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Utilization of Black Soldier Fly (*Hermetia illucens*) Larvae as a Potential Substitute for Fish Meal in the Production of Nile Tilapia (*Oreochromis niloticus* L.)

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Abstract

Utilization of quality aqua-feed relies heavily on fish meal sources of protein because of its nutritional balance. However, due to its limited supply, high cost, and decline of wild fish populations, aquaculture production has shifted focus to cheaper and more readily available alternatives to guarantee sustainable aquaculture productivity. Black soldier fly (*Hermetia illucens*) larvae are a promising replacement for fish meal in fish diets due to their relatively high crude protein, lipid and mineral contents, and the bioactive potential with anti-microbial, and other probiotic properties. This study determined the effect of partially replacing fish meal with black soldier fly meal (BSFLM) on the growth performance of Nile tilapia (*Oreochromis niloticus)*. Four isonitrogenous (30% crude protein) diets in which fish meal protein was gradually substituted with BSFLM were prepared as follows: No BSFLM (control)-T0; 25% (BSFLM25)-T25, 50% (BSFLM50)-T50 and 75% (BSFLM75)-T75. The feeds were fed to the Nile tilapia fingerlings (mean weight 25 ±5 g) which were randomly stocked in 12 cages at a stocking density of 30 fish per cage. The experimental fish were manually fed at 3% of the body weight for 28 days, and 4% of the body weight for 154 days twice a day. The study found that 25% and 50% replacement of fish meal protein with BSFLM resulted in the best growth performance of Nile tilapia, as measured by final mean body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (K). This suggests that BSFLM is a promising alternative to fish meal in aqua-feeds in the production of Nile tilapia.

Keywords: black soldier fly larvae, nutrition, tilapia, sustainability, aquaculture

1. Introduction

The global fish demand has increasingly surpassed the supply (production) due to ballooning population growth, especially in developing countries (FAO, 2022). The capture fisheries are no longer sustainable due to a decline in the amount of fish being caught and a surge in demand for fish products globally. There have been efforts to promote aquaculture as a viable alternative to wild fish stocks (Friend & Funge-Smith, 2012) and also in order to meet the demand for aquatic foods (Nyonje et al., 2018). Aquaculture has grown significantly in the past 50 years and is now comparable to catch fisheries in terms of world food output (Ogello et al., 2014). Developing countries, dominate aquaculture production, with roughly 90% of total aquaculture production coming from these countries (FAO, 2013a). However, despite the availability of rich and diverse natural resources, aquaculture production in most African developing countries is insufficient (FAO, 2010).

Aquaculture relies heavily on providing the necessary nutrients for fish production, which depends on the level of intensification. Properly balanced diets are essential for fish health and increased fish production (Bandara, 2018). However, one of the main obstacles to boosting aquaculture production is the lack of quality and affordable aqua-feeds. The cost of fish feeds accounts for about 60% of production costs and is a hindrance to fish production, especially among smallholder fish farmers (Munguti et al., 2021). Commercial fish feeds are costly and not accessible to most rural African fish farmers, who mostly live below a dollar a day (Charo-Karisa et al., 2013). Therefore, fish meal (FM) can no longer be relied upon as the primary protein source in aquaculture because of its unstable supply and increasing prices (Muin et al., 2017).

Insect-based protein has gained increasing research attention recently, with studies demonstrating their potential as a protein source in aqua-feeds (Barroso et al., 2014; Henry et al., 2015). Insects are a promising source of proteins for farmed animals, and since many fish species feed on insects in their natural habitat, their use as a dietary ingredient in aquaculture is promising. However, their contribution to household fish production remains insignificant (Govorushko, 2019). Currently, only about 20% of the approximately one million known species of insects have been investigated as potential replacements for fish meal in feed (Sanchez-Muros et al., 2014). However, recently there has been a relief in the utilization of insect-derived and insect-derived products in proteins for farmed fish (Tan et al., 2018), due to the lifting of the ban by the European Commission. This has allowed the utilization of seven authorized species of insects. These species include black soldier fly (BSF), *Hermetia illucens*; housefly, *Musca domestica*; yellow mealworm, *Tenebrio molitor*; lesser meal worm, *Alphitobius diaperinus*; three species of crickets, (*Acheta domesticus*;, *Gryllodessigillatus*; and *Gryllus assimilis*) with distinguished required safe rearing conditions for these insects (Gasco et al., 2020; Madau et al., 2020).

