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Complete substitution of fish meal with black soldier flies *Hermetia illucens* (L. 1758) larvae meal at varying incorporation rates for feeding *Oreochromis niloticus* raised in captivity

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Abstract

Background Black soldier fly larvae are exceptional ingredients, often used to replace fish meal. They can be easily cultured using waste and by-products. This study assesses the effect of black soldier fly larvae (BSFL) meal on the growth of *Oreochromis niloticus* raised in captivity, as well as the economic impact of replacing fish meal with BSFL meal in its diet.

Method Black soldier fly larvae (BSFL) were produced for 15 days after egg hatching. The substrate of BSFL production was Soy bran that is a byproduct obtained by processing soy cheese. The larvae of *Oreochromis niloticus* of average initial weight 0.012 ± 0.00 g were randomly distributed in 15 tanks at a rate of 50 larvae per tank. Five different treatments were applied, with TR (imported feed); T0 (diet with 0% incorporation of black soldier fly larvae meal); T40; T50 and T60 are diets with 40%, 50% and 60% incorporation of black soldier fly larvae meal. The feeding frequency was 4 times/day. Each treatment was tested in triplicate for 28 days.

Results The specific growth rate obtained during the experiment varied significantly ($P < 0.05$) with the treatments from 2.88 ± 0.09 (T40) to $4.50 \pm 0.12\%$ /day (TR). The feed conversion rate (FCR), ranged from 2.25 ± 0.05 (T40) to 1.08 ± 0.04 (T60). The protein efficiency ratio (PER) showed a significant difference ($P < 0.05$) with the treatment. It ranged from 0.81 ± 0.07 (T40) to 2.34 ± 0.06 (TR). The survival rates varied from (T0) (92.66 ± 3.52) to (T50) (100.00 ± 0.00). The economic conversion ratio (ECR) presented a significant difference ($P < 0.05$). The best ECR was obtained with T60 (1.62 ± 0.09) and T50 (2.42 ± 0.17). Based on the parameters studied, diet with 40%, 50%, and 60% of BSFL meals showed better performance compared to treatment containing fish meal at 54% (T0). The cost production analyses were used to evaluate the economic impact of utilizing BSFL meal instead of fish meal in *O. niloticus* feeding.

Conclusion It is recommended to use 50% and 60% substitution rates for better economic profitability.

Keywords Black soldier fly larvae, *Oreochromis niloticus*, Fish meal, Substitution, Incorporation rates

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Introduction

Fish is one of the most traded food products in the world [17]. Average annual fish consumption per person has increased significantly from 9.1 kg in 1961 to 20.6 kg in 2021. This growth is mainly due to aquaculture, as production of fishing has remained relatively constant since the late 1980s [17]. In sub-Saharan Africa, fish farming often faces the issue of expensive and unavailable fish feed. This is because of the lack of high-quality fish meal, which is a crucial component in aquaculture feed [2–4, 14, 16]. Several aquaculture researchers and farmers have studied the use of animal by-products as ingredients in aquaculture feeds to replace fish meals [10, 24, 29, 38, 39, 41]. Black soldier fly larvae stand out among the agricultural ingredients and by-products used to replace fish meal. These larvae can be cultured on waste and by-products, are highly efficient in converting food, and pose a low risk of transmitting zoonotic infections [41]. Among insect species capable of rapidly producing significant biomass in controlled breeding conditions, the black soldier fly is currently the main species widely studied for bioconversion and food ingredients [28]. They are insects with a low environmental impact and are a source of protein with a well-balanced profile of essential amino acids (EAAs), almost comparable to fish meal [9, 21, 32, 33]. The protein content of *H. illucens* ranges from 37 to 63% of dry matter while the lipid content varies from 7 to 39% mainly depending on the livestock substrate [8, 21]. The larvae of black soldier flies are saprophagous and are currently being produced on an industrial scale globally due to their abundance and nutritional value [13, 25, 41]. The production of Black Soldier Flies Larvae (LMSN) *Hermetia illucens* (L. 1758) for its use in aquaculture feeding represents a major revolution in the mass production of aquacultural resources for human consumption [4, 19, 20]. Among these aquaculture resources, *Oreochromis niloticus* is a species that requires a high level of protein in its diet during larval stage (35 – 45%). This species is commonly found across Africa and is one of the most cultivated fish in aquaculture. It is known for its rapid growth and is greatly valued by consumers [5]. The mass production of this species in aquaculture requires the feed supply. The cost of fish feed makes up a large portion of the overall production cost, sometimes reaching up to 60% [22]. The present study aims to improve the growth performance and reduce production costs by completely replacing fish meal with black soldier fly larvae meal at varying incorporation rates for feeding *Oreochromis niloticus* larvae raised in captivity. Most studies concerning the use

