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SUBSTITUTING THE FISHMEAL WITH SOLID-STATE-FERMENTED BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) LARVAE MEAL IN GIFT TILAPIA (*OREOCHROMIS NILOTICUS*) FRY DIET: EFFECTS FOR GROWTH PERFORMANCE, CARCASS COMPOSITION AND LIVER HISTOLOGY*

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Abstract

Replacing fishmeal (FM) with an optimal aquafeed alternative remains a significant challenge. Recent research suggests that insect meal is a potential candidate ingredient for a substitute for fishmeal. Simultaneously, the black soldier fly larvae meal (BM) is an intensively researched insect meal with promising results. However, controversial results and the inability to complete FM substitution prompt further investigation into new techniques. Solid-state fermentation (SSF) has been identified as a cost-effective technique to enhance the efficiency of aquafeed ingredients. Therefore, a nine-week experiment was conducted using fermented black soldier fly larvae meal (FB) to replace FM based on the protein content with 0% (0FB), 35% (35FB), 70% (70FB), and 100% (100FB) in the diet of GIFT tilapia (*Oreochromis niloticus*) fry (0.18±0.01 g/fish) applying SSF. Additionally, FM was entirely replaced by the unfermented black soldier fly larvae meal (UB), including diet (100UB). Results of the weight gain (WG), daily weight gain (DWG), relative weight gain (RWG), and specific growth rate (SGR) of FB-fed fish were statistically higher (P<0.05) than those of UB-fed fish. However, there was a decreasing trend in the growth performance when the FB proportion was increased. Simultaneously, the feed conversion ratio (FCR) and protein efficiency ratio (PER) of all the FB-fed fish were lower than the control. Further, 100UB exhibited reduced values in WG, DWG, and PER, underscoring the positive effect of SSF. Results of the carcass composition analysis showed that the SSF did not adversely affect the carcass crude protein, ash, and fiber contents. However, carcass lipid content decreased statistically (P<0.05) with increasing levels of FB. Histopathological alterations in liver tissues of fish fed with the FB-included diets highlight the importance of screening the rearing surfaces of black soldier fly to ensure feed safety. Overall, the SSF of BM is a viable option for substituting the FM in *O. niloticus* fry feed without affecting growth performance and feed utilization.

Key words: insect meal, solid-state-fermentation, alternative animal feed ingredients, Nile tilapia, black soldier fly larvae meal

Fishmeal utilization in the aquafeed industry undermines the attainment of the United Nations Sustainability Development Goals, particularly Goals 1, 2, 3, 9, 12, 13 and 14 (Shahin et al., 2023). Concurrently, 20.4 million tons of the world's fish production is diverted to produce FM and fish oil, disrupting human consumption (FAO, 2022). The fish species employed for FM production are crucial in ensuring food security and sustaining livelihood in some countries (Greenpeace, 2019). Therefore, alternatives are imperative to mitigate marine resource depletion and safeguard food security.

Insect meal has emerged as one of the most successful substitutes for FM owing to its biological, nutritional and sustainable benefits (Nogales-Mérida et al., 2019). Recent research has confirmed that FM can be entirely replaced by insect meal in the diets of certain species such as *Ictalurus punctatus* (Fan et al., 2023), *Catla catla* (Perera et al., 2023 a), *Poecilia reticulata* (Perera and Bhujel, 2022), *Litopenaeus vannamei* (Peh et al., 2021), and *Clarias gariepinus* (Taufek et al., 2017). However, most research results indicate that FM can be partially replaced with insect meals (Fontes et al., 2019; Perera and Bhujel, 2021; Perera et al., 2023 b). The BM is one of the most extensively studied insect species for replacing fishmeal in aquafeeds. However, achieving total FM replacement with BM without compromising fish performance remains a challenge (Gougbedji et al., 2022) for some species and stages, such as *O. niloticus* fry stage due to the unbalanced nutrients, lower digestibility and the availability of the anti-nutrients. The chitin content

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in black soldier fly larvae and prepupae is around 1–9% on dry matter basis (Caligiani et al., 2018), and when it is available in high quantities, chitin performs as an anti-nutrient as demonstrated in tilapia (Eggink et al., 2022). Organic wastes have been used to feed the black soldier fly, and the heavy metals can accumulate in the insects through these rearing substrates (Diener et al., 2015). The health risks of the applicable heavy metals have been identified, even at lower exposure (Tchounwou et al., 2012; Truzzi et al., 2022), raising concerns about these hazardous elements through food or feed chains to humans. Therefore, innovative and cutting-edge techniques such as fermentation must be identified to enhance the performance and mitigate the above issues of BM, rather than solely including the different inclusion levels.

Fermentation has been identified as a cost-effective and environmentally friendly method to enhance the nutritional value and mitigate the issues of anti-nutritional factors in ingredients (Samaddar et al., 2015). The yeast-based SSF is a simple, economical, and efficient approach commonly practiced in conventional food processing. Previous studies have confirmed that yeast serves as a multi-functional feed additive (Qazi et al., 2011).

