D</i>    | <i>E</i>    | <i>F</i>    |
| Methyl Butyrate               | 0.5    | 0.55 ± 0.03 | 0.63 ± 0.07 | 2.88 ± 0.05 | 1.66 ± 0.04 | 0.88 ± 0.06 | 0.76 ± 0.03 |
| Methyl Hexanoate              | 0.7    | 1.47 ± 0.05 | 1.54 ± 0.04 | 1.93 ± 0.03 | 3.52 ± 0.02 | 1.89 ± 0.04 | 3.62 ± 0.07 |
| Methyl Undecanoate            | 0.9    | 0.89 ± 0.08 | 1.04 ± 0.02 | 2.25 ± 0.09 | 3.47 ± 0.08 | 3.09 ± 0.02 | 3.70 ± 0.04 |
| Methyl Laurate                | 0.23   | 1.78 ± 0.04 | 1.88 ± 0.05 | 1.90 ± 0.03 | 2.82 ± 0.03 | 1.83 ± 0.09 | 2.80 ± 0.05 |
| Methyl Tridecanoate           | 0.89   | 2.45 ± 0.09 | 3.23 ± 0.08 | 2.78 ± 0.02 | 3.05 ± 0.02 | 3.22 ± 0.05 | 4.75 ± 0.01 |
| Methyl Pentadecanoate         | 2.27   | 2.88 ± 0.02 | 3.46 ± 0.04 | 4.99 ± 0.05 | 3.50 ± 0.06 | 3.86 ± 0.09 | 4.03 ± 0.09 |
| Methyl Palmitate              | 0.73   | 2.74 ± 0.05 | 3.25 ± 0.03 | 3.52 ± 0.08 | 3.70 ± 0.04 | 3.85 ± 0.03 | 4.59 ± 0.04 |
| Methyl Arachidate             | 4.75   | 3.69 ± 0.08 | 3.73 ± 0.06 | 3.85 ± 0.04 | 3.90 ± 0.03 | 4.07 ± 0.08 | 4.23 ± 0.07 |
| Methyl Tricosanoate           | 1.26   | 1.29 ± 0.03 | 1.35 ± 0.09 | 1.93 ± 0.02 | 2.09 ± 0.06 | 1.85 ± 0.09 | 2.39 ± 0.02 |
| <b>Unsaturated Fatty Acid</b> |        |             |             |             |             |             |             |
| Linoleic                      | 2.2    | 0.65 ± 0.08 | 1.62 ± 0.04 | 2.15 ± 0.02 | 2.79 ± 0.04 | 3.22 ± 0.08 | 3.38 ± 0.08 |
| Linolenic                     | 3.5    | 2.66 ± 0.02 | 3.04 ± 0.04 | 3.58 ± 0.03 | 3.74 ± 0.02 | 4.26 ± 0.08 | 5.79 ± 0.05 |
| Eicosapentaenoic              | 1.7    | 1.03 ± 0.07 | 1.08 ± 0.09 | 2.07 ± 0.08 | 2.67 ± 0.09 | 2.98 ± 0.02 | 3.27 ± 0.09 |
| Docosahexaenoic               | 3.2    | 2.89 ± 0.04 | 3.10 ± 0.06 | 3.17 ± 0.04 | 3.38 ± 0.07 | 3.17 ± 0.03 | 4.55 ± 0.03 |

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**Table 6.****Fatty acid profile analysis of goldfish (*C. auratus*) of after 49 days of maintenance**