Table 2 Nutritional values of (BSFL) produced

Parameters	Dry Matter (%)	Protein (%)	Lipid (%)
BSFL	38.62±0.56	41.54±038	29.55±0.22

of BSFL in the feeding of *O. niloticus* focus on the fry and juvenile stages. The use of BSFL on the larval stage allows us to conclude if BSFL can be used throughout the production cycle of *O. niloticus*.

Materials and methods

Study area

The research was carried out at the Aquaculture and Fisheries Management Research Unit (URAGeP) of the School of Aquaculture (EAq) at the National University of Agriculture (UNA) in the Benin Republic. URAGeP is situated in Adjohoun, Ouémé Department in southern Benin (6° 46′ 18.73″ N | 2° 30′ 2.32″ E).

Black soldier fly larvae production

Black soldier fly larvae (BSFL) were produced for 15 days after egg hatching. The substrate of BSFL production was Soy bran that is a byproduct obtained by processing soy cheese. The soy bran was dried and analyzed for chemical profile before using (Table 1). At the end of the production, the larvae were harvested and weighed. These larvae were dried in an oven at 50 °C for 6 h [20]. The harvested and dried larvae were assayed in proteins, lipids and dry matter (Table 2).

Experimental design

Diet formulation

The nutritional requirements of *O. niloticus* [30, 35] were taken into account when formulating the diets (Table 3). Four iso-protein, iso-lipid and iso-energy diets were formulated for the study. Three of these diets included varying levels of BSFL meal (40%, 50%, and 60%). While one diet served as a control without adding BSFL meal (0%) but containing 54% of fish meal, another diet was a reference composed of imported commercial floating feed (Gouessant®). For each diet, the ingredients were weighed, mixed to obtain a homogeneous powder and then soy oil was added and mixed further. Water was then added 20% of dry matter to obtain a paste. This paste was introduced in extruder using 2 mm mesh. The prepared feeds were then dried, fragmented using a 0.1 mm sieve. The feeds were stored

Table 1 Chemical parameters of the rearing substrates

Parameters	Dry Matter(%)	Organic Matter (%)	Ash (%)	Carbon (%)	Nitrogen(%)	P ₂ O ₅ (mg/l)
Soy bran	91.44±0.11	67.45±0.18	32.54±0.17	34.54±0.22	6.15±0.61	0.72±0.25

Table 3 Feed formulations containing BSFL meal in the diet of *O. niloticus* and theoretical chemical values

Ingredient (%)	TR	T0	T40	T50	T60
Fish meal	-	54	0	0	0
BSFL meal	-	0	40	50	60
Soybean meal	-	19	33	24	14
Wheat bran	-	2	2	2	3
Corn flour	-	13	13	16	17
Methionine	-	3.5	3.5	2	1
Lysine	-	3.5	3.5	2	1
Dicalcium phosphate	-	1	1	1	1
Premix (Vit. + Min.) ^a	-	2	2	2	2
Soy oil	-	2	2	1	1
Total (%)	-	100	100	100	100
Protein (%)		40.86	40.55	40.78	40.94
Lipid (%)		10.18	10.02	10.21	10.48
Carbohydrate (%)		29.14	29.08	29.36	29.86
Energy (kcal/100 g)		448.06	444.55	448.79	454.29

TR: reference diet composed of commercial feed (Gouessant[®]); T0: Diet without BSFL meal, T40: Diet with 40% of BSFL meal, T50: Diet with 50% of BSFL meal, T60: Diet with 60% of BSFL meal