GIFT tilapia is an improved strain of Nile tilapia (*Oreochromis niloticus*), and it fits climate-smart aquaculture (Bhendarkar and Kalbande, 2022). Additionally, *O. niloticus* ranks third in global fish production and is used by seventeen nations (FAO, 2022). Further, it has been estimated that the global percentage of commercial aquafeed usage will be 85% by 2025 (Tacon and Metian, 2015). Simultaneously, FM is a critical ingredient in tilapia feed formulations due to its high nutritional value (El Sayed and Tacon, 1997). The above facts reveal that FM consumption for GIFT tilapia farming is higher, and sustainable ingredients and effective techniques are required to replace FM. Therefore, this experiment aimed to find the replacement potential of FM by FB in the *O. niloticus* fry diet.

Material and methods

The experiment was conducted at the Indoor Aquarium located within the Regional Research Center of the National Aquatic Resources Research and Development Agency (NARA), Panapitiya, Waskaduwa, Sri Lanka, from January 2024 to March 2024.

Experimental setup

Fifteen fiberglass aquaria, each with a capacity of 275 L (74 cm (length) × 61 cm (width) × 61 cm (height)) were used to stock *O. niloticus* fry. Those tanks were filled with conditioned freshwater to a depth of 30.5 cm. Each aquarium was aerated using a RESUN GF-750 air blower (Guangzhou Puyangli Aquarium & Accessories Co. Ltd, Guangdong, China). The light intensity was maintained at 842±31 lux. The photoperiod was maintained as 12 hours (light)/12 hours (dark) using LED bulbs as a light source throughout the experimental period. The illumination was regulated by a Smart Sensor AS803 digital lux meter (Arco Electronics Ltd, Dong Guan City, China).

The study comprised five treatments, including the control and three replicates per treatment; thus, the experimental design was completely randomized.

Fish and culture management

A total of eight hundred *O. niloticus* fry (initial weight, 0.14±0.02 g/fish; initial length, 2.1±0.14 cm/fish) were purchased from the Tilapia Breeding Center, National Aquaculture Development Authority, Udawalawa, Sri Lanka. From this stock, four hundred and fifty fish were randomly selected and stocked in fiberglass tanks at a density of 176/m^3 (30 fish per tank). Each tank was filled with 170 L of water, maintaining a water level of 30.5 cm. The fish fry were acclimatized for one week. During the above period, a feed containing 38.2±0.2% crude protein was administered twice daily at 5% of the body weight of the fish. Water quality parameters were monitored and maintained at the optimum ranges.

The biochemical composition of FM and BM

The chemical composition of the ingredients was analyzed before preparing the feeds, as were the proximate composition of the research diets, pepsin digestibility of FM and BM, and amino acid profiles.

Table 1. Proximate composition of the FM, UB, and FB (%, dry weight basis)

Parameter	FM	UB	FB	P value	Test Method/Reference
Dry matter content	90.9 a	94.2 c	93.5 b	0.000	By calculating = (100-moisture)
Protein	67.1 c	45.7 a	47.0 b	0.000	AOAC 2001.11/AOAC (2001)
Lipid	8.2 a	17.6 b	18.5 c	0.000	AOAC 920.39/AOAC (1995 a)
Moisture	9.1 c	5.8 a	6.5 b	0.000	AOAC 930.15/AOAC (1996)
Ash	14.7 c	9.5 b	7.3 a	0.000	AOAC 942.05/AOAC (1995 b)
Fiber	0.6 a	15.5 b	15.8 b	0.000	AOAC 978.10/AOAC (1995 c)
NFE	9.4 a	11.7 b	11.5 b	0.001	Sibbald et al. (1980)

All values are mean ± SE, calculated from three replicates. a, b, c – means with different letters are significantly different ($P<0.05$) from each other. FM, fishmeal; UB, unfermented black soldier fly meal; FB, fermented black soldier fly meal; NFE, nitrogen-free extract.

Testing the proximate composition of the FM, BM and gross energy

The proximate analysis tests (Table 1) were conducted at the Chemical Laboratory of Ceylon Grain Elevators PLC, Rock House Lane, Colombo 15. Sri Lanka. The protein-to-nitrogen conversion factor for BM was used as 5.95 for crude protein calculations following the recommendation of Smets et al. (2021).

The gross energy was tested at the Palmyrah Research Institute, Jaffna, Sri Lanka, using the e2K Combustion Calorimeter (Digital Data Systems (Pvt) Ltd, Gauteng, South Africa).

Determining the pepsin digestibility of FM, UB, and FB

The pepsin digestibility of FM, UB and FB was tested at the Chemical Laboratory of Ceylon Grain Elevators PLC, Rock House Lane, Colombo 15, Sri Lanka, following the AOAC 971.09 Test Method (AOAC, 1999).

Table 2. Pepsin digestibility of FM, UB, and FB

Parameter	FM	UB	FB	P value
Pepsin digestibility	93.1±1.1	70.1±0.6	75.0±0.7	0.001

All values are mean ± SE, calculated from three replicates. a, b, c – means with different letters are significantly different (P<0.05) from each other.

FM, fishmeal; UB, unfermented black soldier fly meal; FB, fermented black soldier fly meal.

