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Effect of replacing Black soldier fly (*Hermetia illucens*) larvae meal with fish meal in diets for African catfish (*Clarias gariepinus*) reared in earthen ponds

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Abstract

The effect of replacing fish meal for black soldier fly larvae meal (BSFLM) on growth and feed utilization of African catfish (*Clarias gariepinus*) was evaluated in earthen ponds at Sagana research Centre. 240 fish were distributed in a completely randomized design with four treatments in triplicates. Fish were fed increasing inclusion levels of BSFLM replacing fish meal (FM) at 0%, 33%, 66%, and 100 designated as D1, D2, D3 and D4 respectively. Fish were fed to satiation and sampling for growth assessment taken every 28 days while physico-chemical parameters were measured weekly. Growth performance results for *C. gariepinus* showed significant variation ($p < 0.05$) between treatments. The highest mean final weight (MFW) (91.30 ± 51.39 g) was exhibited by fish fed 33% BSFLM diet and the lowest (68.13 ± 40.45 g) for fish fed the 66% BSFLM inclusion. Recorded mean weight gain was highest on D1 (83.00 ± 34.50 g), but no significant difference was reported between the formulations. In summary, it was demonstrated that BSFLM can replace FM in the diets of African catfish by up to 33% and that a further increase in the amount of BSFLM in the diets translates to lower growth performances as reflected in the decrease in mean weight gain.

Keywords: body weight gain, growth performance, organic waste

Introduction

Food security continues to be of great concern globally due to rapid population growth which is projected to continue increasing from the current 7 billion to 9.7 billion individuals by the year 2050 (Nadathur *et al.*, 2017). Climate change, increasing water pollution, and competition for scarce natural resources are making it increasingly difficult to feed the population. An additional 70-100% of food needs to be produced to address the imbalance between supply and demand (Wang *et al.*, 2018) and the average global temperature is predicted to rise due to the increasing greenhouse gases (GHGs) emissions.

The ever-increasing demand for fish protein has led to overfishing of capture fisheries, which poses a serious threat to resource-dependent communities. With a ceiling of 110.2 million tons in 2016, aquaculture production has been one of the primary sources of high-quality fish and fish products for rural areas (FAO, 2020). Although, the industry's expansion has been characterized as modest, its expansion has been judged to be gradual which is attributed to high taxation of feed inputs, poor quality of the fish feed, limited supply, and overreliance on fish meal as a protein source (Opiyo *et al.*, 2018).

Any aquaculture business must start with high-quality fish feed (Munguti *et al.,* 2021). However, fish feeds have been noted to account for more than 60% of aquaculture production costs (Munguti *et al.,* 2012, Nairuti *et al.,* 2021). Protein is considered the most imperative and the most expensive nutrient in fish diets (Lee *et al.,* 2020). Over the years, fish meal has been preferred as a major protein source in fish feed formulation (Shukla *et al.,* 2019). This is primarily due to the high-quality protein it contains, lack of anti-nutritional factors, high digestibility and palatability and balanced amino acid and fatty acid profile, among other attributes which promote fish immunity, health, and feed efficiency (Golden *et al.,* 2016). Overreliance on fishmeal poses challenges to fish farmers in Kenya due to its escalating costs resulting from its scarcity as a result of the Lake Victoria fisheries' periodical closures, climate change and competition from other value chains, especially other animal feed suppliers (Munguti *et al.,* 2014, Adeoye *et al.,* 2020).

Sustainability in aquaculture may not be achievable with fish meal as the sole protein source, thus diversification of protein sources must remain a key priority as recommended by fish nutritionists. Studies have been conducted on fish diets with FM replaced with plant-based protein derivatives. Some of these plant-based protein sources include pea seed, sunflower seed, lupin seed, cotton seed, soybean meal, and rapeseed meal (Nasr *et al.,* 2021). However, these components do not contain major amino acids that are important for the development and growth of fish (Nagel *et al.,* 2012). For example, cotton seed cake contains high levels of gossypol, an antinutritional molecule that prevents feed from being effectively digested, and is deficient in cysteine, lysine, and methionine amino acids, which are key ingredients necessary for fish growth and development (Munguti *et al.,* 2014).

