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Effects of Replacement of Fish Oil with Black Soldier Fly (*Hermitia illucens*) Oil on the Growth Performance and Biochemical Composition of the Flesh of Nile Tilapia (*Oreochromis niloticus*) Fry

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Abstract

The black soldier fly (*Hermetia illucens*) is a promising insect species for use in aquaculture feeds due to its high protein and fat content, with a fatty acid profile primarily consisting of saturated and medium-chain fatty acids. In this experiment, fish oil was replaced with black soldier fly (BSF) oil in varying proportions to evaluate its effect on the growth and biochemical composition of Nile tilapia flesh. Three experimental diets designated R1, R2, and R3 were formulated, replacing 50% and 100% of fish oil with BSF oil. The best results in terms of growth performance and feed efficiency were observed with the R2 diet (50% fish oil replacement), which yielded the following average values: final mean weight (0.42 ± 0.05 g), absolute mean weight gain (0.34 ± 0.05 g), specific growth rate (4.14 ± 0.31%/day), feed conversion rate (2.45 ± 0.74), and protein efficiency coefficient (1.07 ± 0.15). Regarding the biochemical composition of the flesh, there were no significant differences in protein and ash content across the experimental diets, with the following respective values: 17.89 ± 1.49%, 17.29 ± 1.81%, and 16.78 ± 1.83% for protein, and 5.30 ± 0.39%, 4.99 ± 0.47%, and 4.59 ± 0.59% for ash content in diets R1, R2, and R3. However, a significant difference in lipid content was observed between the R2 diet and the R1 and R3 diets, with values of 4.85 ± 0.74, 3.44 ± 1.42, and 3.14 ± 1.27, respectively.

Keywords: *Oreochromis niloticus*, fish oil, Black soldier fly oil, growth

1. Introduction

Tilapia has become one of the most significant farmed fish species worldwide, with production doubling from 2005 to 2016, reaching 5.37 million tons (Deluzarche, 2021). This growth can be attributed to several factors: its nutritional value, profitability, adaptability to various environments, and its role in food security and economic development. Tilapia farming provides employment and income opportunities in rural areas, stimulates exports, and diversifies animal protein sources. Sustainable tilapia production also alleviates pressure on wild fish stocks, helping to meet the increasing demand for seafood products.

However, efficiently feeding fish remains a major challenge. Fish feed accounts for 40-70% of total production costs (Rana et al., 2009). Therefore, low-quality or expensive feed can directly impact fish yield and profitability. The primary components of fish feed are fishmeal and fish oil. Fishmeal serves as the main source of protein, while fish oil provides long-chain polyunsaturated fatty acids (LC-PUFAs), which are essential for human health. There are growing concerns about the sustainability of these resources. A significant portion (about 10-12% in 2016) of the world's fish production is used for fishmeal and fish oil (FAO, 2020), which are derived from the industrial fishing of pelagic forage fish, such as sardines and anchovies (FAO, 2018). The ecological damage caused by overfishing is undeniable, and efforts to prevent it have led to the search for alternative feed ingredients. Despite the ongoing demand for these resources, a downward trend has been observed in the use of fishmeal and fish oil in fish feed (Tacon et al., 2008).

The potential of using insects in fish feed has been extensively studied (Van Huis, 2013a;

Barroso et al., 2014; Nogales-Mérida et al., 2019a; Alfiko et al., 2022; Moyo et al., 2022). Insects are a rich source of lipids and proteins, offering several advantages, including rapid reproduction, growth, efficient feed conversion, and the ability to consume biological waste. Notably, certain farmed fish species readily consume insects in the wild. Currently, insect farming does not directly compete with food production. For instance, on a dry matter basis, black soldier fly larvae contain approximately 35% fat and 42% protein, and their amino acid profile is more similar to that of fish than to soybean meal (Barroso et al., 2014).

Using insect meal in fish feed presents several key benefits. Nutritionally, it provides essential proteins and lipids for fish growth, enhancing the overall quality of their feed. Environmentally, it serves as a sustainable alternative to traditional fish oils, reducing pressure on marine resources and lowering the carbon footprint of aquaculture. Economically, insect oil could reduce production costs and diversify supply sources while meeting the rising demand for sustainable proteins. Moreover, adopting insect-based feed allows fish farmers to differentiate themselves by employing innovative and environmentally friendly practices.

