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Short Communication

Evaluation of Fatty Acid Profile in Red Tilapia Fed Black Soldier Fly Larvae and Tofu Waste-Based Diets

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Abstract

Despite growing interest in sustainable aquaculture, studies on the combined use of BSFL and tofu waste as alternative protein sources in tilapia diets, particularly their effects on fatty acid profiles, remain limited. This study evaluates the effects of fish feed formulated with BSFL and tofu waste on the growth performance, proximate composition, and fatty acid profile of red tilapia (*Oreochromis niloticus*). Experimental diets included: commercial feed, 100% dried BSFL, and a combination of 40% dried BSFL:20% tofu waste. Fish were reared for eight weeks under controlled conditions. Treatment with BSFL:tofu waste exhibited superior growth performance, with the highest weight gain (102.88%) and length gain (33.75%). Proximate analysis revealed significant variations across treatments, with a combination of BSFL:tofu waste achieving the highest caloric value (110.71 cal/g) and fat content (3.75%) while maintaining protein levels comparable to commercial feed. Fatty acid analysis showed a combination of BSFL:tofu waste enhanced total fatty acid content (77.92%), predominantly polyunsaturated fatty acids (PUFAs), increasing from 37.34% in commercial feed to 47.04%. Essential fatty acids, including DHA and linoleic acid, were elevated in a combination of BSFL: tofu waste, indicating improved nutritional quality. Cholesterol levels remained unaffected among treatments. These findings suggest that combining BSFL and tofu waste can enhance fish feed quality, optimize growth performance, and improve the nutritional value of red tilapia. Future research should explore refining feed formulations to maximize nutrient stability and sustainability for broader aquaculture applications.

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1. Introduction

Fish is a major source of protein in the diet of Indonesian people and plays a crucial role in national food security. According to the Indonesia Ministry of Maritime Affairs and Fisheries (MMAF), there has been a steady increase in fish consumption, rising from 54.56 kg per capita in 2020 to 57.61 kg per capita in 2023, with an average annual increase of 1.84%. As fish consumption has increased, aquaculture production has also risen, from 14.8 million tons in 2020 to 15.36 million tons in 2023. Fish farming relies heavily on the availability of feed to support fish growth and survival. Feed costs account for 50-70% of the total production cost per kilogram of fish (Cicilia and Susila, 2018). In 2018, fish meal imports reached 99,220 tons, increasing to 122,419 tons by 2020, accounting for 47.66% of total fisheries imports (Ministry of Marine Affairs and Fisheries, 2022). Aquaculture continues to face challenges related to the high cost and sustainability of conventional feed ingredients, driving the search for alternative protein sources. Among the most promising candidates are Black Soldier Fly Larvae (BSFL) and tofu waste, both of which are increasingly recognized for their nutritional value, availability, and potential to reduce environmental impact. While individual applications of BSFL or tofu waste in aquafeeds have been explored, studies evaluating their combined use are still scarce. In particular, there is a notable lack of research investigating how this combination influences the fatty acid profile of red tilapia (*Oreochromis* sp.), a commercially important species. Addressing this gap is crucial for developing cost-effective and nutritionally balanced aquafeeds that support sustainable aquaculture practices.