Testing the amino acid profile of FM and UB

The amino acid compositions of FM and UB were analyzed using the Biochrom 30^{+} amino acid analyzer (Biochrom Ltd, Cambridge, United Kingdom) following the AOAC 994.12 Test Method (AOAC, 2005). The values are mentioned in Table 3.

Table 3. Amino acid profiles of FM and UB used in the experimental diets (mg/100 g, dry weight basis)

Amino acid	FM	UB	P value
EAA			
Arginine	5690.8 b	2232.3 a	0.000
Isoleucine	2105.3 b	598.0 a	0.000
Leucine	2520.3 b	1325.4 a	0.000
Lysine	757.2 b	777.6 a	0.019
Methionine	3120.0 b	1819.7 a	0.000
Threonine	6187.6 b	3182.3 a	0.000
Valine	1180.0 b	609.6 a	0.000
NEAA			
Alanine	4099.9 b	1865.9 a	0.000
Cystine	3470.2 b	1942.4 a	0.000
Glutamic acid	2784.3 b	1635.0 a	0.000
Glysine	2602.5 b	2164.4 a	0.001
Proline	9160.6 b	4208.8 a	0.000
Serine	2806.4 b	1438.3 a	0.000
Tyrosine	4762.2 b	2346.6 a	0.000

All values are mean ± SE, calculated from three replicates. a, b – means with different letters are significantly different (P<0.05) from each other.

Phenylalanine, tryptophan, and tyrosine were not detected.

FM, fishmeal; UB, unfermented black soldier fly meal.

Table 4. The formulation and biochemical composition of the test diets (%, dry weight basis)

Ingredient	0FB	35FB	70FB	100FB	100UB
1	2	3	4	5	6
Fish meal	20.00	13.01	6.00	0.00	0.00
Black soldier fly meal	0.00	9.99	20.01	28.58	29.37
Soybean	48.00	48.73	49.25	49.00	49.75
Rice bran	15.23	12.00	7.97	5.65	4.65
Corn	4.00	5.00	8.00	10.00	9.46
Lysine	0.15	0.15	0.15	0.15	0.15
Methionine	0.08	0.08	0.08	0.08	0.08
Choline chloride	0.04	0.04	0.04	0.04	0.04
Fish oil	8.00	6.50	4.00	2.00	2.00
Vitamin mixture[1]	1.00	1.00	1.00	1.00	1.00
Mineral mixture[2]	1.00	1.00	1.00	1.00	1.00
CMC[3]	2.00	2.00	2.00	2.00	2.00
DCP[4]	0.50	0.50	0.50	0.50	0.50
Crude protein (%)	38.2	38.3	38.4	38.3	38.7
Crude lipid (%)	10.6	8.9	7.6	6.8	7.4
Moisture (%)	8.4	7.4	7.5	7.7	8.7
Ash (%)	9.8	10.2	10.3	10.2	10.1

Table 4. – contd.

1	2	3	4	5	6
Fiber (%)	3.3	2.2	2.4	2.6	2.4
NFE (%)	29.7	33.0	33.8	34.4	32.7
Energy (KJ/g)	18.9	18.7	18.2	18.6	18.0

[1]Composition of vitamin mixture per 1 kg: vitamin A – 1,000,000 IU, vitamin D_3 – 100,000 IU, vitamin E – 10,000 IU, vitamin C – 10,000 mg, vitamin K – 800 mg, vitamin B_1 – 1500 mg, vitamin B_2 – 1200 mg, vitamin B_6 – 750 mg, vitamin B_{12} – 20 mg, pantothenic acid – 3000 mg, niacin – 2150 mg, folic acid – 300 mg, inositol – 25,000 mg, biotin – 25 mg.

[2]Composition of mineral mixture per 1 kg: selenium – 30 mg, iron – 20,000 mg, zinc – 32,000 mg, copper – 2,000 mg, cobalt – 150 mg, iodine – 325 mg, magnesium – 6,000 mg, potassium – 100 mg, sodium – 5.9 mg, manganese – 1500 mg.

[3]CMC – carboxyl methyl cellulose.

[4]DCP – di-calcium phosphate.

0FB, 0% of fishmeal-replaced diet with fermented black soldier fly larvae meal; 35FB, 35% of fishmeal-replaced diet with fermented black soldier fly larvae meal; 70FB, 70% of fishmeal-replaced diet with fermented black soldier fly larvae meal; 100FB, 100% of fishmeal-replaced diet with fermented black soldier fly larvae meal; 100UB, 100% of fishmeal-replaced diet with unfermented black soldier fly larvae meal.

Fermenting the BM

Commercial BM was purchased from a local supplier. DCL dry instant yeast (*Saccharomyces cerevisiae*, CFU 5×10^8/ml), manufactured in DCL, SIL-41, Zone Portuaire 59211 Santes, France, was purchased, and a previous method practiced by Plaipetch and Yakupitiyage (2012) was used to ferment the BM.