| <i>Fatty acid profile (%)</i> | <i>Treatments</i>      |                        |                        |                        |                        |                        |
|-------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
|                               | <i>A</i>               | <i>B</i>               | <i>C</i>               | <i>D</i>               | <i>E</i>               | <i>F</i>               |
| C14:0 (Myristic)              | 3.20±0.03 <sup>a</sup> | 3.66±0.05 <sup>a</sup> | 4.75±0.05 <sup>a</sup> | 3.75±0.04 <sup>a</sup> | 5.57±0.05 <sup>b</sup> | 5.88±0.05 <sup>b</sup> |
| C15:0 (Pentadecanoic)         | 2.16±0.05 <sup>a</sup> | 2.08±0.03 <sup>a</sup> | 1.72±0.03 <sup>a</sup> | 2.45±0.06 <sup>a</sup> | 2.38±0.03 <sup>a</sup> | 3.10±0.03 <sup>b</sup> |
| C16:0 (Palmitic)              | 3.76±0.06 <sup>a</sup> | 4.17±0.08 <sup>a</sup> | 5.14±0.02 <sup>b</sup> | 4.60±0.02 <sup>a</sup> | 4.77±0.02 <sup>a</sup> | 6.99±0.01 <sup>b</sup> |
| C18:0 (Stearic)               | 4.20±0.04 <sup>a</sup> | 2.55±0.04 <sup>a</sup> | 2.71±0.08 <sup>a</sup> | 4.98±0.08 <sup>b</sup> | 5.75±0.08 <sup>b</sup> | 6.23±0.09 <sup>b</sup> |
| C18:1 n-9 (Oleic/ω9)          | 1.47±0.05 <sup>a</sup> | 1.75±0.02 <sup>a</sup> | 2.97±0.04 <sup>b</sup> | 3.08±0.03 <sup>b</sup> | 3.90±0.07 <sup>a</sup> | 4.95±0.04 <sup>b</sup> |
| C18:2 n-6 (Linoleic/ω6)       | 1.30±0.07 <sup>a</sup> | 1.25±0.05 <sup>a</sup> | 4.60±0.09 <sup>b</sup> | 5.18±0.07 <sup>b</sup> | 5.30±0.04 <sup>a</sup> | 6.37±0.02 <sup>b</sup> |
| C18:3 n-3 (Linolenic/ω3)      | 4.47±0.05 <sup>a</sup> | 4.76±0.09 <sup>a</sup> | 6.15±0.05 <sup>a</sup> | 6.45±0.02 <sup>b</sup> | 6.90±0.03 <sup>a</sup> | 7.75±0.08 <sup>b</sup> |
| C20:0 (Arachidic)             | 1.56±0.02 <sup>a</sup> | 1.78±0.08 <sup>a</sup> | 2.30±0.06 <sup>a</sup> | 3.23±0.08 <sup>a</sup> | 2.50±0.07 <sup>a</sup> | 4.23±0.03 <sup>b</sup> |
| C20:4 n-6 (Arachidonic)       | 3.20±0.08 <sup>b</sup> | 3.39±0.04 <sup>b</sup> | 4.07±0.03 <sup>a</sup> | 4.14±0.07 <sup>a</sup> | 4.36±0.08 <sup>a</sup> | 4.97±0.02 <sup>a</sup> |
| C20:5 n-3 (EPA)               | 2.60±0.05 <sup>a</sup> | 2.73±0.08 <sup>a</sup> | 4.53±0.09 <sup>b</sup> | 4.79±0.06 <sup>b</sup> | 4.83±0.05 <sup>a</sup> | 5.02±0.05 <sup>b</sup> |
| C22:6 n-3 (DHA)               | 4.25±0.03 <sup>a</sup> | 4.46±0.09 <sup>a</sup> | 4.53±0.03 <sup>b</sup> | 4.75±0.02 <sup>b</sup> | 4.96±0.04 <sup>b</sup> | 5.15±0.04 <sup>b</sup> |

Note: different superscripts in the same row indicate significant differences (p&lt;0.05)

824 **Table 7.**  
825 **HSV for goldfish fed by black soldier fly oil for 49 days.**

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| Sample | Mean Hue (°)           | Mean Saturation (%)   | Mean Value (Brightness) (%) |
|--------|------------------------|-----------------------|-----------------------------|
| A      | 36.1±0.2 <sup>a</sup>  | 18.8±0.1 <sup>b</sup> | 68.3±0.2 <sup>b</sup>       |
| B      | 31.5±0.3 <sup>c</sup>  | 22.2±0.3 <sup>a</sup> | 72.5±0.3 <sup>a</sup>       |
| C      | 33.9±0.3 <sup>b</sup>  | 21.5±0.1 <sup>a</sup> | 72.1±0.1 <sup>a</sup>       |
| D      | 30.3±0.2 <sup>d</sup>  | 22.7±0.3 <sup>a</sup> | 67.3±0.4 <sup>c</sup>       |
| E      | 33.9±0.2 <sup>b</sup>  | 21.2±0.2 <sup>a</sup> | 69.2±0.3 <sup>b</sup>       |
| F      | 31.1±0.3 <sup>cd</sup> | 18.8±0.5 <sup>b</sup> | 72.6±0.2 <sup>a</sup>       |

