



Fortification of Black Soldier Fly Oil and Squid Oil in Diets on the Nutritional Quality, Growth, and Survival of Juvenile Giant Gourami (*Osphronemus gouramy*)

Febry Ferdiyanto Purba¹, Netti Aryani^{2*}, Niken Ayu Pamukas², Indra Suharman², Henni Syawal²

¹Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru-Riau 28292, Indonesia

²Department of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru-Riau 28292, Indonesia

*Corresponding Author: netti.aryani@lecturer.unri.ac.id

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ABSTRACT

Fortification of black soldier fly oil (BSFO) has the potential to improve feed quality through its saturated fatty acid (SFA) content, such as lauric acid and palmitic acid, which play a role in supporting juvenile fish growth. Combining it with squid oil (SQO), which is rich in unsaturated fatty acids, is expected to optimize the growth of juvenile giant gourami. Both oils are still rarely used in juvenile giant gourami feed formulations. This study evaluates the performance of black soldier fly oil and squid oil fortification as a substitute for commercial fish oil in juvenile giant gourami (*Osphronemus gouramy*) feed. The experiment was carried out under a completely randomized design (CRD) with one factor and four treatments, namely P1 fortified with 6% corn oil and three feed treatments fortified with BSFO and SQO with compositions of 3%:3% (P2 diet), 2%:4% (P3 diet) and 1.5%:4.5% (P4 diet). The parameters observed included feed fatty acid profile, absolute weight gain, survival rate, viscerosomatic index (VSI), hepatosomatic index (HSI), and feed conversion ratio. The results showed that fortifying feed with maggot oil and squid oil could improve nutritional quality and support the growth of juvenile gourami fish. The use of these two oils has the capacity to enhance growth outcomes and survival rate while also supporting the principle of resource circularity through the utilization of environmentally friendly alternative resources as a sustainable source of oil in aquaculture feed formulations.

INTRODUCTION

Giant gourami (*Osphronemus gouramy*) is one of Indonesia's leading freshwater fishery commodities, especially in Riau Province. This commodity has high economic value and serves as a key contributor to strengthening community nutritional resilience (Azrita *et al.*, 2021). Market demand for giant gourami continues to increase every year, but production is still unable to optimally meet community needs (Aryani *et al.*, 2020). This condition requires more effective and efficient aquaculture strategies.

Several approaches have been undertaken to enhance the productivity of juvenile giant gourami, such as stocking density management (**Kustiana *et al.*, 2024**) and variation of feeding rates in floating net cage systems (**Aryani *et al.*, 2021**). Although these efforts have yielded fairly good results, feed efficiency remains a key factor that needs to be optimized, particularly through improvements in feed nutritional quality. Commercial feed used for juvenile gurami fish contains 30–35% crude protein, 2–8% fat, and 5–12% ash (**Dirmansyah *et al.*, 2022**; **Nurhudah *et al.*, 2023**). However, this composition does not fully support optimal growth. Therefore, fortifying feed with high-quality fat sources such as SFA and MUFA as energy sources is one strategy that needs to be explored.

The use of commercial fish oil rich in essential fatty acids such as EPA and DHA has been shown to increase the growth of several fish species, for example in juvenile green snapper (**Yadav *et al.*, 2020**), juvenile tilapia (**Duarte *et al.*, 2020**), juvenile Asian redbtail catfish (*Hemibagrus wyckioides*) (**Deng *et al.*, 2021**), and juvenile Asian redbtail catfish (*Hemibagrus nemurus*) (**Aryani *et al.*, 2022**). However, commercial fish oil is very expensive, its availability is limited, and the fact that it is also consumed by humans (**Hossain *et al.*, 2020**; **Ahmadkaleyah *et al.*, 2022**; **Nita *et al.*, 2022**) makes it less relevant for continued use in aquaculture feed.