Among the insect species used in aquaculture, the black soldier fly (*Hermetia illucens* L.) stands out as a particularly valuable source of fish feed. Black soldier fly larvae are a promising feed option, especially because this method allows for the use of organic waste (Diener et al., 2009). On a dry matter basis, black soldier fly larvae contain 30-35% fat, 40-45% protein, 4.8-5.1% calcium, 0.6% phosphorus, and 11-15% ash. Additionally, the larvae are rich in essential amino acids, particularly leucine, methionine, and lysine, and do not contain nutritionally inhibitory elements (Basto et al., 2020; Baiano et al., 2020; Shumo et al., 2019). Black soldier fly larvae also contain 10-30% essential lipids, 30-58% protein, and amino acids that are comparable to those found in fishmeal (Kourimská et al., 2016). This study aims to investigate the partial or complete replacement of fish oil with black soldier fly oil to assess its impact on the growth performance and biochemical composition of Nile tilapia (*O. niloticus*).

2. Methods

2.1 Food Formulation and Manufacture

Three isoprotein and isolipid diets were formulated using the Pearson-Square method. These diets were created using ingredients of both animal and vegetable origin, with the only variation being the type of oil used: fish oil and black soldier fly oil.

Table 1. Centesimal composition of different diets

Ingredients	**R1 (0% BSF oil)**	**R2 (50% BSF oil)**	**R3 (100% BSF oil)**
Rice polish	10	10	10
Groundnut cake meal	15	15	15
Brewery waste meal	10	10	10
Zoolithes	1	1	1
Soyabean cake meal	20	20	20

Shrimp waste meal	20	20	20
Bread leftover meal	10	10	10
Neocarya pulp meal	7	7	7
BSF oil	**0**	**2.5**	**5**
Fish oil	**5**	**2.5**	**0**
Vitamins mix [a]	1	1	1
Minerals mix [b]	1	1	1
Total	100	100	100
Protein Level (%)	32.21	32.21	32.21
Lipid Level (%)	9.17	9.17	9.17

a=vit A 250000 UI; vit D3 250000UI; vit E 5000mg; vit B1 100mg; vit B2 400mg; vit B3(pp) 1000mg; vit B5 pantode Ca2000mg; vit B6 300mg; vit K3 1000g; vit C 5000mg; H biotin 15mg; choline 100g; anti-oxydant (BHT), crushed and calcined attapulgite qs 1000mg;

b=phosphorus 7%; calcium 17%; sodium 1,5%; potassium 4,6%; magnesium 7,5%; manganese 738mg; zinc 3000mg; iron 4000mg; copper 750mg; iodine 5mg; cobalt 208mg; calcined and ground attapulgite qs 1000g; fluorine 1.5% (approximately),

The formula for the experimental diets is presented in Table 1. We formulated three isoprotein and isolipid diets, each containing 32% crude protein (CP), designed to replace varying amounts of fish oil with 0% (R1), 50% (R2), and 100% (R3) black soldier fly oil. Fish oil and black soldier fly oil served as the lipid sources. The other ingredients (listed in Table 1) were sourced locally, fried, and then ground into a powder. The dietary ingredients obtained from the local market were first ground into small particles using a hammer mill and then passed through a 250 µm mesh sieve. After achieving homogeneity by thoroughly mixing all the dry ingredients, fish oil and peanut oil were added. Distilled water (approximately 30% of the dry weight) was then incorporated to create a wet paste. This paste was extruded through a 3.0mm diameter die to form pellets. The pellets were subsequently dried in a dry air oven at 60°C and stored at -20°C for preservation.

2.2 Rearing Conditions

The experiment was conducted at “Pr Omar Thiom Thiaw” aquaculture station of the University Institute of Fishery and Aquaculture over a period of 45 days. A total of 180 *Oreochromis niloticus* fry, with an average weight of 0.10 g, were stocked in nine 60-liter fiberglass tanks at a density of 20 individuals per tank. The tanks were filled to two-thirds of their capacity.

Physico-chemical parameters were measured twice daily at 8:00 a.m. and 4:30 p.m. The fish were fed twice a day at feeding rates of 10% and 8% of their body weight. Sampling was conducted every 15 days to monitor fish growth.