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Figure 1. Erythrocyte values of goldfish (*C. auratus*) during the study.

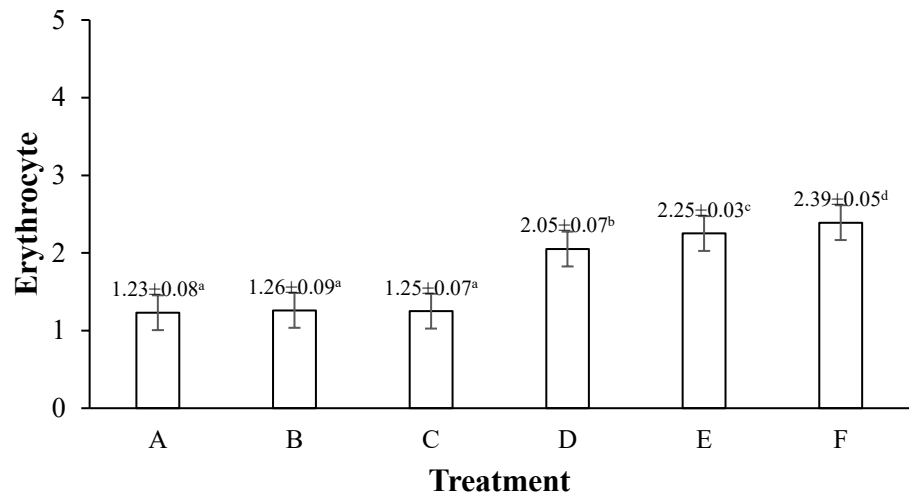


Figure 2. Leukocytes values of goldfish (*C. auratus*) during the study.

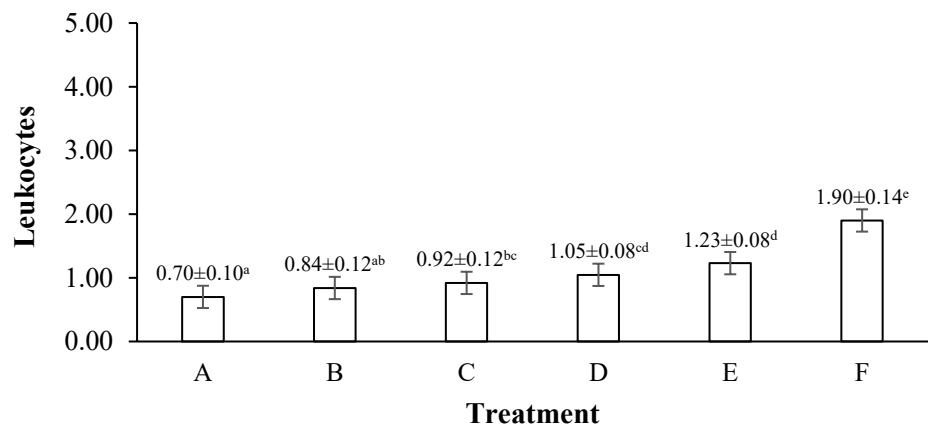


Figure 3. Lymphocytes values of goldfish (*C. auratus*) during the study.