Recent research has shown an increase in interest in the utilization of insect-derived meals as a source of protein in fish feed formulation and production. Studies suggest that insect-based meals can be a sustainable replacement for conventional fish or plant protein meals used in aqua-feeds (Gasco et al., 2016; Henry et al., 2015), this is because insects have the ability to upgrade low-quality organic material, require less resources and have comparatively lower carbon footprint hence lower greenhouse gas emissions (Van Huis, 2013). The shift to insect-based aqua-feed offers bio-circular economy opportunities, enhancing environmental sanitation by recycling bio-waste (Ermolaev et al., 2019; Mertenat et al., 2019). Insect-based meals have a high nutritional content and are environmentally friendly, with low water and carbon footprints and land requirements (Tschirner & Kloas, 2017; Makkar, 2017). The black soldier fly larvae, in particular, contains high-quality amino acids and lipids with fatty acids when grown in substrates of good quality (Rumpold & Schluter 2013; Gasco et al., 2018). The BSF larvae are cheap, easy to rear using domestic organic waste and can provide high-value protein with a better amino acid content that can enhance fish growth (Tran et al., 2015). This study, therefore, investigated the effects of partially substituting fishmeal with partially defatted BSF larvae as protein sources on the growth performance of Nile tilapia (*O. niloticus*).

2. Materials and Methods

2.1 Study Area

This study was carried out at the Kenya Marine and Fisheries Research Institute (KMFRI), Sagana Center (Figure 1) located in Central Kenya (0^0 19 ˈS and 37^0 12 ˈE).

Figure 1. The National Aquaculture Research Development and Training Center, Sagana (Experimental site)

2.2 Ethical Approval

This study was granted approval by the Institutional Animal Care and Use Committee (IACUC) of Kenya Agricultural and Livestock Research Organization (KALRO)-Veterinary Science Research Institute (VSRI); Muguga North, in compliance with all applicable regulations and guidelines, under the reference code KALRO-VSRI/IACUC028/16032022.

2.3 Diet Preparation

Four different diets were prepared for the experiment, all containing approximately 30% crude protein. The control feed was made using fish meal (FM), *Rastrineobola argentea*. Table 1 displays the ingredients and chemical composition of the experimental diets. The experimental feeds were formulated to meet the nutritional and growth requirements for *O. niloticus*. Since the nutritional composition of black soldier fly larvae meal (BSFLM) differed significantly from that of FM, substitution levels were calculated using an Excel spreadsheet to ensure that the other ingredients in the diet were adequate. The ingredients were ground and then mixed thoroughly to make a homogeneous blend. The formulations were made into pellets by adding warm water to the mixture at a rate of 5% and mixed thoroughly to achieve consistency. The mixture was then pelletized using a 2 mm meat mincing equipment. The resultant pellets were properly dried under a shade and stored in airtight dark bags during the period of the experiment.

Table 1. Ingredients and nutritional content of experimental diets fed to tilapia grower/finishing diets

Ingredient	**T0**	**T25**	**T50**	**T75**
Wheat pollard	7.00	7.00	7.00	7.00
Rice polishing	20.00	19.25	19.00	18.75
Maize germ	22.00	22.00	21.50	21.00
Fish meal	7.00	5.25	3.50	1.75
BSFLM	-	2.50	5.00	7.50
Soybean meal	35.00	35.00	35.00	35.00
Sunflower cake	5.00	5.00	5.00	5.00
Lysine	1.00	1.00	1.00	1.00
Methionine	1.00	1.00	1.00	1.00
Fish premix	2.00	2.00	2.00	2.00
Total	**100.00**	**100.00**	**100.00**	**100.00**
Nutrient Level				
CP (%)	30.60	30.52	30.42	30.32
Energy (MJ/kg)	3,037.21	3,049.11	3,049.95	3,050.80

*T0- Diet 1- control (without black soldier fly larvae meal), T25-Diet 2 (25% BSFLM substitution), T50-Diet 3 (50% BSFLM substitution) and T75-Diet 4 (75% BSFLM substitution).