2.3 Growth Parameters

To estimate fish growth during the experiment and assess the efficiency of the tested feeds, the following zootechnical parameters and indices were calculated:

- Absolute average weight gain (aMWG, g) = Final mean weight (FMW, g) - Initial mean weight (IMW, g)

- Relative average mean weight gain (rMWG, %) = (Final weight (g) - Initial weight (g)) *100/ Initial weight (g)

- Specific Growth Rate (SGR, % /d) = [ln (final weight) - ln (initial weight)] x 100 / Duration

- Feed Conversion Ratio (FCR) = Quantity of dry feed ingested / Absolute average weight gain

- Survival rate (SR, %) = Final number of fish x 100 / Initial number of fish

2.4 Flesh Analysis

At the end of the experiment, fish samples from each tank and diet were analyzed at the ENSA bromatology laboratory in Thiès, Senegal, following the AOAC (1995) method. Moisture content was determined by drying the samples at a constant temperature of 105°C. Crude protein content (calculated as total nitrogen multiplied by 6.25) was measured using the micro-Kjeldahl method with a Kjeltec System 1002 distillation unit (Tecator, Hoeganes, Sweden). Crude fat content was analyzed using the Soxhlet method, while ash content was determined by incinerating the samples in a muffle furnace at 550°C for 6 hours.

2.5 Statistical Analysis

A one-way analysis of variance (ANOVA) was conducted to evaluate differences in final mean weight (FMW), absolute average weight gain (AMWG), relative average weight gain (rMWG), specific growth rate (SGR), and feed conversion ratio (FCR) among the three (3) treatments. When significant differences were detected ($P < 0.05$), Duncan's test was applied for multiple comparisons between treatments. All statistical analyses were performed using SAS for Windows (Version 9.3, SAS Institute, Cary, NC, USA).

3. Results

3.1 Physico-chemical Parameters

Table 2. Temperature variation

Temperature (°C)	**R1**	**R2**	**R3**
Min	**26.34**	**26.27**	**26.20**
Mean	**27.32**	**27.24**	**27.28**
Max	**28.18**	**28.07**	**28.04**

The recorded temperature values in this study ranged from 26.20°C to 28.18°C, with an average of 27.28°C.

Table 3. pH variation

pH	R1	R2	R3
Min	**8.17**	**8.18**	**8.15**
Mean	**8.56**	**8.57**	**8.56**
Max	**8.89**	**8.91**	**8.92**

The pH values were consistent, ranging from 8.15 to 8.92, indicating that the water remained alkaline across all tanks.

Table 4. variation in dissolved oxygen

O2 (mg/L)	R1	R2	R3
Min	**3.71**	**3.69**	**3.46**
Mean	**4.86 ± 0,08[a]**	**4.92 ± 0,03[a]**	**4.95 ± 0,06[a]**
Max	**6.45**	**6.29**	**6.43**

Dissolved oxygen levels ranged from 3.46 to 6.45 mg/L, with an average of 4.9 mg/L. These values fall within the optimal range for tilapia growth.

3.2 Zootechnical Parameters

To evaluate fish growth, analytical methods based on commonly used zootechnical indices were applied. The main results are presented in the table below.

Table 5. Fish growth parameters

Diets / Parameters	R1	R2	R3
IMW (g)	0.10[a]	0.10[a]	0.10[a]
FMW (g)	0.42 ± 0.17[a]	0.42 ± 0.05[a]	0.34 ±0.07[b]
aMWG (g)	0.32 ± 0.17[a]	0.32 ± 0.05[a]	0.24 ± 0.06[b]
rMWG (%)	321.85 ± 169.8[a]	319.64 ± 49.45[a]	242.94 ± 66.72[b]

VI (g/d)	0.38 ± 0.02^a	0.36 ± 0.05^a	0.28 ± 0.03^b
SGR (% /d)	3.68 ± 1.21^a	4.14 ± 0.31^a	3.35 ± 0.77^b
FCR	2.95 ± 1.22^b	2.45 ± 0.74^a	2.83 ± 0.54^b
PER	1.16 ± 0.39^a	1.33 ± 0.34^a	1.13 ± 0.22^a
SR (%)	55 ± 5.00^b	55 ± 8.66^b	76.66 ± 10.40^a

Superscripts a and b indicate a significant difference (P<0.05).