The Black Soldier Fly (BSF), scientifically known as *Hermetia illucens*, is found in tropical, subtropical, and temperate regions. BSFL are easy to cultivate and can thrive on various substrates such as agricultural and livestock by-products (Caligiani *et al.*, 2018). BSFLs are widely recognized for their ability to efficiently break down various types of organic waste, such as fruits, vegetables, agricultural byproducts, and municipal waste. Implementing effective BSF farming techniques not only provides a sustainable source of income for farmers but also helps minimize environmental pollution, contributing to long-term ecological sustainability (Ganvir *et al.*, 2022). The larvae's high reproductive capacity and rapid growth allow them to convert organic waste into nutrient-rich biomass and reduce daily organic waste by 65.5-78.9% (Suryati *et al.*, 2023). BSFL is a potential lipid source for aquaculture feed with a high content of lipid (38.5%). The combination of fish oil and BSFL oil in the diets showed positive effects on red tilapia (Bakar *et al.*, 2021). Research by Supardan *et al.* (2023) found that feed formulated with 50% BSFL and 50% commercial feed produced optimal results in terms of length, weight growth, and specific growth rates in Nile tilapia. Similar research conducted by Herawati *et al.* (2023) concluded that BSF oil for common carp has a significant influence ($P<0.05$) on total feed consumption, feed conversion ratio, feed utilization efficiency, specific growth rate, and survival. Another research by English *et al.* (2021), the method used to produce BSFL meal differs across feeding trials, leading to variations in its overall composition. These differences, in turn, influence the outcomes related to growth performance, fish health, and tissue biochemical composition.

Tofu waste, or okara, is a by-product of the tofu-making industry. The tofu industry typically generates solid waste equivalent to 40% of the total capacity for processing 100 kilograms of soybeans (Faisal *et al.*, 2016). Tofu waste spoils easily, has a short shelf life, and can cause environmental pollution if not properly managed (Putri *et al.*, 2022). The protein content, especially insoluble protein, in tofu waste is relatively high because not all the protein is extracted during the tofu production process (Rahayu *et al.*, 2016; Rahman *et al.*, 2021). Okara contains high moisture, high energy, crude protein, crude fiber, crude fat, and ash. Protein in okara range between 10-40% (Kamble and Rani, 2020; Rahman *et al.*, 2021). Additionally, the inclusion of tofu waste improves protein and starch digestibility, as well as structural integrity. The use of tofu waste as a fish feed ingredient is an attractive alternative due to its simplicity, availability, and high nutritional value. Research by Suwoyo *et al.* (2018) and Voss *et al.* (2021) demonstrated that feed formulation and different pre-treatments of okara could be optimally utilized by Nile tilapia.

As fish is an essential source of protein, it is also very important as a source of fatty acids. Fish and other aquatic foods are known to be the main sources of long chain polyunsaturated fatty acids (LC-PUFA), especially eiocosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Tasbozan and Gokce, 2017; Houguo *et al.*, 2020). Omega-3 polyunsaturated fatty acids (PUFAs) include α-linolenic acid (ALA), stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) (Shahidi and Ambigaipalan, 2018). A large body of evidence has supported the beneficial roles of omega-3 PUFAs in various pathophysiological conditions, including metabolic syndrome, cardiovascular diseases, cancer, depression, and neurodegenerative illnesses. Omega-3 PUFAs have also been shown to function against inflammation and oxidative stress, which are underlying causes of different disorders (Akbar *et al*., 2021). These PUFAs also contribute to various cellular activities like cell signaling, structural integrity, and fluidity of the cell membrane, the regulation of blood pressure, glucose level, the nervous system, inflammatory reactions, and hematic clotting (Kapoor *et al*., 2021).

Fish fat content, type, and quantity of fatty acids influence many factors; these factors include, e.g., fish species, size and age of fish, season, water temperature and quality, as well as geographic location (Sen Ozdemir *et al.,* 2023; Zhang *et al*., 2021). The major factors contributing to fatty acid profiles of the freshwater fish flesh are the diet and the rearing conditions. The fat content and composition vary among different fish species as well as the same species cultured under different conditions (Všetičková *et al*., 2019).

In this study, we focused on providing feed aimed at enhancing fatty acid levels in fish flesh. BSFL and tofu waste, with their abundant nutritional content, availability, and environmentally friendly impact, hold great potential as more efficient and sustainable alternative feed ingredients. However, to date, there is still limited information on the effects of BSFL and tofu waste feed on red tilapia, particularly when compared to dried BSFL feed and commercial feed. Thus, this study aims to analyze the fatty acid profile of red tilapia (*Oreochromis niloticus*) fed with a combination of BSFL and tofu waste, as well as growth performance and proximate composition.