^a premix (vitamin–mineral) contains (%): vitamin A, 4,000,000 U.I.; vitamin D, 800,000 IU; vitamin E, 40,000 IU; vitamin K3, 1600 mg; vitamin B1, 4000 mg; vitamin B2, 3000 mg; vitamin B6, 3800 mg; vitamin B12, 3 mg; vitamin C, 60,000 mg; biotin, 100 mg; inositol, 10,000 mg; pantothenic acid, 8,000 mg; nicotinic acid, 18,000 mg; folic acid, 800 mg; choline chloride, 120,000 mg; colbat carbonate, 150 mg; ferrous sulphate, 8000 mg; potassium iodide, 400 mg; manganese oxide, 6000 mg; copper, 800 mg; sodium selenite, 40 mcg; lysine, 10,000 mg; methionine, 10,000 mg; zinc sulfate, 8000 mg

in boxes in a refrigerator at a temperature of 5 °C until used for the experiment. The protein, lipid, ash, and dry matter contents of the manufactured feeds were analyzed according to the AOAC, 1990 [6]. Carbohydrates are calculated by deduction to the AOAC, 1990 [6] (Table 4).

$$\text{SGR (\%/day)} = 100 \times (\ln (\text{final biomass weight}) - \ln (\text{initial biomass weight})) / \Delta T$$

Experimental design

The larvae of *Oreochromis niloticus* of average initial weight 0.012 ± 0.00 g were randomly distributed in 15 plastic tanks (50 l capacity) indoor at a rate of 50 larvae per tank. Five different treatments were applied, with TR (imported feed); T0 (diet with 0% incorporation of black soldier fly larvae meal); T40; T50 and T60 are diets with 40%, 50% and 60% incorporation of black soldier fly larvae meal. The feeding was at satiety and frequency was 4 times/day. The feed was distributed to apparent satiety. The left over feed and fecal matter were siphoned out every morning. Each treatment was tested for 28 days and

Table 4 Nutritional composition of the experimental diet of *O. niloticus*

Chemical analysis of constituted diets based on analyses					
	TR	T0	T40	T50	T60
Dry matter	93.54	89.83	90.22	90.17	90.03
Ash	9.42	9.84	10.17	10.28	10.34
Protein (%)	40.48	39.75	39.97	40.88	41.94
Lipid (%)	11.66	10.56	10.68	10.87	11.02
Carbohydrate (%)	25.47	24.57	24.95	25.08	25.66
Energy (kcal/100 g)	444.76	426.576	430.516	438.022	447.852

each experimental diet was in triplicate. The water was renewed at a flow rate of 1 l/min. Each tank contained 25 L of water. Control fishing was carried out every 7 days. The physicochemical parameters such as dissolved oxygen, pH, and temperature, were monitored three times daily. These parameters were monitored with an oxygen meter, a pH meter and thermometer respectively.

Zootechnical parameters and feed utilization

To evaluate feed performance, zootechnical and feed utilization parameters such as the survival rate (SR), daily weight gain (DWG), Biomass gain (BG), specific growth rate (SGR), feed conversion rate (FCR), protein efficiency ratio (PER) were calculated.

$$\text{SR (\%)} = 100 \times (\text{final number of fish} / \text{initial number of fish})$$

$$\text{DWG (g/day)} = \text{body mass gain (g)} / \Delta T$$

Δt : the duration of the experiment in the number of days

$$\text{BG (g)} = \text{final biomass weight} - \text{initial biomass weight}$$

ln: natural logarithm.

FCR = dry feed fed (g)/body mass gain (g).

PER = wet body mass gain/crude protein fed.

Chemical analyses

The substrate of BSFL production and feed ingredients were analyzed following AOAC, 1995 [7]. Dry matter (DM) was determined by the sample that had been oven-dried for six hours to constant weight at 105 °C. Crude protein was analyzed by the Kjeldahl method after acid digestion. The nitrogen content was measured and converted to crude protein content using a nitrogen factor

for the crude protein calculation of 6.25. Ash contents were determined by incinerating samples in a muffle furnace heated to 550 °C at a constant rate of 50 °C every 30 min for 4 h and then cooling in a desiccator. Total carbon was measured using the method of [34]. Organic matter content was determined by the following equation: $OM\% = C\% \times 1.724$. The lipid was extracted by heating the sample in diethyl ether under reflux at 105 °C for 30 min in a VELP Solvent Extraction unit. The ether extract was calculated as the difference between the original sample and the ether extract residue. Total phosphorus was analyzed using the colorimetric method with molybdenum in sulphuric acid.