Fish fed the R2 diet, which contained a 50% fish oil-black soldier fly oil mixture, exhibited the highest growth rates. Their growth was significantly higher (P< 0.05) than that of fish fed the R3 diet but not significantly different from those fed the R1 control diet. The mean final weights of fish on the R1 and R2 diets showed no significant differences.

With an average mean weight gain of 0.32 g over 45 days and a specific growth rate of 4.14% per day, fish fed the R2 diet demonstrated the best growth performance among the three diets. Statistically, these results were comparable to those obtained with the control diet (R1).

At the end of the experiment, the feed conversion ratio (FCR) was calculated based on biomass production and total feed consumption. The FCR values were 2.45 for the R2 diet, 2.83 for the R3 diet, and 2.95 for the R1 diet. The lower FCR value of the R2 diet indicates better feed efficiency compared to the other two diets.

Survival rates varied from 55 ± 5.00% to 76.66 ± 10.40%, with the highest survival observed in fish fed the R3 diet and the lowest in those fed the R1 diet. Although fish from the R1 (control) and R2 groups had the same survival rate (55 ± 5.00%), there was a significant difference in survival rates among the diets.

3.3 Results of Biochemical Analysis of Flesh

Table 6. biochemical composition of fish flesh

Paramètres	**R1 (0% BSF oil)**	**R2 (50% BSF oil)**	**R3 (100% BSF oil)**
Ash (% DM)	**5.30 ± 0.39^a**	**4.99 ± 0.47^a**	**4.59 ± 0.59^a**
Crude Protein (%DM)	**17.89 ± 1.49^a**	**17.29 ± 1.81^a**	**16.78 ± 1.83^a**
Crude Lipid (%DM)	**3.44 ± 1.42^b**	**4.85 ± 0.74^a**	**3.14 ± 1.27^b**

DMA= Dry matter analyzed
Superscripts a and b indicate a significant difference (P<0.05).

The results of the chemical composition analysis of the fish flesh indicate that ash content was highest in fish fed the R1 control diet, with 5.30% DMA. However, no significant differences were observed among the diets.

Regarding protein levels, all fish were rich in protein, with values of 17.89 ± 1.49%, 17.29 ± 1.81%, and 16.78 ± 1.83% DMA for diets R1, R2, and R3, respectively. Fish fed the control diet (R1) exhibited the highest protein content.

Fat content in this study ranged from 3.14% to 4.85%, with the highest fat content observed in fish fed the R2 diet (4.85%).

4. Discussion

Water temperature has a significant impact on food intake, metabolism, growth, and survival in fish (Azaza et al., 2008). It also influences incubation and its duration, which are primarily dependent on water temperature. The results of this study showed that temperature ranged between 26°C and 28°C, with an average of 27°C. These values fall within the optimal range for tilapia growth, as recommended by Arrignon (1996), who set the acceptable limits between 21°C and 30°C. Additionally, Suresh (2003) suggests that the ideal temperature for rearing Nile tilapia ranges from 26°C to 32°C.

pH is a key parameter that characterizes the acidity or alkalinity of water, serving as an indicator of the quantity and nature of dissolved mineral ions. It helps determine water suitability for aquatic life (Groga, 2012). The average pH values recorded in this study ranged between 8.56 and 8.57. According to Alcántar-Vázquez et al. (2014), the recommended pH range for freshwater fish is between 6 and 9, with fish generally thriving better in neutral to alkaline water rather than acidic conditions (Bruslé et al., 2004).

Dissolved oxygen is one of the most crucial factors affecting fish survival, food intake, growth, and metabolism (Tran-Duy et al., 2012), particularly in intensive aquaculture systems. In this study, dissolved oxygen levels ranged from 3 mg/L to 6 mg/L, with an average of 4 mg/L. These values are comparable to those reported by Merah (2023), who recorded levels between 4.6 mg/L and 6.1 mg/L in his study on the incorporation of locally available ingredients into Nile tilapia diets.

Survival rate (SR) is a key parameter in planning an effective harvest, as it provides valuable insights into feed rations and biomass distribution among small and large fish. In this study, survival rates ranged from 55% to 76.6%. These results differ from those reported by Herawati et al. (2023), who recorded survival rates between 82.22% and 95.56% in their study on the inclusion of black soldier fly (*Hermetia illucens*) oil (10%, 15%, and 20% in feed) and its effects on the growth performance of common carp (*Cyprinus carpio*).