Black soldier fly oil derived from the black soldier fly (BSF) larvae is a potential alternative source of fat. This oil contains 38.5% fat with a dominant composition of lauric acid (35.2%), palmitic acid (25.1%), and oleic acid (19.6%), which are known to stimulate appetite and support juvenile fish growth (**Simó-Mirabet *et al.*, 2017**; **Nugroho *et al.*, 2024**; **Sangsawang *et al.*, 2024**). However, when compared to commercial fish oil, the unsaturated fatty acid content in BSFO is still relatively low, with EPA 0.39%, DHA 0.25%, linoleic acid 11.1%, and linolenic acid 0.4% (**Mai *et al.*, 2019**; **Khan *et al.*, 2024**; **Yang *et al.*, 2025**).

To overcome these limitations, fortifying BSFO with SQO is considered promising since squid oil is rich in polyunsaturated fatty acids, such as arachidonic acid (2.78%), linolenic acid (3.10%), linoleic acid (5.20%), DHA (15.40%), and EPA (9.60%) (**Asadpour, 2016**). The combination of both components is expected to enhance the nutrient composition of the diet, resulting in a more balanced formulation for the fish.

Thus, research on the fortification of BSFO and SQO in juvenile gurami fish feed is important as an alternative to commercial fish oil, which is becoming increasingly scarce and expensive. It is hoped that the combination of these two oil sources has the potential to improve the growth and survival of juvenile gourami fish, as well as providing an innovative solution for the sustainability of juvenile gourami fish farming in the Riau region.

MATERIALS AND METHODS

The experiment took place between June and September 2025. Feed formulation and performance testing were performed at the Fish Breeding Facility, Faculty of Agriculture, Riau Islamic University. Furthermore, feed proximate analysis and fish carcass quality testing were conducted at the Testing, Calibration, and Certification Laboratory, Bogor Agricultural University. An experimental approach employing a completely randomized design (CRD) was applied in this study, consisting of one factor with four treatment levels. Each treatment was tested in triplicate, resulting in a total of 12 experimental units.

Black soldier fly oil production

Black soldier fly oil is obtained from 4.0kg of fourteen-day-old black soldier fly larvae, which are then placed in a roasting machine. This process takes 30 minutes, after which the dried black soldier fly are placed in an oil extraction machine. After being placed in the oil extraction machine, 1.0kg of roasted black soldier fly produced 550 grams of maggot oil (Supartini *et al.*, 2024). Meanwhile, the squid oil used was commercially available squid oil.

Experimental diets

Three types of test feed fortified with maggot oil (BSFO) and squid oil (SQO) were used with four treatments of three replicates, each referred to as the 6% corn oil control treatment (P1 diet) and three treatments of feed fortified with maggot oil and squid oil in the following ratios (w/w) 3%:3% (P2 diet), 2%:4% (P3 diet), and 1.5%:4.5% (P4 diet).

The fortified test feed was then analyzed for proximate content according to the AOAC (2005) standard procedure. Meanwhile, the fatty acid composition was determined through gas chromatography coupled with mass spectrometry (GC–MS). Details on the test feed formulation are presented in Table (1).

Table 1. The composition of the experimental diet fortified with BSFO and SQO to enhance feed nutrition and the growth performance of giant gourami (*Osphronemus gouramy*)

Bahan	Diets (% dry ingredient)			
	P1	P2	P3	P4
Fish meal	46,85	46,85	46,85	46,85
Soybean meal	23,42	23,42	23,42	23,42
Rice bran	15,82	15,82	15,82	15,82
Corn meal	8,90	8,90	8,90	8,90
CMC	2,00	2,00	2,00	2,00
Vitamin mix ^a	2,00	2,00	2,00	2,00

Mineral mix ^b	1,00	1,00	1,00	1,00
Corn oil	6	0	0	0
BSFO ^c	0	3,00	2,00	1,50
SQO ^d	0	3,00	4,00	4,50

^a Vitamin mix (mg/100 g diet): thiamine 5.0; riboflavin 5.0; Ca-pantothenate 10.0; niacin 2.0; pyridoxine 4.0; biotin 0.6; folic acid 1.5; cyanocobalamin 0.01; inositol 200; *p*-aminobenzoic acid 5.0; menadion 4.0; vitamin A palmitate 15.0; chole-calciferol 1.9; α -tocopherol 20.0; choline chloride 900.0.