Experimentation on different protein sources in fish nutrition has been conducted, with the incorporation of insects in fish feed formulation being recently in focus (FAO, 2020). This is because insects are part of the fish food web in nature, at least for most farmed fish species. The application of black soldier fly (BSF) in substituting FM in diets of different fish species has demonstrated a high potential and this is because the BSFL have high-quality protein, an essential amino acid characterization almost similar to FM, the ability to utilize a variety of local organic waste materials, and they are inexpensive to produce (Wang and Shelomi 2017, Nairuti *et al.,* 2021). These larvae can also be produced in mass at a relatively reduced cost, thus confirming its sustainability as a protein source (Ssepuuya *et al.,* 2017). Black soldier fly larvae meal (BSFLM) has been successfully used in the diets of different fish species, such as redband trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*) and blue St. Peter's fish (*Oreochromis aureus*) (Kroeckel *et al.,* 2012). Significant growth performance was achieved when BSFLM substituted up to 48% of FM in the diets of yellow catfish (*Pylodictis olivaris*) (Xiao *et al.,* 2018). However, there were no significant differences in fish growth in studies conducted by Hu *et al.* (2017), Zhou *et al.* (2018) and Magalhàel *et al.* (2017) between Jian carps and European seabass fed on FM and BSFLM-based diets.

This study was motivated by BSF's ability to convert organic waste into valuable proteins that can be used to provide quality feed for *Clarias gariepinus* and also improve waste management, especially in protocols involving integrated aquaculture systems. In the long-term, this will encourage commercially and environmentally sustainable aquaculture production (Ayoola, 2010). Upon this background, the effect of replacing fish meal for black soldier fly larvae meal (BSFLM) on growth and feed utilization of African catfish (*C. gariepinus*) was evaluated to assess how well *C. gariepinus* grew on diets that replaced FM with BSFLM in increasing proportions.

Materials and methods

Source of ingredients and proximate analysis

Black soldier fly (BSF) larvae were procured from the International Centre for Insect Physiology and Ecology (ICIPE), Nairobi while propagation and mass production were performed at Sagana Research Centre. Other feed components,

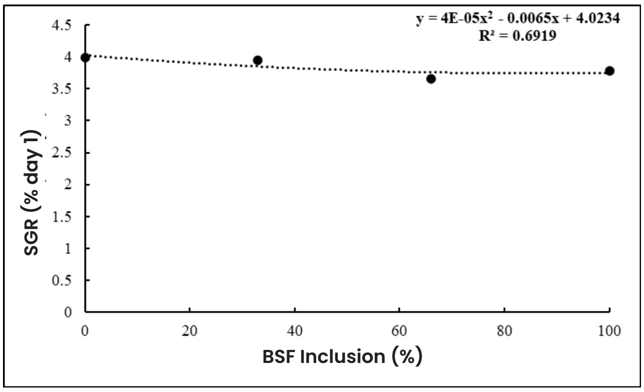

Figure 1. Variation in SGR in different the BSF inclusion levels.

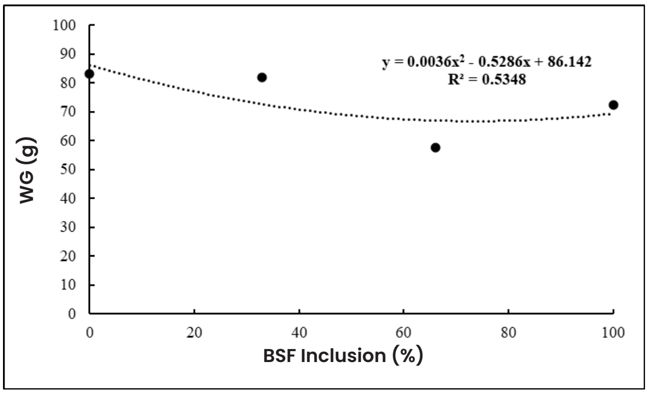

Figure 2. Variation in body weight gain (WG) in the different BSF inclusion levels.

such as fishmeal (Omena, *Rastrineobola argentea*), soybean meal (*Glycine max*), and maize bran (*Zea mays*), were procured from local feed suppliers based on cost and availability.