2.4 Experimental Design

The study utilized twelve cages with dimensions of 2m × 2.5m × 1m, which were set up in a large earthen pond measuring 40m × 20m, with 3m maintained between each cage. Prior to filling the pond with water up to a depth of 1m, agricultural lime was applied at a rate of 100 g/m^2 and was allowed to dry for a period of two weeks. Mixed sex fingerlings of *O. niloticus* numbering three hundred and sixty were procured from KMFRI Sagana Research Centre hatchery and randomly assigned to the cages at a stocking density of 30 fingerlings per experimental cage. The fingerlings were fed on commercial feed for one month to adapt to the experimental environment before the feeding trial started, during which their initial weight was recorded.

The experiment involved feeding four types of isonitrogenous diets to the fingerlings, with varying proportions of defatted BSFLM. These diets were labeled as 0% (BSFLM0)-T0, 25% (BSFLM25)-T25, 50% (BSFLM50)-T50 and 75% (BSFLM75)-T75. Each diet was tested in triplicate, and the fingerlings were fed manually twice a day (at 10:00 hrs. and 16:00 hrs.) at 3% of their body weight over the first 28 days and 4% of their body weight for the following 154 days. The feeding trial lasted for 26 weeks.

2.5 Feeding Trials and Data Collection

Throughout the 26-week experimental period, data was collected every 21 days (3 weeks) to monitor the growth rates of the fish in terms of their length and weight. The length (TL) was measured using a measuring board while their weight was determined using a weighing scale (model EHB-3000, China). The amount of feed given to the fish was adjusted every three weeks according to the changes in their mean body weight. Fish mortality was recorded and replacement was done during the first 30 days of introducing the fish to the test (experimental) diets. The growth performance of the fish; daily weight gain, survival rate, and feed conversion ratio were calculated. The feed conversion ratio (FCR) was calculated by dividing the daily feed intake in grams (g) by the change in fish weight (g). The water parameters particularly pH, conductivity, temperature (℃), total dissolved solids and salinity, were monitored on a weekly basis and during fish sampling using a multi-parameter meter (model H19828, Hanna Instruments Ltd., Chicago, IL, USA).

2.6 Evaluation of Dietary Performance

To determine the growth and fish feed efficiency the following parameters were measured:

Weight gain (WG), percentage WG (%WG), specific growth rate (SGR) and condition factor (K) were calculated following the formulae by Sveier et al (2000) as follows:

1. WG = W_f-W_i, where W_f is final body weight and W_i is initial body weight
2. %WG = [(W_f-W_i)/W_f] × 100
3. SGR = [(ln (W_f) - ln (W_i)/ T] × 100, where T is experimental period (days)
4. K = (TBW/TL^3) × 100, where TBW is total body weight (g) and T length (cm).

The Feed Conversion Ratio (FCR) and survival rate (SR) were calculated based on formulae derived by Qi et al (2012) as follows:

5. FCR = FI/WG, where FI is feed intake (g/day) and WG is weight gain (g/day)
6. SR = $(N_f/N_i) \times 100$, where N_f is the final number of fish and N_i is the initial number of fishes

2.7 Statistical Analysis

The collected data was processed by removing errors or inconsistencies and statistical analysis was conducted using MS Excel and R Studio software. A one-way ANOVA test was conducted to determine the differences among the diets. This was followed by a Tukey HSD post hoc test for pairwise comparisons. Differences were considered significant when $p<0.05$.

3. Results

3.1 Water Quality

The results of selected water quality parameters are summarized in Table 2. All values fell within the recommended range for Nile Tilapia culture.