A total of 1 kg of BM, 33.4 mg of the yeast mentioned above, yeast, and 0.8 L of distilled water were homogenized in a Panasonic MK-GB1 Stand Mixture (Panasonic Taiwan Co., Ltd., New Taipei City, China) for 15 minutes. The mixture was fermented for 24 hours in a 10-liter jar covered with aluminum foil. Then, the fermented BM was soaked with 3 L of distilled water for 5 minutes. After decanting the excess water, a fine mesh cloth was used to squeeze the meal to remove as much water as possible from the fermented meal. The residual meal was dried to constant weight at 70°C.

Test diet preparation

Five iso-protein (38.4±0.09% crude protein) and iso-caloric (18.5±0.2 kJ GE/g) test diets were prepared according to the formulas as outlined in Table 4. FB replaced the FM in the control diet (0FB) at 35%, 70%, and 100% in 35FB, 70FB, and 100FB diets, respectively, based on the crude protein content. Moreover, 100% FM was replaced by UB in the 100UB diet.

All the ingredients were mixed well, warmed distilled water was added, and the formed dough was passed through a Sherry UH-B12MEC-B meat mincer (Sherry Bakery Equipment Suppliers (Pvt) Limited, Malabe, Sri Lanka). After transferring to trays, the feeds were dried at 60°C for 24 hours in a Gemmy YCO-010 hot air sterilizer (Gemmy Industrial Corporation, Taipei, Taiwan). Finally, drying constantly, cooled feeds were transferred to sealed bags and stored in a refrigerator.

The proximate composition and energy of the above research diets were tested following the methods mentioned in Table 1.

Rearing and feeding

After acclimatizing the *O. niloticus* fry for one week, the initial body weight of the randomly selected five fish in each replicate was measured and recorded on the first day of the experiment using the Sartorius BSA-822-CW chemical balance (Sartorius Lab Instruments GmbH & KG, Goettingen, Germany). The fish were hand-fed at 5% of the body weight twice daily at 09:00 and 15:00 hours. The body weights of the randomly selected five fish in each treatment were measured weekly, and the feeding ratio was adjusted. Finally, the body weights of all fish in each treatment were measured after 63 days (9 weeks) using the same balance. The growth performance was evaluated using the parameters and related equations mentioned below.

$$\textit{Survival rate} = (\textit{Final fish number} \div \textit{Initial fish number}) \times 100$$

$$\textit{Weight gain (WG)} = \textit{Final weight (g)} - \textit{Initial weight (g)}$$

$$\textit{Daily weight gain (DWG)} = \textit{Weight gain (g)} \div \textit{Number of days}$$

$$\textit{Relative weight gain (RWG)} = \textit{Weight gain (g)} \div \textit{Initial weight (g)} \times 100$$

$$\textit{Specific growth rate (SGR)} = (\textit{Ln weight (final)} - \textit{Ln weight (initial)}) \div \textit{number of days} \times 100$$

$$\textit{Feed conversion ratio (FCR} = \textit{Feed given (g)} \div \textit{Weight gain (g)}$$

$$\textit{Protein efficiency ratio (PER)} = \textit{Weight gain (g)} \div \textit{Protein consumed (g)}$$

Water quality management

Two-thirds of the water volume was changed from each fiberglass tank every week. Excreta was siphoned out daily, then refilling the tank to a 30.5 cm level. Total ammonia was tested weekly using a standard test kit (NT Laboratories Ltd, Kent, TN 12 9QS, UK). Dissolved oxygen was measured weekly using the EZDO PDO-408 Dissolved Oxygen Meter (GOnDO Electronic Co. Ltd., Taipei City, Taiwan). The pH and water temperature were also measured weekly using the Eutech $PH6^+$ pH meter (Eutech Instruments Pte Ltd., Singapore). Additionally, conductivity was measured weekly using the WTW

Cond 3310 Conductivity Meter (Xylem Analytics GmbH & Company, Weinheim, Germany). Water quality parameters were maintained within the optimal ranges as water temperature 27.2±1.8°C, pH 7.66±0.02, dissolved oxygen 6.63±0.6 mg/l, total ammoniacal nitrogen 0.20±0.10 mg/l, and conductivity 109.0±0.5.

Carcass composition testing

At the end of the experiment, one fish from each replicate was kept for liver histology testing. All remaining fish were euthanized following the aforementioned method and dried in a Gemmy YCO-010 hot air oven (Gemmy Industrial Corporation, Taipei, Taiwan) for 24 hours at 100°C. Subsequently, all the samples were ground and stored in air-tight zipper bags for proximate analysis.

Liver histology

One fish from each aquarium was randomly selected and euthanized, exposed to 250 mg/L of MS-222 for 10 minutes (AVMA, 2007). After sacrificing, fish were preserved in 10% neutral buffered formalin (Mumford, 2004). Subsequently, samples were sent to the Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka, and the slides were prepared following the hematoxylin and eosin staining (NSH, 2001). The prepared slides were analyzed using the Euromex iScope microscope (Euromex iScopes, Arnhem, Netherlands).

Statistical analysis

All measurements were conducted in triplicate. Data were processed and compiled using MS Excel. A T-test was used to compare the amino acid profiles of FM and UB.