The formulated diets and individual ingredients underwent proximate analysis in triplicates using standard methods (AOAC, 1995). Analyses of dry matter (DM), crude protein (CP), crude fiber (CF), crude fat and ash content were performed in triplicate at Cropnuts Laboratory Services, Lim-

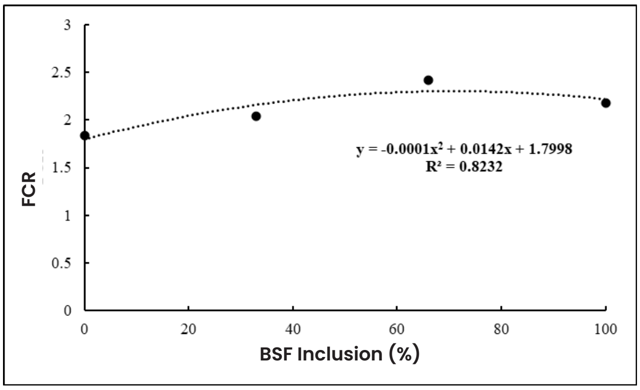

Figure 3. Variation in FCR in the different BSF inclusion levels.

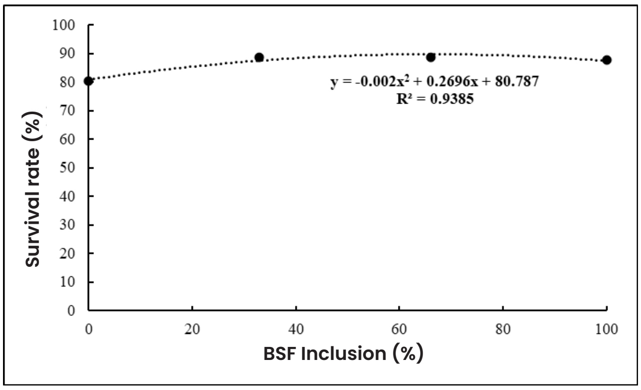

Figure 4. Variation in survival rate in the different BSF inclusion levels.

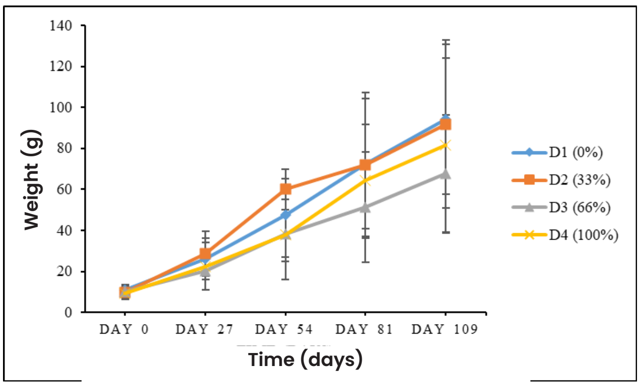

Figure 5. Trends in weight of C. *gariepinus* fed diets with varying levels of BSFLM.

** D1 (0% BSFLM inclusion); D2 (33% BSFLM inclusion); D3 (66% BSFLM inclusion); D4 (100% BSFLM inclusion)

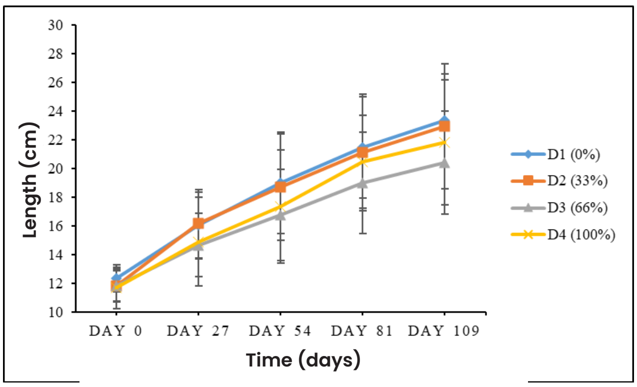

Figure 6. Trends in length of *C. gariepinus* fed diets with varying levels of BSFLM.

** D1 (0% BSFLM inclusion); D2 (33% BSFLM inclusion); D3 (66% BSFLM inclusion); D4 (100% BSFLM inclusion) uru, Kenya. DM was derived by drying the sample in an oven to constant weight for six hours at 105°C. Ash content was measured by heating the samples in a muffle furnace at a temperature of 600°C for three hours. The CP was measured using the conventional micro-Kjeldahl nitrogen analysis, and CF analysis comprised 4 hours of heating at 550°C in a muffle furnace after four consecutive alkaline-acid digestions.