2. Materials and Methods

2.1 Materials

2.1.1 The materials

The main materials used in this study included red tilapia (*O. niloticus*) fingerlings (5-6 cm, 2 weeks of age) (AquaBioFresh Indonesia), de-fatted BSFL flour (53% protein) (Protenusa, Indonesia), and commercial fish pellets (16% protein, 3% fat) (Omega, Newhope Aquafeed Indonesia).

2.1.2 The equipment

The main equipment used are oven (Eyela, Japan), pellet-making machine (Tiger, TPM-125), water tank size 100 cm x 30 cm x 50 cm, aerator set, spectrophotometer UV-Vis (BMG Labtech), Soxhlet and heating mantel (B-One Soxhlet 500 AHM).

2.1.3 Ethical approval

This experiment was performed according to National Standardization Agency of Indonesia (SNI) No. 8228.4:2015-Good Aquaculture Practices (CBIB) Part 4: Freshwater Fish, and Guidelines for Use of Fish in Field Research by the American Fisheries Society (1996).

2.2 Methods

2.2.1 Experimental design

A Completely Randomized Design (CRD) was applied, consisting of three dietary treatments with ten replicates each, as follow: (a). P0: Commercial fish pellet (control), (b). P1: 100% dried BSFL, and (c). P2: 40% BSFL flour + 20% tofu waste flour.

The independent variable of this experiment is the type of feed given to the subject red tilapia (*O. niloticus*) as mentioned above (P0, P1, and P2). The response or outcome measured in this study is the growth rate of fish (body weight and length), proximate, cholesterol, and fatty acid content analysis of fish flesh. We also keep the conditions, such as environmental conditions (water temperature, pH, and feeding schedule), fish species, initial length, and duration of the experiment, constant.

2.2.2 Feed preparation

The de-fatted BSFL flour was used as a main ingredient for fish feed, mixed with tofu waste as outlined in the experimental design. The tofu waste was dried in an oven at 60°C for 1–2 days until completely dry, then ground using a blender and stored at room temperature with silica gel as a desiccant prior to use as a feed component. The prepared raw materials were weighed according to the respective treatment formulations for fish feeding. The first treatment (P0) consisted of 100% commercial standard fish feed. The second treatment (P1) used 100% dried BSFL. The third treatment (P2) was formulated using 40% BSFL flour, 20% tofu waste, 25% rice bran, 10% corn flour, and 5% tapioca flour, mixed with 30% water per kilogram of feed mixture. Specifically, treatment P3 was prepared in a 1 kg batch using a pelletizing machine.

2.2.3 Feeding trial

The growth trial used red tilapia fingerlings with an average body length of 5–6 cm. Red tilapia was chosen for its rapid growth, consumer preference, high economic value, and resilience to environmental changes. After a seven-day acclimatization period, the fish were randomly distributed into tanks and fed the experimental diets twice daily (7:00 and 16:00) at 5% of biomass weight. The temperature of water was set to 30˚C, and the pH was maintained around 7.0-8.5 (Indonesian National Standard, 2009). The trial lasted eight weeks, with three treatments and ten replicates (Rawski *et al*., 2020).

2.2.4 Sampling and measurements

Fish growth metrics were recorded weekly, including weight and length. Additional growth parameters measured included final body weight and length (FBW), body weight and length gain (BWG), and percentage of weight and length gain (PWG, PLG), Feed Conversion Ratio (FCR), and Specific Growth Rate (SGR) according to Rawski *et al*. (2020). On the final day of the experimental period, each fish was euthanized, followed by the separation of flesh from the internal organs and other body parts. The flesh samples were then stored at -10°C for laboratory analysis.