Treatments were compared using one-way ANOVA followed by Tukey's post hoc test after confirming the normality of the data and homogeneity of the variance using the Levene test. Statistical analysis was performed using SPSS software version 22.0. All means were expressed as the mean ± standard error at a significant level of $P<0.05$.

Results

Biochemical composition of FM and BM

The proximate compositions of FM, UB, and FB were tested and compared before the experiment. The crude protein, lipid, moisture, and ash values of FM were significantly higher than UB and FB, while the fiber level of FM was lower than that of UB and FB ($P<0.05$) (Table 1). However, the enhanced crude protein and lipid contents were observed in the FB. The EAA profile of FM is higher than BM; i.e., seven EAAs were tested, and six EAAs were higher in FM than in BM, except for lysine (Table 3). Simultaneously, all the tested seven NEAA contents in FM were higher in FM than in the BM ($P<0.05$).

Growth performance and feed utilization of the fish

Table 5 illustrates the growth performance, feed utilization, and survival of *O. niloticus* during the experimental period.

Results of the growth performance of FB-fed fish are comparable to FM-fed fish in terms of WG and DWG ($P<0.05$). However, when the FB percentage was increased, a decreasing trend of the WG and DWG can be observed. Simultaneously, 100UB-fed fish showed lower growth performance ($P<0.05$) when compared with the control. Though all the growth-related parameters of FB-fed fish were statistically similar to UB-fed fish, a non-significant lower performance can be observed in UB-fed fish.

Table 5. Growth performance, feed utilization, and survival of *O. niloticus* fry at the end of the feeding trial

Parameter	0FB	35FB	70FB	100FB	100UB	P value
Initial weight (g)	0.17±0.04	0.16±0.03	0.20±0.0.03	0.18±0.03	0.16±0.02	0.92
Final weight (g)	1.72±0.09	1.53±0.06	1.25±0.22	1.03±0.27	0.97±0.19	0.09
WG (g)	1.65±0.16 b	1.13±0.10 ab	1.09±0.20 ab	1.07±0.16 ab	0.84±0.19 a	0.04
DWG (mg/day)	26.13±2.55 b	17.91±1.57 ab	17.30±3.22 ab	16.92±2.49 ab	13.38±3.07 a	0.04
RWG	1017±205 b	681±29 a	686±154 a	634±49 a	515±54 a	0.11
SGR	3.42±0.27	3.11±0.06	3.10±0.26	3.05±0.05	2.90±0.03	0.26
FCR	0.71±0.02 b	1.04±0.04 a	1.04±0.10 a	1.06±0.03 a	1.12±0.04 a	0.00
PER	5.57±1.23 c	3.82±0.13 b	2.87±0.70 b	2.81±1.22 b	1.98±0.52 a	0.00
Survival (%)	80.0±1.9	94.5±4.0	83.3±5.8	81.1±2.2	85.6±0.8.0	0.30

All values are mean ± SE, calculated from three replicates. a, b – means with different letters are significantly different ($P<0.05$) from each other.

WG, weight gain; DWG, daily weight gain; RWG, relative weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio.

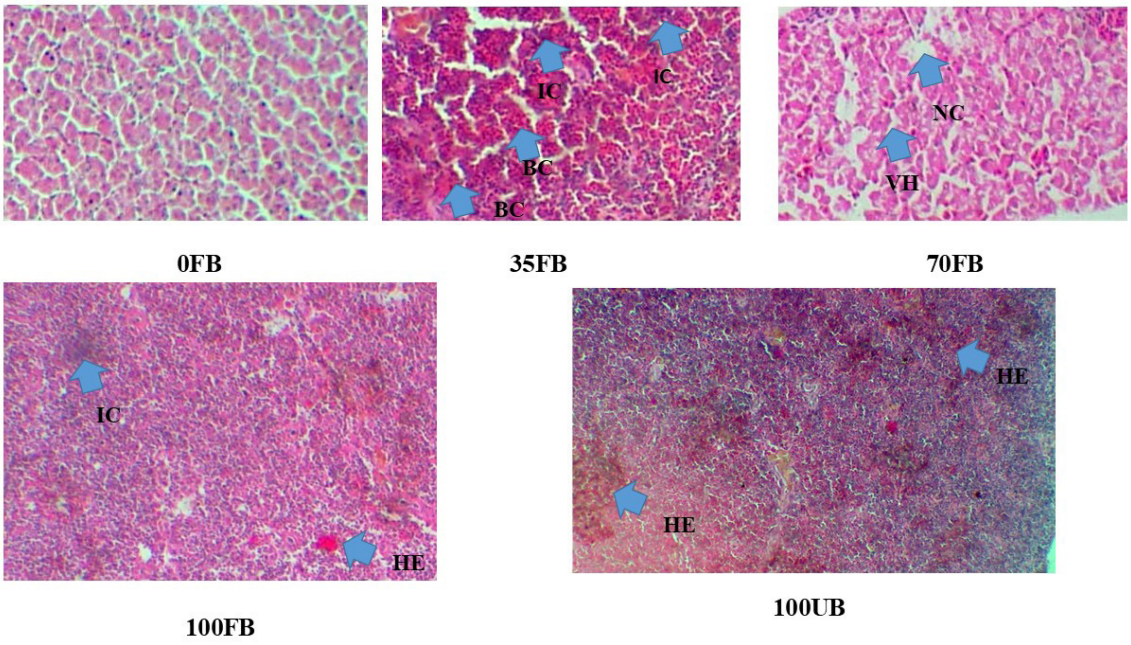
0FB, 0% of fishmeal diet replaced with fermented blacksoldier fly larvae meal; 35FB, 35% of fishmeal diet replaced with fermented blacksoldier fly larvae meal; 70FB, 70% of fishmeal diet replaced with fermented blacksoldier fly larvae meal; 100FB, 100% of fishmeal diet replaced with fermented blacksoldier fly larvae meal; 100UB, 100% of fishmeal diet replaced with unfermented blacksoldier fly larvae meal.