^b Mineral mix (mg/100 g diet): KH₂PO₄ 412; CaCO₃ 282; Ca (H₂PO₄) 618; FeCl₃.4H₂O 166; ZnSO₄ 9,99; MnSO₄ 6,3; CuSO₄ 2; CuSO₄.7H₂O) 0,05; KJ 0,15; Dekstrin 450; Selulosa 553,51.

^c BSFO (Black Soldier Fly Oil);

^d SQO (Squid Oil).

Preparation and proximate analysis

The test diet for juvenile giant gourami was prepared by first combining small quantities of micronutrients, including vitamin and mineral premixes, followed by incorporating corn flour, fine bran, soybean meal, and fish meal as the primary ingredients. Black soldier fly oil (BSFO) and squid oil (SQO) were measured based on the designated treatment levels and then thoroughly blended with the feed dough to ensure uniform distribution. Next, the feed was extruded into 2mm pellets and dried (Afriyanti *et al.*, 2020). Proximate analysis of the test feed and fatty acid profile are shown in Tables (2, 3, respectively).

Table 2. The chemical composition of the experimental diets used to evaluate the nutritional content and growth performance of giant gourami (*Osphronemus gouramy*)

Chemical composition	P1	P2	P3	P4
Crude protein	29.36	28.86	29.50	28.49
Crude fat	10.71	11.20	11.01	11.10
Crude fiber	6.7	6.35	6.14	6.77
Ash	8.94	8.94	9.15	9.75
Moisture	11.06	10.27	9.4	10.27
NFE ^a	33.23	34.38	34.80	33.62
Energy ^b	403.34	409.86	413.40	403.71

^a NFE is calculated based on the difference (100 – protein – fat – fiber – moisture – ash) (Hasan *et al.*, 2019).

^b Gross energy was calculated according to Chandan *et al.* (2021).

Experimental units and fish acclimation

Twelve round tarpaulin ponds (80cm in diameter and 63cm in height) were filled with 200 liters of fresh water and equipped with aeration. A total of 240 juvenile gurami fish with an average initial weight of 7.4±0.52 grams were obtained from the Kampar

district hatchery. Upon arrival at the laboratory, they were acclimatized in a pond measuring $2 \times 1 \times 0.5$ m for 30 minutes and were then transferred to the tarpaulin ponds. The fry were stocked at a density of 1 fish per 10 liters of water (**Tambunan *et al.*, 2025**).

Rearing and feeding of experimental fish

The experimental fish were acclimatized for one week and fed commercial PF 78-1 feed containing 30% protein, 4% fat, 5% fiber, 10% moisture, and a test diet fortified with black soldier fly oil (BSFO) and squid oil (SQO). After one week of adaptation, the test diet was administered for 60 days. The feeding protocol followed the procedure described by **Syandri *et al.* (2021)**, with feed distributed three times daily at 08.00 a.m, 01.00 p.m and 05.00 p.m. The feeding rate was adjusted according to the method described by **Tambunan *et al.* (2025)** at 3% of the fish's body weight per day.

Water quality management

Water quality was maintained through daily filtration. The parameters observed consisted of temperature, pH, dissolved oxygen, and ammonia, which were recorded at the start, midpoint, and conclusion of the study. Temperature and pH were analyzed using a pH meter (H198108, Romania), while DO was determined with a DO meter (Lutron PDO-519, Taiwan), while ammonia was analyzed using ammonia MR (HI715, Romania). The range of measurement results for these parameters was temperature 27.0–29.10°C, pH 5.32–6.60, DO 5.5–6.4 mg L⁻¹, and ammonia 0.012–0.320 mg L⁻¹, which is still within the optimal range for the growth and survival of juvenile giant gourami.

Experimental design and data analysis

The experiment employed a completely randomized layout consisting of three replicates per treatment. All fish from each treatment and replicate group were individually weighed every 15 days, for a total of five sampling periods (**Tambunan *et al.*, 2025**). Absolute weight gain and survival rate was computed following **Effendi (1979)**, the feed conversion ratio (FCR) was determined following **Effendi (1997)**, viscera somatic index (VSI) according to **Sulistyo (1998)**, and hepatosomatic index (HIS) based on **Effendi (2002)**.