2.2.5 Laboratory analysis

Proximate, total cholesterol, and fatty acid analyses were conducted on the fish flesh. Proximate analysis included measurements of dry weight, crude protein, crude fat, and crude fiber using the Kjeldahl method (Rawski *et al*., 2020). Proximate analysis is conducted to determine the nutritional composition of a feed material, including moisture content, ash, crude protein, and crude fat. Carbohydrate content and total caloric value are calculated by difference. Total cholesterol measured based on the quantitative Liebermann-Burchard method with cholesterol as the standard (R^2 = 0.9992; y=0.0058x+0.0004). Fatty acid composition was analyzed using Gas Chromatography (GC) according to AOAC (2012): 991.36 and AOAC (2012): 969.33. Condition of GC as follow: column cyanopropyl methyl siloxane; N_2 flow rate = 30 mL/second , H_2 flow rate = 40 mL/second, He flow rate = 30 mL/second, air flow rate = 400 mL/second, injector temperature = 220ºC , detector temperature = 240ºC; injection volume = 1 µL; split ratio = 1 : 80.

2.3 Analysis Data

The total cholesterol data were statistically analyzed using ANOVA at a 5% significance level. If significant differences were detected, a post-hoc Least Significant Difference (LSD) test was performed.

3. Results and Discussion

3.1 Results

3.1.1 Fish growth and proximate analysis

Based on Table 1 shows the composition of the feed based on BSFL and tofu waste, as well as the commercial fish feed. The data shows that both types of feed, namely BSFL-based feed and commercial feed, have a relatively similar nutritional composition. The nutrient composition is predominantly made up of protein and carbohydrates. We also measured the percentage of body weight and body length as presented in Table 2. In terms of body weight, P2 demonstrated the highest increase, with an average weight gain of 25.00 grams, corresponding to a growth percentage of 102.88%. This was followed by P0, which achieved an increase of 22.90 grams, while P1 exhibited the lowest growth, with an increase of 18.40 grams. For body length, P2 again showed the most significant improvement, with an increase of 3.50 cm. P0 also exhibited a comparable increase of 3.10 cm. However, P1 displayed the least growth in length.

The proximate composition of *O. niloticus* flesh following different treatments (P0, P1, P2) is shown in Table 3. Water content showed the highest value in P1 compared to P0 and P2. The ash content decreased in P1 but increased in P2 relative to P0, suggesting variations in mineral retention. Protein content was reduced significantly in P1 but remained relatively consistent in P0 and P2. Fat content displayed a marked increase in P2 compared to P0 and P1, indicating differential lipid metabolism. Carbohydrate levels were minimal across all treatments, with P2 exhibiting the lowest value and P1 showing a slight increase over P0.

Caloric value followed a trend consistent with fat and protein content, where P2 recorded the highest caloric density, followed by P0 and P1, respectively. The observed changes in proximate composition across treatments suggest that the feed treatment significantly influenced the macronutrient profiles of *O. niloticus* flesh. Treatment P2 appeared to enhance fat content and caloric value while maintaining comparable protein levels to P0, which may enhance its nutritional appeal.

3.1.2 Cholesterol content and fatty acid profile

The level of cholesterol in this study is also being measured. A linear regression analysis of cholesterol standard curves using six concentrations (0, 50, 75, 100, 125, and 150 ppm) yielded an R^2 value of 0.9992, meeting normal standards. The cholesterol concentrations in dried red tilapia samples are detailed in Table 4. Fish fed commercial feed exhibited the highest cholesterol levels, followed by dried BSFL and BSFL:tofu waste combination, respectively. One-way ANOVA statistical analysis indicated no significant differences among the three treatment groups (p-value > 0.05).