Table 6. Carcass composition of *O. niloticus* fry at the end of the experiment (%, wet weight basis)

Parameter	0FB	35FB	70FB	100FB	100UB	P value
Crude protein (%)	59.6±0.10 b	61.2±0.10 c	60.4±0.10 bc	60.1±0.10 bc	58.0±0.10 a	0.001
Crude lipid (%)	13.8±0.20 c	10.6±0.20 b	12.2±0.20 a	9.8±0.20 a	10.2±0.20 a	0.000
Moisture (%)	78.0±0.20 a	78.3±0.20 a	79.0±0.20 ab	79.7±0.20 bc	80.2±0.20 c	0.002
Ash (%)	15.6±0.20 ab	15.8±0.20 ab	15.0±0.20 a	15.1±0.20 a	16.4±0.20 b	0.021
Fiber (%)	0.40±0.10	0.40±0.10	0.50±0.10	0.80±0.10	0.50±0.10	0.127
NFE (%)	10.6±0.20 a	12.0±0.20 b	11.9±0.20 b	14.2±0.20 c	14.9±0.20 c	0.000

All values are mean ± SE, calculated from three replicates. a, b, c – means with different letters are significantly different (P<0.05).

0FB, 0% of fishmeal diet replaced with fermented blacksoldier fly larvae meal; 35FB, 35% of fishmeal diet replaced with fermented blacksoldier fly larvae meal; 70FB, 70% of fishmeal diet replaced with fermented blacksoldier fly larvae meal; 100FB, 100% of fishmeal diet replaced with fermented blacksoldier fly larvae meal; 100UB, 100% of fishmeal diet replaced with unfermented blacksoldier fly larvae meal.



Pointed arrows show the symptom; 0FB, typical hepatocytes; 35FB, inflammatory cell aggregation (IC) and blood congestion (BC); 70FB, vacuoles in hepatocytes (VH) and necrosis (NC); 100FB, inflammatory cell aggregation (IC) and hemorrhages (HE); 100UB, hemorrhages (HE). 0FB, 0% of fishmeal diet replaced with fermented blacksoldier fly larvae meal; 35FB, 35% of fishmeal diet replaced with fermented blacksoldier fly larvae meal; 70FB, 70% of fishmeal diet replaced with fermented blacksoldier fly larvae meal; 100FB, 100% of fishmeal diet replaced with fermented blacksoldier fly larvae meal; 100UB, 100% of fishmeal diet replaced with unfermented blacksoldier fly larvae meal.

Figure 1. Representative images of the histology of hepatocytes of *O. niloticus* as seen under the light microscope at 10×magnification (Euromex iscope, Holland)

The FM-fed fish showed the best FCR and PER. All the other FB-fed fish had higher FCR values. No significant FCR difference could be observed between the FB-fed fish and UB-fed fish. However, FB-fed fish showed better PER values than UB-fed fish. There is an increasing trend of FCR, and a decreasing trend of PER can be observed when the FB content is increased.

The inclusion of FB or UB did not affect the survival rate of the fish.

Testing of the pepsin digestibility of FM, UB, and FB

The pepsin digestibility of FM is significantly higher (P<0.05) than that of UB and FB (Table 2). However, the pepsin digestibility of FB was higher than that of UB.

Analysis of the carcass composition of the fish

Table 6 shows that the carcass crude protein content of 35FB-fed fish was significantly higher (P<0.05) than that of the control and rest of the groups. Carcass crude protein contents of the 70FB- and 100FB-fed fish were not significantly different (P<0.05) from the control, and it was statistically lower (P<0.05) in the unfermented feed-fed fish than the control and the fermented feed-fed fish. The lipid contents of FB and UB-fed fish were lower than the FM -fed fish. When the FB content was increased, the lipid contents of the fish were decreased. Fiber and ash contents were not affected by the fermentation of BM.

Histopathological analysis of the liver of the fish

The normal morphology was observed in the histopathological architecture of the liver tissues in fish fed the 0FB. When the FB level was gradually increased from 35% to 100%, replacing the FM, hepatocytes showed abnormalities such as necrosis, inflammatory cell aggregation, blood congestion, vacuoles, and hemorrhages. When 100FB and 100UB hepatocytes were compared, the intensity of the damage for both groups was not different. Therefore, liver cell alterations can be identified not because of the fermentation but because of BM's inclusion level.