Cost production analyses

The cost production analyses were used to evaluate the economic impact of BSFL meal utilization instead of fish meal utilization in *O. niloticus* feeding. The cost of formulated diets was calculated based on the cost of the ingredients in each diet. The ingredient costs were based on the prevailing market prices within the experiment (Table 5). The US dollar exchange rate against FCFA currency was pegged at 600 FCFA. We calculated the cost of feed required to produce 1 kg of biomass. The study assumed that all other costs of production were constant for all dietary treatments and thus not considered. The economic conversion ratio (ECR) was calculated with the following equation:

$$ECR = \text{feed conversion rate} \times \text{feed cost}$$

Data processing

Data were collected and encoded in Excel software. Physico-chemical parameters, zootechnical parameters, and feed utilization parameters were calculated. The mean and range of each parameter were calculated and

graphs were drawn. The data were analyzed using a one-way analysis of variance (ANOVA) with the facilities of STATVIEW version 5.01 software, after the verification of variance homogeneity, using Hartley's test. Significant differences among means were determined using Fisher's test $p=0.05$ significance level.

Results

Water quality

Throughout the experiment, the physicochemical parameters of the water were recorded. The average temperature during the experiment varied from 26.78 (T0) to 27.07 °C (TR). The average pH was ranging from 6.87 (T60) to 7.24 (TR). The average level of dissolved oxygen was ranging from 6.89 (TR) to 6.95 (T60) mg/L.

Zootechnical and feed utilization parameters

The biomass production rate over time differed significantly among the treatments (Fig. 1). Table 6 shows that final biomass varied significantly with the treatment ($P<0.05$). Diet T60 presented the highest final biomass. No significant difference was observed between these diet and the diet composed of commercial feed (Gouessant[®]) (TR). Final biomass observed with treatments T0 (1.37 ± 0.03 g) and T40 (1.31 ± 0.03 g) were not significantly different ($P>0.05$) and presented the lowest final biomass. The same trend was observed for the daily weight gain (DWG) which varied from 0.02 ± 0.00 g (T0, T40) to 0.05 ± 0.00 g (TR, T60).

The specific growth rate obtained during the experiment varied significantly ($P<0.05$) with the treatments from 2.88 ± 0.09 to $4.50 \pm 0.12\%$ /day (Table 6). While the highest SGR was obtained with diets TR and T60 and T40 showed the lowest results of this parameter. About feed conversion rate (FCR), a significant difference was observed ($P<0.05$) between treatments. It ranged from

Table 5 Feed cost of experiment diet

Ingredients	Cost USD/kg	TR	T0	T40	T50	T60
Fish meal	3,5	-	1,89	0,00	0,00	0,00
BSFL meal	1,66	-	0,00	0,66	0,83	1,00
Soybean meal	0,83	-	0,16	0,27	0,20	0,12
Wheat bran	0,25	-	0,01	0,01	0,01	0,01
Corn flour	0,5	-	0,07	0,07	0,08	0,09
Methionine	7,5	-	0,26	0,26	0,15	0,08
Lysine	5,83	-	0,20	0,20	0,12	0,06
Dicalcium phosphate	1,5	-	0,02	0,02	0,02	0,02
Premix (Vit. + Min.)	5,83	-	0,12	0,12	0,12	0,12
Soy oil	3,33	-	0,07	0,07	0,03	0,03
Feed cost (USD)/kg		3.5^a	2.78	1.67	1.55	1.50

^a Cost of kg of commercial feed (Gouessant[®])