The data in Table 5 indicate that the fat content in the red tilapia flesh under treatments P0 and P2

Table 1. Proximate analysis of floating fish feed based on black soldier fly (BSF) larvae and tofu waste compared to commercial fish feed

	Water Content (%)	Ash Content (%)	Crude Protein (%)	Crude Fat (%)	Crude Carbohydrate (%)
Larvae BSF Feed	8.47	10.17	30.35	11.63	39.34
Commercial Fish Feed	11.00	11.00	32.00	8.00	38.00

Table 2. Morphological characteristics of *O. niloticus* treated with black soldier fly (BSF) larvae and tofu waste feed

Parameter	Control (P0)	Dried BSFL (P1)	BSFL:Tofu Waste (P2)
Initial Body Weight (g)	23.10	23.30	24.30
Final Body Weight (g)	46.00	41.70	49.30
Percentage of Body Weight gain (%)	99.13	78.96	102.88
Initial Body Length (cm)	9.25	10.25	10.37
Final Body Length (cm)	12.37	12.23	13.86
Percentage of Body Length gain (%)	33.51	19.50	33.75
Feed Conversion Ratio (FCR)	2.26	2.65	2.21
Specific Growth Rate (SGR)	2.30	1.94	2.36

Table 3. Proximate analysis of *O. niloticus* flesh after treatment

Component (%)	P0	P1	P2
Water	75.73	79.21	75.59
Ash	1.56	1.23	1.48
Protein	19.61	16.60	19.24
Fat	2.49	2.29	3.75
Carbohydrate	0.61	0.67	0.06
Calories (cal/gram)	103.29	89.69	110.71

Description: P0 (control/commercial pellet feed), P1 (dried BSF larvae), P2 (BSF larvae and tofu waste formula).

Table 4. Cholesterol levels of *O. niloticus* flesh after treatment

Treatment	Cholesterol content (mg/100g)
P0	299.698 ± 35.05a
P1	198.190 ± 4.87a
P2	193.664 ± 3.96a

Description: P0 (control/commercial pellet feed), P1 (dried BSF larvae), P2 (BSF larvae and tofu waste formula).

was 1.62% and 2.83% of the total weight, respectively. Both treatments utilized feed based on BSFL meal and tofu waste, but with differing formulations that influenced the fatty acid profile. In treatment P0, the total saturated fatty acid (SFA) content reached 30.5%, whereas it decreased to 27.95% in P2. The dominant SFA in both treatments was palmitic acid (C16:0), with concentrations of 16.14% (P0) and 17.42% (P2). Other significant SFAs included stearic acid (C18:0) and myristic acid (C14:0).

The total monounsaturated fatty acid (MUFA) content in P0 was 2.67%, which increased slightly to 2.93% in P2. Palmitoleic acid (C16:1) was the primary MUFA, with concentrations of 2.46% (P0) and 2.72% (P2). The polyunsaturated fatty acid (PUFA) content was higher compared to other categories. In P0, the total PUFA content was 37.34%, while it increased significantly to 47.04% in P2. The dominant PUFAs were oleic acid (C18:1n9t) and linoleic acid (C18:2n6c), with higher concentrations observed in P2. Addition

Tabel 5. Fatty acid profile of *O. niloticus*

Parameter	P0 (% w/w)	P2 (% w/w)
Fat content	1.62	2.83
Saturated Fatty Acid		
Butyric acid (C4:0)	0.09	0.08
Caproic acid (C6:0)	3.25	4.14
Lauric acid (C12:0)	3.25	0.47
Myristic acid (C14:0)	3.44	1.91
Pentadecanoic acid (C15:0)	0.14	0.14
Palmitic acid (C16:0)	16.14	17.42
Heptadecanoic acid (C17:0)	0.17	0.15
Stearic acid (C18:0)	3.87	3.48
Arachidic acid (C20:0)	0.15	0.16
Total	30.5	27.95
Monounsaturated Fatty Acid		
Myristoleic Acid (C14:1)	0.11	0.09
Palmitoleic acid (C16:1)	2.46	2.72
Cis-10-heptadecanoic acid (C17:1)	0.10	0.12
Total	2.67	2.93
Polyunsaturated Fatty Acid		
Elaidic acid (C18:1n9c)	0.27	0.28
Oleic acid (C18:1n9t)	23.88	28.23
Linolelaidic Acid (C18:2n9t)	0.06	0.04
Linoleic acid (C18:2n6c)	9.36	14.19
γ-Linolenic acid (C18:3n6)	0.38	0.64
cis-11-Eicosenoic acid (C20:1)	0.86	1.02
Linolenic acid (C18:3n3)	0.27	0.41
cis-11,14-eicosedioneic acid (C20:2)	0.54	0.55
cis-8,11,14-eicosetrienoic acid (C20:3n6)	0.62	0.69
Arachidonic acid (C20:4n6)	0.72	0.68
Cis-4,7,10,13,16,19-Docosahexaenoic Acid (DHA) (C22:6n3)	0.38	0.31
Total	37.34	47.04
Grand Total	70.51	77.92