Absolute weight gain = Average final weight of study – Average initial weight

Survival rate = Number of fish at end / Number of fish at start $\times$ 100

HIS = Liver weight (g) / Fish body weight (g) $\times$ 100

VSI = Viscera weight (g) / Fish body weight (g) $\times$ 100

FCR = Feed supply (kg) / Total harvest weight (kg)

Data were subjected to the one-way ANOVA, and treatment means showing statistical variation were further compared through Duncan's multiple range test at a 95% confidence interval using SPSS software.

RESULTS

The experimental diets fortified with BSFO and SQO with different compositions (P2, P3, P4) were proven to improve nutritional quality and growth of juvenile gurami fish compared to diet P1. All experimental diets enhanced feed nutrient composition and the growth outcomes of juvenile giant gourami. Nutritional quality parameters, absolute weight gain, and feed conversion showed a significant effect ($P < 0.05$). Meanwhile, the highest absolute weight gain and FCR were achieved in treatment P3 compared to treatments P1, P2, and P4. Meanwhile, the average HSI and VSI values did not show significant differences ($P > 0.05$) between treatments P1 and P4 (Table 4). The survival rate in each treatment did not differ significantly ($P > 0.05$).

Table 3. Fatty acid profile of experimental diets fortified with BSFO and SQO used to evaluate the nutritional composition and growth performance of giant gourami (*Osphronemus gouramy*)

Parameter	Diets			
	P1	P2	P3	P4
<i>Fatty acid</i>				
Caproic acid (C6:0)	0,07 ^d	0,06 ^d	0,04 ^b	0,01 ^a
Caprilic acid (C8:0)	0,04 ^a	0,09 ^a	0,07 ^a	0,05 ^a
Capric Acid (C10:)	0,05 ^a	0,23 ^d	0,19 ^c	0,12 ^b
Lauric Acid (C12:0)	0,41 ^a	7,53 ^d	6,22 ^c	3,44 ^b
Myristic Acid (C14:0)	0,53 ^a	3,06 ^d	2,59 ^c	1,68 ^b
Palmitic Acid (16:0)	22,41 ^d	18,1 ^a	18,7 ^b	20,42 ^c
Palmitoleic Acid (16:1)	0,19 ^a	0,37 ^c	0,38 ^c	0,31 ^b
Heptadecanoic Acid (C17:0)	0,07 ^a	0,1 ^b	0,14 ^c	0,11 ^b
Stearic Acid (C18:0)	3,15 ^c	2,87 ^a	3 ^b	3,06 ^b
Total SFA	26,92	32,41	31,33	29,2
Cis-11-Eicosenoic Acid (C20:1)	0,08 ^b	0,06 ^a	0,05 ^a	0,06 ^a
Arachidic Acid (C20:0)	0,33 ^c	0,27 ^a	0,27 ^a	0,3 ^b
Behenic Acid (C22:0)	0,17 ^b	0,13 ^a	0,19 ^c	0,19 ^c
Lognocerac Acid (C24:0)	0,08 ^b	0,07 ^{ab}	0,08 ^b	0,06 ^a
Elaidic Acid (C18:1n9t)	0,27 ^d	0,05 ^b	0,09 ^c	0,02 ^a
Oleic Acid (C18:1n9c)	31,03 ^d	24,91 ^a	26,12 ^b	27,66 ^c

Total MUFA	31,96	25,43	26,75	28,23
Linoleic Acid (C18:2n6c)	17,03 ^a	18,26 ^b	22,18 ^d	21,03 ^c
Linolenic Acid (C18:3n3)	0,72 ^a	0,99 ^b	1,34 ^d	1,12 ^c
Total PUFA	17,75	19,25	23,52	22,15

Note: Values within a row bearing different superscript notations represent significant variation ($P < 0.05$), whereas those marked with identical superscripts within the same row show no statistically significant difference ($P > 0.05$).