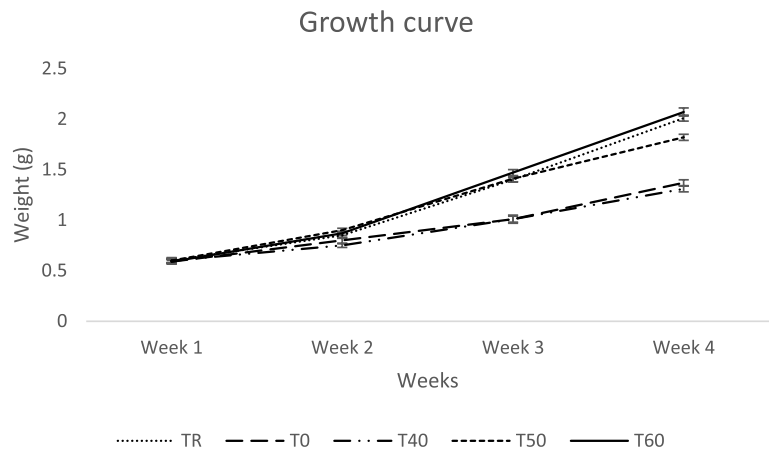


Fig. 1 Growth evolution of *O. niloticus* larvae during experimental period

Table 6 Zootechnical and feed utilization performance of *O. niloticus* fed with the experimental diets

Parameter	TR	T0	T40	T50	T60	F-Value	P-Value
IBW (g)	0.59±0.01 ^a	0.60±0.01 ^a	0.59±0.01 ^a	0.60±0.01 ^a	0.60±0.02 ^a	0.36	0.83
FBW (g)	2.01±0.03 ^a	1.37±0.03 ^b	1.31±0.03 ^b	1.82±0.04 ^c	2.07±0.03 ^a	294.26	0.00
DWG (g/day)	0.05±0.00 ^a	0.02±0.00 ^b	0.02±0.00 ^b	0.04±0.00 ^c	0.05±0.00 ^a	226.01	0.00
PER	2.34±0.06 ^a	1.11±0.02 ^b	0.81±0.07 ^c	1.55±0.05 ^d	2.20±0.08 ^a	103.92	0.00
SGR (%/day)	4.50±0.12 ^a	3.23±0.12 ^b	2.88±0.09 ^c	3.94±0.07 ^d	4.49±0.07 ^a	54.70	0.00

TR: reference diet composed of commercial feed (Gouessant[®]); T0: Diet without BSFL meal, T40: Diet with 40% of BSFL meal, T50: Diet with 50% of BSFL meal, T60: Diet with 60% of BSFL meal

IBW Initial Body Weight, FBW Final Body Weight, DWG Daily Weight Gain, PER Protein Efficiency Ratio, SR Survival Rate

The values are expressed as the means ± standard deviations. Values with the same alphabetical letters in the same row are not significantly different at $p > 0.05$

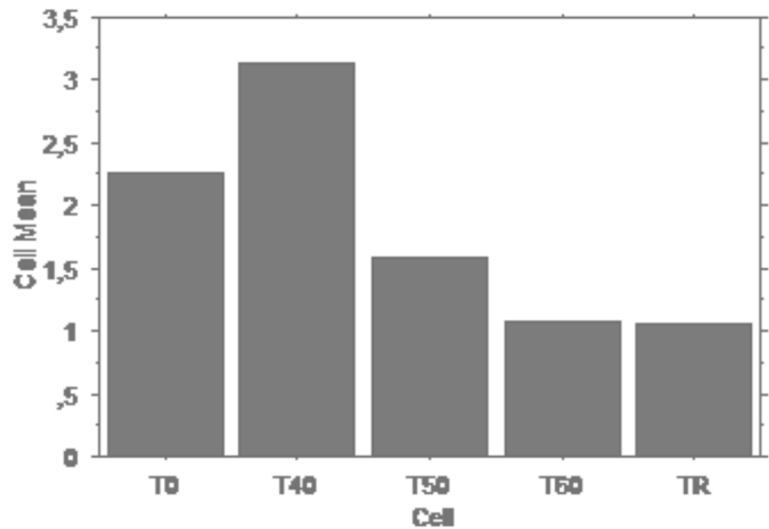


Fig. 2 Variation of feed conversion rate of *O. niloticus* during the experimental period

2.25 ± 0.05 (T40) to 1.08 ± 0.04 (T60). However, no significant difference was observed between T60 and the reference diet (TR) (Fig. 2). The protein efficiency ratio (PER) showed a significant difference ($P < 0.05$) with the treatment. It ranged from 0.81 ± 0.07 (T40) to 2.34 ± 0.06 (TR). But no significant difference was observed between TR and (T60) (Table 6). The box plot of biomass gain (BG) showed that a significant difference ($P < 0.05$) was observed between the treatments. It varied from 0.71 ± 0.02 (T40) to 1.47 ± 0.03 (T60) (Fig. 3).