ally, DHA (C22:6n3), an essential fatty acid for human health, was present at levels of 0.38% (P0) and 0.31% (P2).

3.2 Discussion

3.2.1 Fish growth and proximate analysis

The findings indicate that BSFL flour is a viable alternative to fish meal, although minor changes in body fatty acid composition were observed. According to Belghit *et al.* (2019), the complete substitution of fish meal with BSFL flour does not negatively affect growth performance, digestibility, nutrient utilization, liver health, or the sensory quality of salmon fillets. The defatted BSF larvae flour variant provides the longest float time for floating fish feed. Research by Bußler (2016) shows that BSF larvae processing is limited by their high fat content. Direct processing of frozen or dried larvae into flour is often impractical, as the fat melts during grinding, impacting the quality and consistency of the resulting flour. BSF larvae flour typically contains around 20% crude fat and 36% crude protein. Defatting reduces the crude fat content to 8.8% and increases crude protein to 47%.

The required feed composition for Nile tilapia (*Oreochromis* spp.) based on Indonesian National Standard (2024) includes a minimum protein content of 25-35%, a minimum fat content of 5-7%, a maximum crude fibre content of 7%, a maximum ash content of 12%, and a maximum moisture content of 12%. Generally, feed formulations using BSFL and tofu waste in this experiment meet the quality requirements.

According to Asghar *et al.* (2023), livestock feed based on tofu waste offers beneficial nutrients and bioactive compounds for improving animal digestion and flesh quality. Tofu waste nutritional composition includes 74-80.25% moisture, 9.91-32.8% protein, 6.22-21.98% fat, and 4.1-23.4% crude fiber (Kamble and Rani, 2020). Essential fatty acids such as linoleic, palmitic, stearic, oleic, and linolenic acids are commonly found in tofu waste. It also contains carbohydrates like arabinose, glucose, galactose, fructose, stachyose, raffinose, sucrose, and starch, along with minerals such as calcium (126 mg/100g), iron (4.45 mg), copper (0.77 mg), phosphorus (313 mg), potassium (286 mg), and zinc (3.14 mg) (Asghar *et al*., 2023). The dried tofu waste is ground and sieved, producing a low-moisture, shelf-stable flour with nutritional values of approximately 24.7% protein, 35.8% carbohydrates, 25.7% fat, 58.27% fiber, and 6.3% ash (Santos *et al*., 2019).

Additional ingredients are also essential in feed production. In this study, we added other ingredients such as corn flour, rice bran, and starch. Corn flour is used as an alternative ingredient in fish feed due to its high carbohydrate content (73.7%) (Sikumbang *et al*., 2024). It contains 17% moisture, 4.2% ash, 11% crude protein, and 4.6% crude fat (Lapui *et al.*, 2021). According to Safitri *et al.* (2020), fish pellets with over 20% corn flour have reduced floatability and water stability compared to those with less than 20%.