Discussion

Black soldier fly larvae meal has completely replaced FM in the fingerling stage of Nile tilapia (Muin et al., 2017; Tippayadara et al., 2021), blue tilapia (Bondari and Sheppard, 1981) and other fish species such as Jian carp (Li et al., 2017), channel catfish (Bondari and Sheppard, 1981), Atlantic salmon (Bruni et al., 2019) and common carp fry (Jahan et al., 2021). However, previous research results on the total replacement of FM with BM in the diet of the fry stage of *O. niloticus* are hardly found.

The proximate analysis of FM and UB of this study confirmed an increase in crude protein and crude lipid contents with SSF, and the results confirmed that SSF positively affected growth performance. Fermentation enhances the nutrient's protein digestibility, micro-nutrient availability, and bioavailability by hydrolyzing the by-products (Karlund et al., 2020).

The chitin content in black soldier fly larvae and pupae ranges from 1% to 9%, and the availability of chitin in higher quantities acts as an anti-nutrient to tilapia (Eggink and Dalsgaard, 2023). It has been identified that the presence of chitin in higher levels in aquafeeds negatively affects the growth performance, protein and lipid digestibility of fish (Pascon et al., 2024). The pepsin digestibility value of BM was comparatively lower than the FM (Table 2), and this result is also comparable to the above finding.

The pepsin digestibility of the UB was 70.1% increasing to 75.0% when the UB was fermented. Qazi et al. (2011) have explored that the SSF improved the crude protein percentage up to 32.90% and decreased total carbohydrate by 54.54% in aquafeeds after 48 hours. Furthermore, a wide range of enzymes formed during this process degrade carbohydrates and enhance protein levels (Pandey et al., 1999; Iyayi and Losel, 2001). Therefore, the proximate composition, pepsin digestibility, and the above-mentioned previous research results confirmed that the changes in the chemical composition of FB have been positively affected by the fermentation.

Several experiments have been conducted to test the effect of fermented plant-based ingredients. The protein and total hydrolyzed amino acids percentages increased by 13.65% and 16.27%, respectively, through the SSF of soybean meal while decreasing unfavorable phytic acid and trypsin inhibitors (Hassaan et al., 2015). Furthermore, beneficial results were observed when replacing the FM with mulberry leaves in the diet of *Labeo rohita* (Kaviraj et al., 2012). However, replacing FM with yeast-fermented canola meal in Asian seabass feed succeeded only up to a 50% level, and further replacement led to a reduction in the growth performance, nutrient digestibility, body crude protein, ash, and mineral content (Plaipetch and Yakupitiyage, 2012). This reduction was related to the increased dietary phytic acid. Nevertheless, a 50% replacement level did not affect overall growth.

There is a research gap on the fermentation of animal-based ingredients compared with plant-based materials. However, Ardra et al. (2024) recently found that FM can be totally replaced with formic acid-fermented BM in the *Pangasianodon hypophthalmus* fingerling diet without affecting growth, nutrient utilization, hepatic health, immunity and protease activity. Samaddar et al. (2015) found that by replacing the FM with fermented slaughterhouse blood and fish offal blend in *Labeo rohita* fingerlings, increased the apparent digestibility coefficients of protein and lipids were increased as the fermented ingredient contents increased. Simultaneously, the above researchers confirmed an increased amino acid absorption rate. When FM was substituted with acid-fermented chicken waste silage, the growth performance and activity of digestive enzymes improved as the level of fermented silage increased without affecting the intestine and liver histology of Mozambique tilapia (*Oreochromis mossambicus*) juveniles (Mbokane et al., 2022). Fungi have the potential to synthesize and excrete hydrolytic enzymes extending and infiltrating inside the solid substrate, thus predominating in the SSF process (Manpreet et al., 2005).

Kari et al. (2023) found that when the inclusion level of BM was increased above 13% in *B. splendens*, the growth performance was significantly reduced. The authors suggested that the indigestible and higher chitin level of BM was the reason for this effect. The SSF promotes microbial growth and enhances the lactic/ethanol contents, resulting in a higher protein percentage (Sedanza et al., 2016). Han et al. (2012) mentioned that indigestible fiber content can be degraded, converting it into monosaccharides, polysaccharides, and prebiotics essential to fish. Results of the growth parameters of this experiment confirmed the above findings because the FB-fed fish obtained significantly higher and enhanced growth results. Further, PER value has been significantly enhanced in the FB-fed fish group due to the above reasons. The result of this experiment consolidates evidence confirming that the SSF can improve the growth performance of fish due to higher availability of protein, lipid, amino acids and enzymes through the SSF process. Therefore, it could be assumed that the above-mentioned facts are the reasons for the enhanced growth performance of solid-state-fermented BM diets. However, the decreasing trend of the growth parameters (WG, DWG, RWG, and SGR)

of fish can be observed, when the FB inclusion level was increased though these reductions were not statistically different. In addition to that, a significant increase of the FCR and a significant decrease of the PER can be identified when the FB content is enhanced. This study showed that the pepsin digestibility (Table 2) and amino acid profile (Table 3) of BM were lower when compared with FM, and these poor nutrient quality and content have led to its reduced growth performance. Moreover, higher FCR values have been recorded from fermented and unfermented feed-fed fish groups, and this suggests that the efficiency of the BM should be further enhanced using different techniques in addition to the SSF.