The relative average weight gain (rMWG), specific growth rate (SGR), and feed conversion ratio (FCR) of fish fed the R2 diet showed no significant differences ($P > 0.05$) compared to the R1 control diet. However, a significant difference was observed between these two diets and the R3 diet (100% black soldier fly (BSF) oil).

Although summarized, the highest specific growth rate (SGR) values were recorded in fish fed the R2 and R1 diets, with 4.14 ± 0.3%/day and 3.68 ± 1.21%/day, respectively. The SGR values obtained in this study align with those reported by Xu et al. (2024), who recorded

SGRs of 4.16 ± 0.06%/day and 4.21 ± 0.01%/day in their study on the effects of BSF oil in different lipid diets for juvenile mirror carp. However, our results surpass those of Herawati et al. (2023), who reported SGRs ranging from 0.86 ± 0.08%/day to 1.27 ± 0.19%/day in their study on the inclusion of BSF oil (10%, 15%, and 20%) in the diet of common carp (*Cyprinus carpio*).

The feed conversion ratio (FCR) is a key indicator of fish feed quality, as it evaluates feed utilization and absorption. A lower FCR indicates better feed efficiency, reducing feed wastage (Opiyo et al., 2014). The FCR values observed in fish fed the R1 and R2 diets were 2.45 and 2.83, respectively. These values do not align with those reported by Herawati et al. (2023), who recorded FCRs of 1.52 ± 0.03, 1.92 ± 0.14, and 2.81 with the inclusion of 10%, 15%, and 20% BSF oil in common carp diets. Similarly, our results differ from those of Ndione et al. (2022), who reported FCR values of 2.4 ± 0.36 and 2.8 ± 0.36 in diets R3 and R5 (50% and 100% cricket meal), respectively, in their study on the partial or total replacement of fish meal with cricket meal in Nile tilapia diets.

The results of the biochemical analyses of fish fed diets are presented in Table 3. The biochemical composition of the flesh of fish fed diets R1 and R2 (17.89 ± 1.49% and 17.29 ± 1.81% protein, respectively) showed significantly higher protein retention compared to fish fed diet R3 (16.78 ± 1.83%). This suggests that a combination of 25% fish oil and 25% black soldier fly (BSF) oil represents a nutritionally balanced proportion. These protein values are higher than those reported by Xu et al. (2024), who obtained 13.09 ± 0.18%, 13.19 ± 0.02%, and 13.20 ± 0.05% in their study on BSF oil inclusion (25%) in different lipid diets for juvenile mirror carp.

In contrast, body lipid content appeared to be more clearly influenced by the rate of BSF oil incorporation. The highest lipid content was recorded in fish fed the R2 diet (4.85 ± 0.74%), which was significantly different from the other diets, while the lowest lipid content was observed in fish fed the R3 diet (3.14 ± 1.27%). These values are lower than those reported by Xu et al. (2024), who recorded lipid contents of 7.54 ± 0.01% and 7.71 ± 0.02% in juvenile mirror carp.

Ash content did not appear to be significantly affected by dietary treatments, as no statistical differences were observed. The highest ash content was found in fish fed the R1 diet (5.30 ± 0.39%), while the lowest was in fish fed the R3 diet (4.59 ± 0.59%). These values are higher than those reported by Xu et al. (2024), who recorded ash contents ranging from 2.62 ± 0.23% to 2.66 ± 0.29% in juvenile mirror carp.

5. Conclusion

The complete replacement of fish meal with insect-based meals and oils remains an open question; however, achieving high levels of substitution without negatively affecting fish performance appears feasible. The results of this study indicate that fish fed the R2 diet (50% BSF oil) demonstrated the best growth performance and feed efficiency, although no significant differences were observed among the three diets.

Furthermore, economic considerations could play a key role in guiding future research toward optimizing the profitability of fish diets incorporating black soldier fly. The nutritional outcomes of this study are promising and suggest the potential for the total replacement of fish meal and fish oil in aquafeeds. However, since alternative sources to fish oil are limited, it would be more justified to aim for partial substitution, since the best results were obtained with fish fed a diet containing 25% fish oil and 25% black soldier fly oil.

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