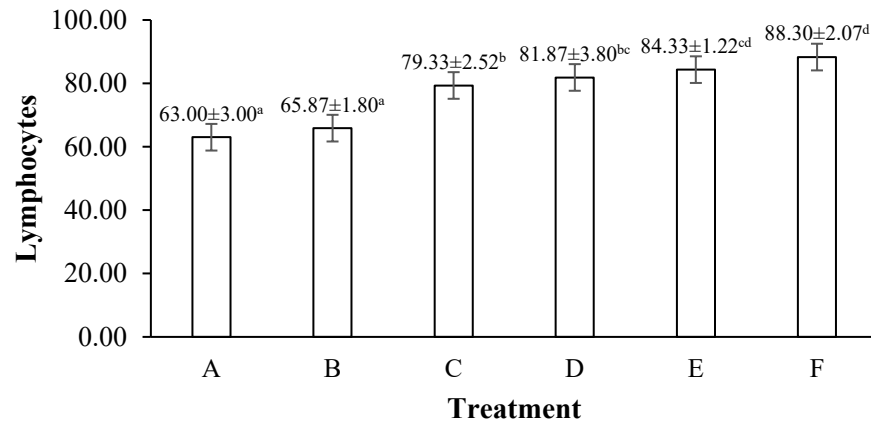


Figure 4. Monocytes values of goldfish (*C. auratus*) during the study.

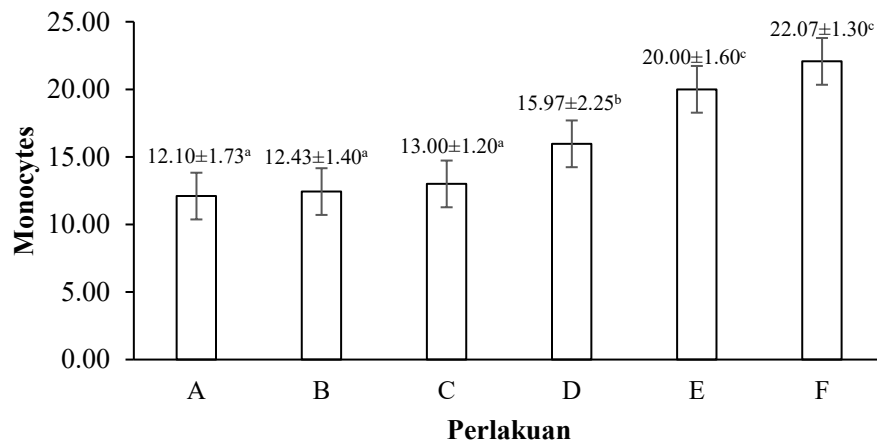


Figure 5. Neutrophils values of goldfish (*C. auratus*) during the study.

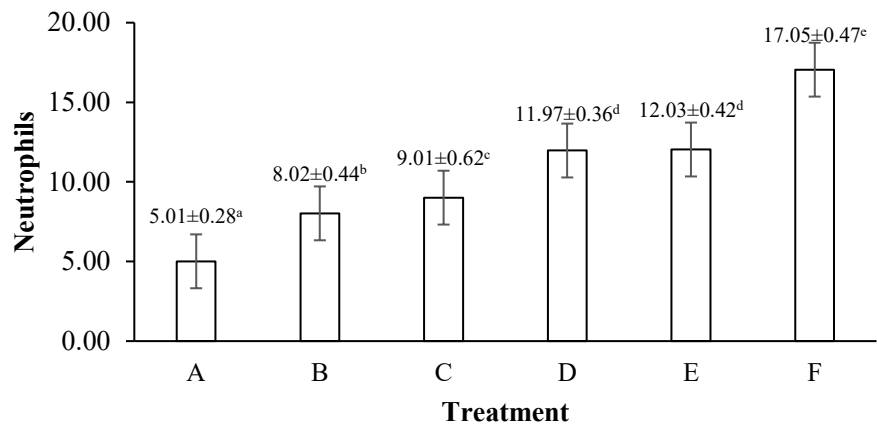


Figure 6. Hemoglobin values of goldfish (*C. auratus*) during the study.