Table 2. Mean variation (Mean ±SD), the minimum and maximum values of physio-chemical water quality parameters during the experimental period in the earthen pond where the experimental cages were mounted

Parameter	Mean ±SD	Minimum	Maximum
Temperature (^{o}C)	21.81 ±0.66	17.200	26.500
Dissolved Oxygen (DO) (mg/L)	8.76 ±0.15	7.050	9.870
pH	7.57 ±0.09	6.9200	8.2000
TDS (mg/L)	67.64 ±3.42	54.25	98.20
Salinity (ppt)	0.05 ±0.002	0.04000	0.06000
Conductivity (µS/cm)	83.17 ±2.36	60.09	92.15

3.2 Fish Growth Trends over the Experimental Period

Figure 2 shows the growth trends of mean weights for *O. niloticus* over the course of the experiment. There were similarities and overlaps between the treatments throughout the period of the experiment. At the end of the experiment, the growth curve for T25 (25% BSFLM inclusion) had the highest weight (77.08 ±2.45g), followed by T50 (50% BSFLM inclusion) with 76.95 ±2.52g, while T75 (75% BSFLM inclusion) had the lowest weight at 70.1 ±2.08g.

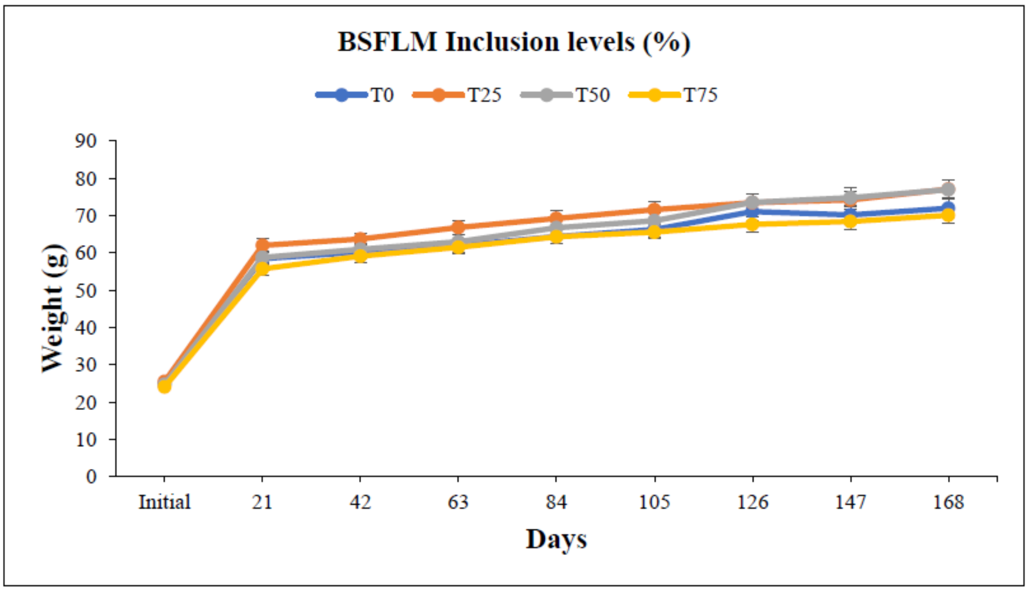

Figure 2. Growth rate (weight) of *O. niloticus* fed on varying levels of BSFLM inclusion during the culture period

3.3 Growth Parameters and Feed Utilization

The growth performance parameters presented in Table 3 showed no significant differences in growth performance among the treatments (diets) (P>0.05). However, experimental diets T50 and T25 recorded slightly higher weight gain (BWG) and specific growth rate (SGR) (BWG; 64.71±1.30; 63.88±1.28, SGR; 0.60±0.02; 0.59±0.02 respectively) than the other treatments, although the differences were not significant. The lowest values for BWG and SGR were recorded in the control diet T0 (62.69±1.36; 0.57±0.02 respectively). The survival rates during the experimental period of Nile tilapia fed on experimental diets T50, T25 and T75 were significantly higher (100%, 96.7% and 96.6% respectively) than those of the control diet T0 (85.6%). The feed conversion ratio was lowest for T0 and T50 (1.36±0.14; 1.46±0.16 respectively) but highest for T75 (1.67±0.17). Diet T25 exhibited the highest condition factor (1.73±0.02), while diet T50 recorded the lowest condition factor (1.67±0.01).