Rice bran, a byproduct of rice milling, is produced from the outer layer of broken rice grains, contributing about 8–12% of rice milling output (Nadimin and Fitriani, 2019). It contains approximately 8.43% crude protein, 18.47% crude fat, 11.28% moisture, 10.44% ash, 22.17% crude fiber, 88.23% organic matter, and 34.97% nitrogen-free extract (Azizah *et al.*, 2022). The recommended inclusion rate of rice bran in omnivorous fish feed is 15%, while for herbivorous/omnivorous fish it is 35% (Abidin *et al*., 2015). The abundance and low cost of corn flour and rice bran make them ideal as basal ingredients in fish feed production.

The results showed that the feed formula of BSF larvae and tofu waste provides the most effective treatment for enhancing growth in both weight and length parameters. Commercial feed, while slightly less effective than larvae feed, still shows substantial growth compared to dried larvae, which consistently produced the lowest results across all metrics. Subandiyono and Hastuti (2020) also found that protein content significantly affected the growth performances (weight, survival rate, and growth rate), nutritional compositions of the fish body, and amino acid profiles. This indicates that the formulation of BSF larvae and tofu waste may offer optimal nutritional or environmental benefits for growth performance in the fish.

According to Djunaedi *et al.* (2016), several factors influence fish growth, including feed, cultivation containers, salinity, season, physical activity, and recirculation systems. A well-functioning recirculation system can maintain the stability of water quality in the rearing medium, ensuring its suitability for cultivation. Fish growth is influenced by two major factors. The first is internal factors, such as genetic traits, disease resistance, and the ability to utilize food. The second consists of external factors, including the physical, chemical, and biological properties of the aquatic environment (Mulqan *et al.*, 2017). Effendi (1997) stated that growth occurs when there is a change in fish size, either in weight or length, due to tissue changes resulting from the division of muscle and bone cells, which constitute the largest composition in fish.

The proximate composition of fish flesh reflects the influence of dietary macronutrients on biochemical pathways governing protein turnover, lipid deposition, and energy metabolism. Water content was highest in P1 (79.21%), which correlates with lower protein and fat levels, indicating possible inefficient protein retention or increased catabolism. Protein content in P2 (19.24%) was comparable to the control (P0: 19.61%), reinforcing the hypothesis that a mixed larvae-tofu formulation supports optimal nitrogen retention. The elevated fat content in P2 (3.75%) relative to P0 (2.49%) and P1 (2.29%) suggests that the presence of tofu waste promotes lipid deposition, potentially through enhanced lipid biosynthesis. The caloric value trend (P2 > P0 > P1) aligns with the proximate findings, where higher fat content contributes to increased energy density. The lower caloric content in P1 (89.69 cal/g) could reflect inefficient lipid metabolism or limited fatty acid synthesis, which may negatively impact overall growth.

The proximate analysis of the formulated feed showed a protein content of 30.35%. However, digestible protein in Nile tilapia fed commercial feed was higher compared to those fed with larvae-based feed and soybean pulp. According to Ediwarman *et* al. (2008), the protein content in Nile tilapia fed larvae-based feed tends to be lower due to the presence of chitin. Protein digestion becomes more difficult in the presence of chitin in the feed. Chitin, which is found in larvae, is insoluble in strong acid solutions, making it difficult to be fully digested by fish. Several factors significantly affect protein levels, including fish species, age, size, feed protein quality, feed digestibility, and environmental conditions (Pramono *et al.,* 2007). The amount of protein provided, absorbed, and utilized for building or repairing fish body cells, as well as for daily metabolism, is referred to as protein retention. When fish are given excessive protein, not all of it is synthesized into body protein; instead, the excess protein undergoes catabolism, ultimately resulting in ammonia production.

3.2.2 Cholesterol content and fatty acid profile

The differences in cholesterol and fatty acid profiles across treatments highlight the role of dietary lipids in modulating lipid metabolism in *O. niloticus.* Fish fed commercial feed (P0) exhibited the highest cholesterol content (299.698 mg/100 g), while those on 100% BSFL feed (P1) and the larvae-tofu formulation (P2) had significantly lower cholesterol levels (198.190 mg/100 g and 193.664 mg/100 g, respectively). Despite these differences, ANOVA analysis indicated no statistically significant variation ($p > 0.05$), suggesting that dietary lipids influenced cholesterol metabolism but did not drastically alter overall cholesterol homeostasis.