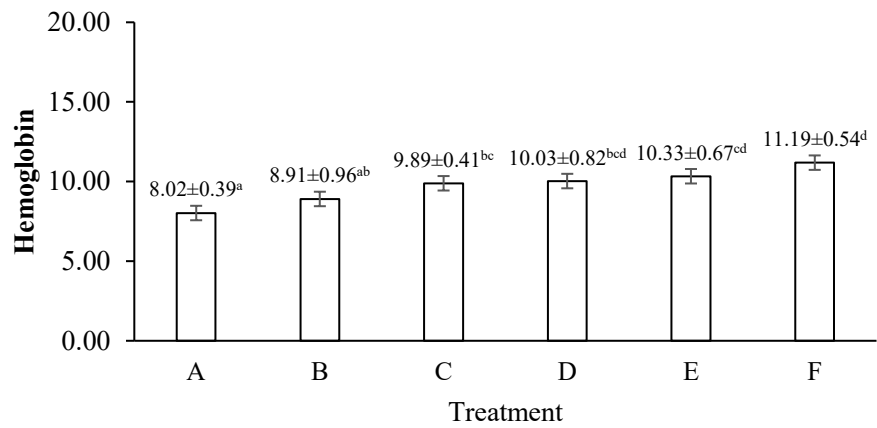


Figure 7. Hematocrit values of goldfish (*C. auratus*) during the study.

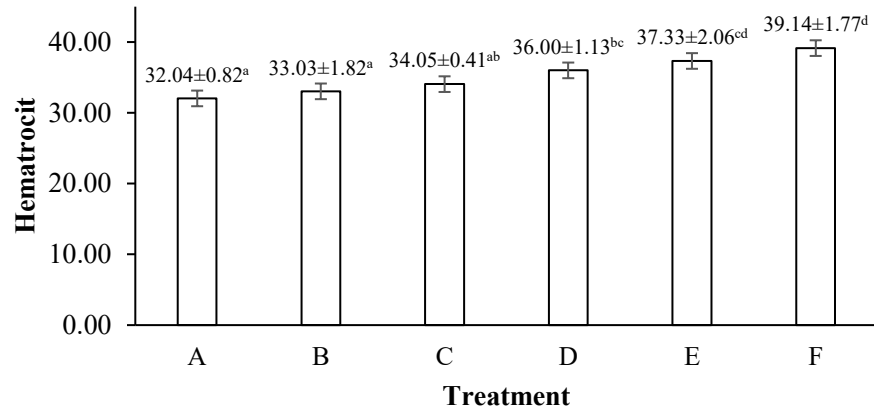


**Figure 8. The color quality of goldfish fed by black soldier fly oil for 49 days for each treatment (A-F)**

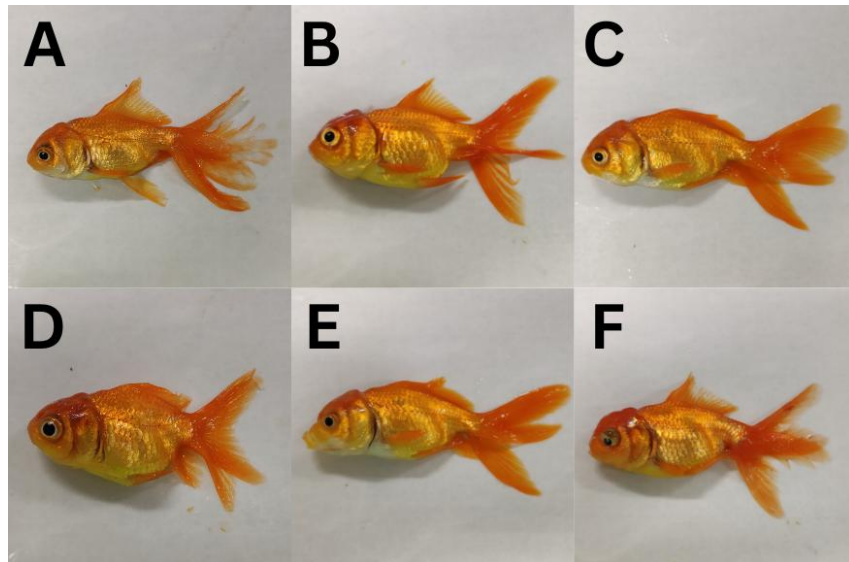


**Figure 9. Histological cross section of goldfish for each treatment (A-F).**