Table 3. Growth performance of *O. niloticus* fed on diets with varrying BSFLM proportions

	Dietary Treatment				**ANOVA test**	
Parameter	T0	T25	T50	T75	F-Value	P-value
Mean initial weight (g)	24.49 ± 0.37^{ab}	25.48 ± 0.32^{a}	24.72 ± 0.39^{ab}	24.02 ± 0.36^{b}	2.94	0.033
Mean final weight (g)	71.96 ± 2.63^{a}	77.08 ± 2.45^{a}	76.95 ± 2.52^{a}	70.10 ± 2.08^{a}	2.17	0.092
Mean weight gain (g)	47.46 ± 2.62^{a}	51.60 ± 2.45^{a}	52.22 ± 2.58^{a}	46.08 ± 2.13^{a}	1.56	0.199
BWG (%)	62.69 ± 1.36^{a}	63.88 ± 1.28^{a}	64.71 ± 1.30^{a}	63.01 ± 1.29^{a}	0.48	0.694
SGR (%)	0.57 ± 0.02^{a}	0.59 ± 0.02^{a}	0.60 ± 0.02^{a}	0.57 ± 0.02^{a}	0.66	0.578
Condition factor (K)	1.68 ± 0.02^{ab}	1.73 ± 0.02^{a}	1.67 ± 0.01^{b}	1.70 ± 0.01^{ab}	2.57	0.054
Survival rate (%)	85.6	96.7	100	96.6	-	-
Feed conversion ratio (FCR)	1.36 ± 0.14^{a}	1.66 ± 0.25^{a}	1.46 ± 0.16^{a}	1.67 ± 0.17^{a}	0.73	0.536

**Means on the same row with different superscript letters are significantly different at $p< 0.05$. SGR - Specific growth rate. T0- Diet 1- control; T25-Diet 2 (25% BSFLM), T50-Diet 3 (50% BSFLM) and T75-Diet 4 (75% BSFLM).

4. Discussion

Farmers in aquaculture have a major challenge of over-reliance on fish meal and fish oil in their feed formulations. This necessitates a need for a more sustainable, cost-effective, and environmentally friendly alternative protein source. Finding such alternatives is difficult because the substitutes need to contain the necessary components required to maintain fish health and welfare standards are adhered to. Insect-based feeds have become more popular because of their relatively low impact on the environment. Black Soldier Fly (BSF) larvae meal is particularly attractive as it meets the growth requirements of both terrestrial and aquatic animals, making it a viable alternative protein source in animal feeds. A study by Katya et al., (2017) reported that as much as 28.4% of fish meal replacement by BSF larvae meal without any adverse effect on growth performance in juvenile barramundi (*Lates calcarifer*). According to Nairuti et al., (2021), Nile tilapia achieved the best growth performance with an inclusion rate of 33% of BSF larvae meal in its diet.

Physico-chemical parameters in this study were at optimum levels for biochemical reactions that enhance growth, as recommended by Kirimi et al., (2022). The length coefficient was less than 3, indicating a negative allometric growth. This contrasts with Shati et al., (2022) findings that Nile tilapia fed BSFM-based diets exhibited isometric growth with a length coefficient of 3. Fish fed on BSFLM50% had the highest body weight increase at 64.71%, followed by BSFLM25% at 63.88%, BSFLM75% at 63.01% and BSFLM0% at 62.69%. Other studies on *O. niloticus* have reported varying results, such as a lower body weight gain of 30% as reported by Tippayadara et al., (2021), and higher body weight gains of 64% (Devic et al., 2018), 73% (Muin et al., 2017) and 89% (Ogello et al., 2022). These differences may be due to varying fish development stages and study periods, with previous trials lasting between 32 and 96 days compared to the current study with a duration of 182 days.