Experimental test diets

Experimental diets were generated by substituting FM protein with BSFLM protein at substitution rates of 0, 7.5, 15, and 22.5% of BSFLM, or 0, 33, 66, and 100%, respectively. The ingredients were thoroughly mixed before diet formulation. A soft dough for pelletizing was prepared by addition of clean water to the feed mixture then pulverized several times and pelletized using a commercial 2-4 mm diet pelletizing machine to produce a homogeneous diet. The pellets were evenly spread and exposed to the sun to achieve proximate moisture content. Table 1, indicates the chemical composition and proximate composition of different test diets while Table 2 indicates the proximate composition of BSFLM.

Table 1. Ingredient formulation and proximate composition of test diets. D1- Diet 1- control (without black soldier fly larvae meal inclusion), D2-Diet 2 (33% substitution rate), D3-Diet 3 (66% substitution rate) and D4-Diet 4 (100% substitution rate, i.e., maximum BSFLM inclusion).

Ingredient inclusion (%)	D1 (0%)	D2 (33%)	D3 (66%)	D4 (100%)
Fish meal	20	13.3	6.6	0
Black soldier fly larvae meal	0	7.5	15	22.5
Soybean meal	60.9	59	63	64.1
Maize bran	4.7	10	7	6.7
Lipid	7.3	5.1	3	0.9
Monocalcium phosphate	0.5	0.5	0	0.5
Vitamin premix	0.5	0.5	0	0.5
Carbohydrate	6.1	1.7	1.4	0
Filler	0	2	4	4.8
Total	100	100	100	100
Proximate composition of test diets (%)				
Moisture	7.38	6.86	9.46	8.64
Ash (dry)	15.9	12.8	11.1	7.27
Crude protein	37.9	44.6	45.4	47.8
Crude fat	8.93	6.20	4.60	4.05
Crude fibre	4.86	3.93	5.48	5.22
Nitrogen free extract (NfE)	24.9	25.6	23.9	26.9
Dry matter	92.6	93.1	90.5	91.36

Table 2. Black soldier fly larvae meal (Whole crushed) proximate composition.

Parameter	Abbrev.	Unit	Result
Energy	E	MJ/kg	14.4
Protein	CP	%	51.1
Total Ash	Ash	%	15.6
Fat	Lipid	%	11.8
Fibre	Fibre	%	7.10
Nitrogen free extract	NfE	%	14.3
Dry Matter	DM	%	93.8

Even though the diets were intended to be iso-proteinous and isolipidous, analysis of the diets' crude protein (CP) levels revealed that they varied among diets but were frequently greater than the estimated 36% CP. However, there were overarching patterns throughout the test diet regimens, showing that the measured crude protein rose proportionately with increasing inclusion on BSFLM. In contrast to diet 1 (D1) with 0% BSFLM, which recorded the least amount of proximate crude protein (37.96 ± 0.02), diet 4 (D4) with 100% BSFLM had the highest CP (47.83 ± 0.03). The proximate crude fat showed the similar trend, with diet D1 having the greatest level (8.93 ± 0.00) and diet D4 having the lowest level (4.05 ± 0.01). the ash content decreased with increasing BSFLM in the diets.

Experimental design and trial

This study was carried out at Sagana Research Centre (0°19´S and 37°12´E) for 109 days. 352 one-month old *C. gariepinus* of uniform sizes were obtained from Sagana research station hatchery. The fish were stocked in hapa nets (4 x 4 m) installed in earthen ponds for 1 week to acclimatize, while being fed with commercial floating feeds. Thereafter, two hundred and forty fish were selected from the acclimatized stock and distributed in a completely randomized design with four treatments in triplicates. Fish were fed to satiation, increasing inclusion levels of BSFLM, replacing Fishmeal (FM) at 0%, 33%, 66%, and 100% designated as D1, D2, D3 and D4 respectively. To control predators, the cages were covered with predator nets. Daily records of fish mortality were kept. To track the growth performance criteria that guided feed quantity adjustment, fish samples were taken after every 28 days.