Table 4. Growth performance and body indices of giant gourami (*Osphronemus gouramy*) during the 60-day feeding trial

Parameter	P1	P2	P3	P4
Weight gain (g)	7,64±0,60 ^a	9,75±0,36 ^b	9,94±0,69 ^b	9,76±1,03 ^b
Survival rate (%)	95±5,00 ^a	100±0,00 ^a	100±0,00 ^a	98,33±2,88 ^a
HSI (%)	0,96±0,34 ^a	1,37±0,05 ^a	1,45±0,27 ^a	1,40±0,14 ^a
VSI (%)	7,25±4,14 ^a	7,64±0,40 ^a	7,73±0,86 ^a	7,65±0,56 ^a
FCR	2,76±0,46 ^b	2,19±0,80 ^{ab}	2,00±0,69 ^a	2.16±0,37 ^a

Description: The values shown are the mean ± SD. Superscripts that differ within the same column denote statistically significant variation among treatments ($P < 0.05$).

DISCUSSION

Fortification of black soldier fly oil (BSFO) and squid oil (SQO) with different compositions in juvenile giant gourami feed has been proven to improve the nutritional composition of the feed in accordance with the needs of this species. The total SFA in the test feed fortified with P2, P3, and P3 was higher (29.20–32.41%) than in feed P1 (22.41%) (Table 3). This increase mainly came from the contribution of lauric acid (C12:0) and myristic acid (C14:0), with the highest levels of 7.53% and 3.06%, respectively, in treatment P2. The increase in fatty acid content is in line with the characteristic of black soldier fly oil (BSFO), which is rich in lauric acid, known to play an important role in rapid energy metabolism and has antibacterial properties (Barragan-Fonseca *et al.*, 2017; Ewald *et al.*, 2020; Kim *et al.*, 2021; Nugroho *et al.*, 2024). Conversely, the palmitic acid (C16:0) content actually decreased in feed fortified with P2 (18.1%), P3 (18.7%) and P4 (20.42%) compared to P1 (22.41%). This can be explained since palmitic acid is commonly found in vegetable oils, including corn oil used in feed P1. This is in line with research of Murru *et al.* (2022), Abrante-Pascual *et al.* (2024) and Rosqvist *et al.* (2024).

The total MUFA in the BSFO- and SQO-fortified experimental diets obtained lower P2 (25.43%), P3 (26.75%), and P4 (28.23%) values compared to P1 (31.96%). This decrease was mainly due to oleic acid (C18:1n9c), which reached 31.03% in experimental diet P1, while in experimental diets fortified with BSFO and SQO (P2, P3, P4) it only

ranged from 24.91 to 27.66%. Oleic acid is known to be one of the main MUFA contents found in vegetable oils such as corn, soybean, sunflower, and olive (**Murru *et al.*, 2022; Rosqvist *et al.*, 2024**). Although the total MUFA decreased, there was an increase in palmitoleic acid (C16:1) in experimental diet P2 (0.37%), P3 (0.38%), and P4 (0.31%) compared to P1 (0.19%). Palmitoleic acid plays an important role as the main precursor in fatty acid synthesis and is one of the main sources in fish metabolism, therefore its presence continues to support energy and physiological needs (**Carta *et al.*, 2017; Nadia *et al.*, 2020**).

The increase in PUFA levels in the experimental diet fortified with BSFO and SQO shows that the combination of these two oil sources can improve feed nutritional quality. The dominant increase in linoleic acid (C18:2n – 6) and linolenic acid (C18:3n-3), particularly in the P2 diet treatment, indicates the role of SQO as the main contributor of PUFA, both n-6 and n-3. This higher PUFA content has positive implications for fish growth, the immune system, and cell membrane synthesis (**Hossain *et al.*, 2020; Goda *et al.*, 2024**).

The experimental diet in treatments P2, P3, and P4 showed a significant increase in the absolute weight growth of juvenile giant gourami compared to P1. The highest absolute weight of 9.94 ± 0.69 g was obtained in treatment P3, which was significantly higher than P1, which was 7.64 ± 0.60 g ($P < 0.05$). This increase indicates that BSFO and SQO provide a balance of fatty acids in the form of linoleic and linolenic acids that support energy metabolism and tissue synthesis. As reported by **Hossain *et al.* (2020)** and **Mickael *et al.* (2025)**, freshwater fish can meet their essential fatty acid requirements through the conversion of PUFAs, namely linoleic acid (C18:2n6c) and linolenic acid (C18:3n3), to support growth. This is in line with reports postulating that increasing fatty acids in feed, particularly linolenic and linoleic acids, can increase the growth of the Nile tilapia fingerlings (**Ayisi *et al.*, 2017**), juvenile pacu (**Goncalves *et al.*, 2021**), coho salmon larvae (**Yu *et al.*, 2022**), and juvenile golden pompano (**Zhang *et al.*, 2025**).