Survival rate and economic analyses

About survival rate (SR), no significant difference ($p > 0.05$) was observed between the treatments. Excepted (T0) (92.66 ± 3.52) which presented a significant difference ($P < 0.05$) with (T50) (100.00 ± 0.00). The economic conversion ratio (ECR) presented a significant difference ($P < 0.05$) between the treatment. The best ECR (Table 7) was obtained with T60 (1.62 ± 0.09) and T50 (2.42 ± 0.17) which presented no significant difference ($p > 0.05$) among these treatment groups.

Discussion

Water quality

The temperature (26.78 °C to 27.07 °C), the pH (6.87 to 7.24), and the dissolved oxygen levels (6.89 to 6.95 mg/L)

remained within the acceptable range for the species during the experiment [1].

Growth and nutrient usage performance

It has been generally proven that insect meal can be utilized as a source of animal protein in aquaculture feeds [23, 33]. Among these, black soldier fly larvae are increasingly used in aquaculture feed because of their nutritional quality for partial or complete replacement of fish meal. [18, 23]. The complete replacement of fish meal with BSFL meal at various incorporation rates in this study confirms that fish meal in aquaculture feed for *O. niloticus* can be entirely avoided. [11, 25, 42]. The growth rate of fish during experiment period differed significantly ($P < 0.05$) among the treatment groups. The use of 60% BSFL meal in the feed (T60) resulted in the best zootechnical performance and was not significantly different ($p > 0.05$) from the reference feed (TR), which is a commercial feed (Gouessant[®]). The treatments T0 and T40 achieved the lowest zootechnical performance. No significant difference ($p > 0.05$) was observed between T0 and T40 treatments. The results show that adding 40% BSFL meal to a non-fish meal feed promotes the growth of *O. niloticus* larvae. Additionally, incorporating 50% (T50) and 60% (T60) of BSFL meal improves the zootechnical performance of *O. niloticus* larvae. All non-fish meal treatments

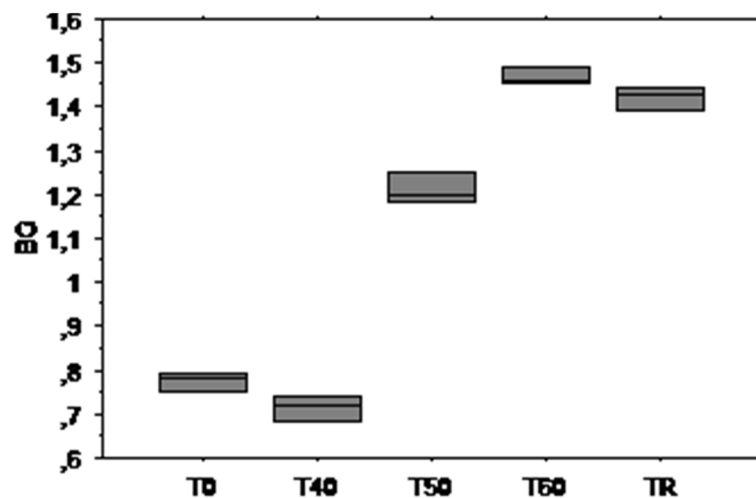


Fig. 3 Box plot of biomass gain of *O. niloticus* during the experimental period

Table 7 Survival rate and economic conversion ratio *O. niloticus* larvae fed with the experimental diets

Parameters	TR	T0	T40	T50	T60
SR (%)	96.66 ± 1.76^{ab}	92.66 ± 3.52^a	98.00 ± 1.15^{ab}	100.00 ± 0.00^b	98.00 ± 1.15^{ab}
ECR	3.67 ± 0.18^b	6.26 ± 0.21^c	5.23 ± 0.19^d	2.42 ± 0.17^a	1.62 ± 0.09^a

^{abc} The values are expressed as the mean \pm standard deviation. The values of the same line with a common letter are not significantly different ($p > 0.05$)