Liver function is used as an indicator for evaluating the quality of aquafeed. Previous experiments have shown that higher levels of BM in aquafeeds have led to hepatic steatosis (Zarantoniello et al., 2019 a). Abnormalities in liver cells were observed when the BM level exceeded 13% in the diets of *B. splendens* (Kari et al., 2023). Furthermore, zebrafish fed diets containing higher levels (75% and 100%) of BM exhibited severe liver cell infiltration with fat (Zarantoniello et al., 2019 b). In contrast, no alterations were found in liver cells when FM was replaced with BM in 50% of rainbow trout (14.6±0.2 g) diet by weight basis (Melanchon et al., 2022).

Alterations in liver cells were observed in all BM-fed fish regardless of the inclusion levels in this study. Significant hepatic alterations, such as inflammatory cell aggregation compared with the control, can be observed in the hepatic cells when FM was replaced with 35FB. However, this type of observation is categorized as mild and less severe damage. When the FM was replaced with 70FB, 100FB and 100UB severe and irreparable damages such as necrosis and hemorrhages were found in the liver cells. Siddek et al. (2018) observed severe and irreversible hepatic changes in juvenile *Lates calcarifer* when the researchers replaced the FM with 75% fermented tuna hydrolysate compared to the unfermented feed-fed fish. The authors further assumed that this was due to the acceleration of lipid peroxidation caused by boosting tocopheroxyl radicals. This situation may happen due to unsuccessful fat metabolism in the liver cells (Caballero et al., 1999) or excessive energy intake (Spisni et al., 1998). In contrast to the above findings, no severe alterations in the liver cells of the FM-fed fish (100FM) were observed when compared with UB-fed fish (100UB), though the intensity of the damage increased when the FB inclusion level was increased. Some findings have confirmed that the fermentation improves typically the structure of the hepatocytes in fish (Xie et al., 2021). However, some experiments have proved that it was not because of the fermentation but because of the fermented ingredient's inclusion level that influences the fish's liver morphology (Roslan et al., 2024). Thus, the above findings apply to researched species, stages, biochemical composition of the used ingredients, and fermented techniques and could not be generalized. Further research is required to fill the above research gaps and to settle the controversial findings.

The juvenile stage of *O. niloticus* was not affected by the total replacement of FM with UB for liver histology in terms of ALT content (de Oliveira et al., 2024). Sangsawang et al. (2024) and Kariuki et al. (2024) have confirmed that there were no detrimental impacts for the fingerling stage of *O. niloticus* at levels when FM was replaced by UB up to 75% in terms of liver histology. Ouko et al. (2024) and Limbu et al. (2022) have recommended that the replacement levels from 50 to 75% and 50% of UB with FM are ideal for the liver functions in the fry stage of Nile tilapia diets, respectively. These results are comparable to the findings of the hepatic alterations in this experiment. The above results reveal that when the stage of fish is nearer from the adult stages to the nursery stages, adverse effect on the liver is intensified. Therefore, a 50–75% replacing level is optimum for replacing FM with UB in the diet of *O. niloticus* fry. It is suitable to conduct future research using SSF for fingerling and juvenile stages to explore the effect on liver functions and growth performance.

However, the above findings underscore the need for further study on the effect of FB on the fish liver. Results based on other ingredients indicate that the adverse effects on the liver are due to the fermented feed ingredients. which apply only to some species. Further, Feng et al. (2021) attempted to minimize excessive fat deposition in the liver of Chinese perch using two probiotics (*Bacillus subtilis* and *Lactobacillus plantarum*). It was reported that these probiotics produced acetate, which activates AMPK and accelerates oxidative decomposition of the liver lipids. This perspective highlights exploring alternative fermentation methods to the SSF for the relevant fish species in future research.

The substrate composition of black soldier fly larvae highly impacted the growth performance, nutrient composition (protein and lipid) and digestibility (Kieβling et al., 2022; Yakti et al., 2022). The presence of chitin hindered protein digestibility in UB (Bonomini et al., 2024). Further, the fat level of the UB affects the digestibility. The facts mentioned above have not been covered in this study, and future research on the applicable topics may provide further insight into the optimization of UB as a practical feed ingredient.

The BM is one of the most frequently researched insect meals to replace FM. However, the application of BM in practical aquafeeds has been limited due to its inability to completely replace FM. The SSF is a simple, economical, and sustainable method. Therefore, SSF is a better approach to applying BM in practical and commercial aquafeeds.

Conclusions

This experiment confirms that the SSF is a successful technique to enhance the nutrient performance of BM when replacing fishmeal in the *O. niloticus* fry diet in terms of growth and feed utilization. However, suitable

techniques or optimum inclusion levels need to be identified to minimize potential risk to liver functions in fish.

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