The survival rate of juvenile giant gourami in this study was very high, ranging from 95–100%, and there was no significant difference between treatments ($P > 0.05$). This condition can be attributed to the significant increase in lauric acid (C12:0) in the treatment with BSFO. Lauric acid is known to be easily digestible, quickly metabolized as energy, and has antimicrobial properties that can strengthen the immune system of fish (**Ewald *et al.*, 2020; Kim *et al.*, 2021; Barragan-Fonseca *et al.*, 2022**). Research by **Fontinha *et al.* (2025)** also shows that lauric acid supplementation in juvenile seabass feed can enhance immune response and reduce mortality. Thus, the presence of lauric acid from BSFO plays an important role in maintaining the physiological stability of juvenile giant gourami, thus supporting optimal survival.

In this study, the HSI values for treatment P1 ($0.96 \pm 0.34\%$), P2 ($1.37 \pm 0.05\%$), P3 ($1.45 \pm 0.27\%$), and P4 ($1.40 \pm 0.14\%$) were obtained, while the VSI values for P1 ($7.25 \pm 4.14\%$), P2 ($7.64 \pm 0.40\%$), P3 ($7.73 \pm 0.86\%$), and P4 ($7.65 \pm 0.56\%$) were relatively

consistent and did not show significant differences between treatments ($P > 0.05$). This indicates that feed fortification with BSFO and SQO did not cause excessive fat accumulation in the liver or visceral organs. The stability of these two parameters indicates that lauric acid from BSFO is more likely to be utilized as a quick energy source than stored in the liver. The present results are consistent with the study of **Fotinha et al. (2025)**, which demonstrated that lauric acid supplementation in juvenile seabass (*Lates calcarifer*) enhanced growth and immune responses without promoting hepatic fat accumulation. Similar results were also reported by **Farias et al. (2025)** in juvenile hybrid catfish *Ictalurus punctatus* $\times$ *I. furcatus* fed BSF oil, where there was no increase in HSI or VSI despite the high oil substitution rate. Thus, oil from BSFO and SQO plays a greater role as a source of metabolic energy and growth support, rather than as a reserve stored in hepatosomatic or visceral tissues.

The experimental diet fortified with BSFO and SQO showed the most significant difference in the FCR parameter ($P < 0.05$). Treatment P1 produced the highest FCR value (2.76), while experimental diets fortified with BSFO and SQO produced lower FCR values of 2.19 (P2 diet), 2.00 (P3 diet), and 2.16 (P4 diet), with the best value in treatment P3 at $2.00 \pm 0.69\%$. These findings are in line with **Eom et al. (2024)**, who reported that increasing the fat content in feed can significantly reduce FCR, as fat functions as a non-protein energy source so that protein can be directed more toward tissue synthesis and growth rather than energy requirements. A comparable investigation conducted by **Fan et al. (2021)** in adult common carp (*Cyprinus carpio*) also revealed that fish receiving oil-enriched diets exhibited the lowest FCR, further confirming that lipid inclusion contributes substantially to enhancing feed utilization efficiency.

CONCLUSION

Fortification of black soldier fly oil (BSFO) and squid oil (SQO) in juvenile giant gourami feed can improve nutritional quality, particularly SFA and PUFA levels, and support optimal growth. The best treatment was obtained in feed P3, which had the best absolute weight and feed conversion ratio (FCR) compared to the control (P1). Survival rates and HSI and VSI values did not differ significantly. Thus, fortification of maggot oil (BSFO) and squid oil (SQO) in feed has the potential to be an alternative to commercial fish oil in giant gourami feed formulations and can promote a circular economy in advancing aquaculture.

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