Fatty acid composition analysis further elucidates metabolic adaptations to dietary variations. The reduction in total saturated fatty acid (SFA) content in P2 (27.95%) compared to P0 (30.5%) indicates that the larvae-tofu blend promotes a more favorable lipid profile, potentially enhancing cardiovascular benefits in human consumers. The primary SFA, palmitic acid (C16:0), was slightly elevated in P2 (17.42%) compared to P0 (16.14%), possibly due to differential fatty acid synthesis or absorption rates.

Monounsaturated fatty acids (MUFAs), particularly palmitoleic acid (C16:1), showed a modest increase in P2 (2.93%) compared to P0 (2.67%), indicating a shift towards lipid desaturation. The most notable change was observed in polyunsaturated fatty acid (PUFA) content, where P2 exhibited a substantial increase (47.04%) over P0 (37.34%). The elevated levels of linoleic acid (C18:2n6c) and oleic acid (C18:1n9t) in P2 suggest enhanced lipid metabolism pathways favoring PUFA synthesis and incorporation into tissues.

The level of DHA (C22:6n3), a key omega-3 fatty acid, was slightly reduced in P2 (0.31%) compared to P0 (0.38%), suggesting that the dietary formulation may not significantly enhance omega-3 deposition in fish muscle. However, the overall fatty acid composition shift in P2 supports a lipid profile conducive to better nutritional quality and health benefits.

This finding is supported by Jacob *et al.* (2015), who stated that fish fat content is not only influenced by the type of feed but also by fish species, age, and rearing environment. Treatment with commercial feed resulted in a relatively low-fat content, close to that of the control group, at approximately 2.49%. Fat content has an inverse relationship with protein content—higher protein levels correspond to lower fat content in a sample (Isa *et al.,* 2015). Although fish contain relatively high fat levels (0.1–2.2%), this is not harmful to the body, as 25% of the total fat consists of unsaturated fatty acids, which are essential for human health and have very low cholesterol levels. During the cold stress, saturated fatty acids (SFA) significantly decreased, while unsaturated fatty acids (UFA) tended to increase. Thus, this study demonstrates, to a certain level, that high dietary lipid levels have a positive effect on the cold tolerance of Nile tilapia fingerlings (Abdel-Ghany *et al*., 2021).

4. Conclusion

Combination of de-fatted Black Soldier Fly larvae (BSFL) and tofu waste showed its nutritional composition met the standards for high-quality fish feed. While the use of dried larvae feed increased calorie content in fish flesh rather than protein or fat, it did not significantly affect cholesterol levels. Based on the fatty acid profile, the combination of de-fatted BSF larvae and tofu waste has the potential to enhance the nutritional quality of red tilapia flesh. To further improve feed quality, the processing of tofu waste and BSF larvae should focus on reducing moisture and fat content to produce more stable and superior fish feed. Future research should investigate the long-term effects of defatted BSF larvae flour on various aquaculture species and its impact on fish flesh's nutritional composition to ensure optimal product quality, as well as explore enzymatic activities and amino acid profiling to further elucidate the metabolic mechanisms underlying these observations.

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Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author as follow, UMSP, SPS, and RN; conducted the experiment, collected the data, and drafted the manuscript. DA and MS; devised the main conceptual ideas and critical revision of the article. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

Declaration of Artificial Intelligence (AI)

The author(s) acknowledge the use of ChatGPT for language refinement and summarization in preparing this manuscript. All AI-generated content was rigorously reviewed, edited, and validated to ensure accuracy and originality. Full responsibility for the manuscript's final content rests with the author(s).

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