Fish that were fed with a diet consisting of 50% BSFLM (T50) had comparatively lower FCR among the test diets. The FCR values observed in this study were lower than those found in previous studies like Muin et al. (2017) and Tippayadara et al. (2021) which also used BSFLM as a feed ingredient for Nile tilapia. However, Limbu et al. (2022) reported even lower FCR values in a similar study. A lower FCR indicates better feed utilization. The comparatively low FCR observed in the fish fed with T50 may be attributed to the efficient utilization of defatted BSFLM. Insect meals that have been defatted have been shown to improve digestibility and nutrient utilization, although the chitin content is similar between defatted and non-defatted insect-based meals (Basto et al., 2020). The relatively high fat content in full-fat BSFLM may manifest in the diet increasing dietary energy density, leading to decreased feed intake by the fish. The high FCR values recorded in fish fed with T75 suggest that a high content of BSFLM negatively influenced feed utilization, possibly due to inhibition of chitin. Besides protein, BSFLM has been reported to have higher chitin content which has been observed to increase the fiber content of feeds. This could be due to low levels of fish meal in the diet, compromising the amino acid profile. Fish meal contains high-quality, digestible amino acids and fatty acids (Cho & Kim, 2011).

The utilization of BSF larvae meal has been linked to a decrease in FCR in other fish species like the Atlantic salmon post smolt (Lock et al., 2016) and Siberian sturgeon (Rawski et al., 2020). Studies have shown that de-fatting of insect meals can improve digestibility and nutrient utilization, thereby increasing feed efficiency (Basto et al., 2020). In Nile tilapia, the growth performance was improved when soybean meal was replaced with BSFLM at 100% (Shati et al. 2022). The experimental fish in this study had high survival rates, which could be attributed to the control of experimental conditions as well as the increased feed efficiency of the defatted BSFLM. Recent studies have also reported similar outcomes in Nile tilapia, including those conducted by Abdel-Tawwab et al., (2020), Wachira et al., (2021), Limbu et al., (2022), and Shati et al., (2022).

5. Conclusion

Identifying cost-effective fish feeds is a major problem impeding aquaculture production today. In recent years, the utilization of insect meal as an alternative source of protein in aqua-feed has gained popularity. Our research has shown that BSF larvae meal can replace fish meal by up to 50% without compromising growth quality. This is particularly important as fish meal is expensive and its market price is very competitive due to scarcity and overfishing in the African water bodies. By substituting fish meal with BSF larvae meal at 25%, the cost of producing well-balanced pelletized aqua-feed for Nile tilapia could be significantly reduced. However, it is important to conduct further research to optimize the aqua-feed and validate the quality of fish carcasses fed these diets to promote adoption among fish farmers and increase their profitability.

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Authors contributions

JM-Conceptualization, Funding acquisition, Investigation, Methodology, Project Administration, Writing-original draft; FW- Conceptualization, Methodology, Project administration, Validation, Writing-original draft; IO-Conceptualization, Investigation, Methodology, Project administration, Writing-review & editing; MK-Methodology, Project administration, Writing - review & editing; RY-Investigation, Methodology, Validation, Writing- review & editing, DM- Methodology, Project administration, Writing-review & editing; DK-Conceptualization, Funding acquisition, Methodology, Project administration, Writing-review & editing; JA-Investigation, Project administration, Writing-review & editing; MO-Conceptualization, Methodology, Project administration, Writing-review & editing; KO- Conceptualization, Methodology, Validation, Writing-review & editing; NO-Methodology, Project administration, Validation, Writing - review & editing; EO-Conceptualization, Methodology, Writing - review & editing; JI- Methodology, Project administration, Writing-original draft, Writing - review & editing; JGK- Methodology, Validation, Writing-review & editing; AM-Conceptualization, Methodology, Writing-review & editing; DL- Validation, Writing - review & editing; CMT- Methodology, Validation, Writing- review & editing.

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Competing interests

The authors declare that they have no conflicts of interest.

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education. The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and peer review

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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