(T50 and T60) showed better zootechnical performance compared to the treatment using only fish meal (T0), except for treatment T40, which did not significantly differ from treatment T0. The same trend was achieved with the other zootechnical parameters considered (DWG, SGR, FCR and PER). However, studies by Kariuki et al. [23] suggest a partial replacement of fish meal with BSFL (Black Soldier Fly Larvae) meal up to 75% for *O. niloticus*. This study showed that there is no significant difference in growth parameters when substituting 0 to 75% of fish meal with BSFL meal in the feeding of *O. niloticus*. Our results support the findings of Shati et al. [36], which focused on a total replacement of fish meal with BSFL meal in the feeding of *O. niloticus*. The best weight gain was achieved with the 100% replacement treatment using 30% of BSFL meal in the feed. Similarly, studies by Tippayadara et al. [37] have also shown that completely replacing fish meal with BSFL meal does not affect the growth of *O. niloticus* juveniles. This study shows that there was no significant difference in growth, food consumption, and blood parameters between the subjects that were fed a diet containing fishmeal and those fed with a diet containing black soldier fly larvae (BSFL) meal. Additionally, the subjects fed with BSFL meal showed a better immune response compared to those fed with fish meal. In contrast, studies by [12] and [27] do not recommend a complete replacement of fish meal with BSFL meal in the feeding of *O. niloticus* and carp *Ctenopharyngodon idellus*. Indeed, these studies highlight the high level of fiber (chitin) and amino acid imbalance in BSFL meals compared to fish meals. The high levels of chitin and the imbalance of amino acids in BSFL meal would hinder weight gain in fishes fed with BSFL meal-based treatments. Studies by [31] and [43] showed that a 50% replacement of fish meal with BSFL meal would be ideal for feeding *O. niloticus* and the carp *Cyprinus carpio*.

Survival rate and economic analyses

The study demonstrated that completely replacing fish meal with BSFL meal had no negative impact on the survival rate of *O. niloticus*. The survival rate ranged from 92.66 ± 3.52 (T0) to 100.00 ± 0.00 (T50). All treatments that do not contain fish meal T40, T50 and T60 have a higher survival rate than treatments containing fish meal (92.66 ± 3.52 , T0). These results confirm the findings of several authors who have assessed the use of BSFL meal in feeding *O. niloticus* [23, 37] and other species such as *Clarias gariepinus*, *ctenopharyngodon idellus*, *Pelteosbaenus fulvidraco* [15, 27, 42] does not adversely affect their survival rates. Good survival rates also indicate favorable livestock conditions during breeding. A high survival rate depends not only on the diet but also on breeding

conditions and fish handling [13]. In terms of economic analysis, the current study demonstrates that using BSFL (Black Soldier Fly Larvae) meal in the feed for *O. niloticus* (Nile tilapia) has a positive impact on the economic profitability of production. This is attributed to the efficient dietary conversion of feeds containing BSFL meal, similar to those based on fish meal. BSFL meal has a good nutritional profile as well as fish meal and is cheaper than fish meal. Based on economic profitability, our study shows that the incorporation of 50 (T50) and 60% (T60) of BSFL meal into *O. niloticus* feed provides the best profit. These results support the findings of Wachira et al. 2022 [40], who achieved the highest economic profitability by completely replacing fish meal with BSFL meal to feed *O. niloticus* fry. Similarly, the work of Limbu et al. 2022 [26]; Shati et al. 2022 [36] and Kariuki et al. 2024 [23] also promotes the use of BSFL meal in feeding *O. niloticus* for partial or total substitution to make the production profitable.

Conclusion

The present study shows that it is possible to produce aquaculture feed using BSFL meal as a complete substitute for fish meal to feed the larvae of *O. niloticus*. The results indicate that 40%, 50%, and 60% of BSFL meals outperform fish meal-based feed in terms of biomass gain, consumption index, and specific growth rate. For better economic profitability, 60 and 50% of BSFL meal in a total substitution is recommended.

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Authors' contributions

Author contributions VJV., I.R., O.G., K.D., and A.S. carried out the conceptualization, conducting the research, data analysis and data interpretation. V. JV. and M.J.-C. carried out developing methods. VJV. and I.R. wrote the main manuscript text and carried out the figures and tables. All authors reviewed the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Note applicable.

Consent for publication

Note applicable.

Competing interests

The authors declare no competing interests.

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