Analysis of dietary performance

The following metrics were evaluated, and these formulas were applied, to assess the efficiency of growth and feeding:

Specific growth rate (SGR, %) = 100 × [(Ln BW final (g) – Ln BW initial (g) / days of experiment]

Body weight gain (BWG, g) = Final weight (g) - Initial weight (g)

Feed conversion ratio (FCR) = Feed provided/ Live weight gain (g)

Survival rate (%) = (Number of fish harvested)/ (Number of fish stocked) × 100

Statistical analysis

Statistical analysis was done using MS Excel and SPSS statistics (Version 21). For normality checks, Shapiro-Wilk test was applied to assess normality of the data obtained and then ANOVA was used to test for significant at $\alpha = 0.05$. Tukey-HSD post hoc was used to determine the variance amongst the means.

Results

Water quality

The results of the water quality parameters analyzed are shown in Table 3. There were no significant variations in physico-chemical parameters during the study period ($p > 0.05$). All the values were within the acceptable levels for African catfish culture.

Table 3. Water quality parameters recorded during the study period. Mean, minimum and maximum values are presented.

Parameter	Mean	Minimum	Maximum
Temperature (°C)	24.5	22.0	29.4
Dissolved oxygen (DO) (mg/L)	4.75	2.54	7.07
Conductivity (µS/cm)	71.6	51.1	103
Total dissolved solids (TDS) (mg/L)	67.6	53.7	81.9
pH	7.92	7.82	9.97

Growth performance

The growth performance of *C. gariepinus* fed on diets with differing BSFLM proportions showed no major variances in specific growth rates (SGR) (Fig. 1), mean body weight gain (WG) (Fig. 2), feed conversion ratio (FCR) (Fig. 3), and survival rates (Fig. 4) during the study period ($p > 0.05$). Diet D1 had the lowest FCR (1.84 ±1.01) while the fish fed on D3 (66% inclusion) had the highest FCR (2.42±1.08). In comparison to the control diet (D1), the survival rates of the African catfish fed with D2, D3, and D4 were significantly higher ($p < 0.05$). Fish fed diet D2 had the highest survival rate (88.62 ± 9.23%).

Growth trend (weight and length) of *C. gariepinus* over the study period is shown in Fig. 5 and 6 respectively. The growth trend from day 0 to day 109 showed overlap and similarity between treatments. However, there appeared a clear segregation on the 81st day between the diets, up until the last day of the study. At the end of the culture period, diet D1 (0% BSFLM inclusion) and D2 (33% BSFLM inclusion) showed the best growth performance of *C. gariepinus* in terms of final mean weight and length.

Discussion

During the study period, there were no observed incidences of fish deformity or ill health. The survival rate of fish amongst the dietary treatment was generally high (above 75%) perhaps due to the stability of the trial conditions but higher survival rates (above 98%) were reported by Adeoye *et al.* (2020) when *C. gariepinus* fingerlings were fed diets with increasing inclusion levels of BSFLM. The difference in survival rates could be attributed to the adoption of the tank system hence ensuring more control as opposed to the cage system in the current study. Further, in comparison to previous studies, lower survival rates were realized in the present study than those of Rainbow trout (Caimi *et al.,* 2021), Nile tilapia (Tippayadara *et al.,* 2021; Limbu *et al.,* 2022), Jian carp (Zhou *et al.,* 2018), rainbow trout (Cardinaletti *et al.,* 2019), zebrafish (Zarantoniello *et al.,* 2019; Ordoñez *et al.,* 2022) and African catfish (Njoroge, 2020) when fed on diets containing varying proportions of the BSFLM.

Fish fed diet D2 with 33% BSFLM substitution of fishmeal exhibited improved final mean weight and length though not significantly different from control diet 1, (0% BSFLM). Studies by Kroeckel *et al.* (2012) and Wachira *et al.* (2021) reported a higher mean weight gain of 15% at an inclusion level of 33% in juvenile turbot (*Psetta maxima*) and Nile tilapia (*Oreochromis niloticus*), respectively. Increasing inclusion levels of BSFLM beyond 50% resulted in lower growth as observed in fish fed diet D3, which agrees with the study by Tippayadara *et al.* (2021) who reported significant decreases in BWG and SGR of *O. niloticus* with substitution levels of FM by BSFLM above 50%.

The final weight, BWG and SGR in this study showed that fish fed on a diet with 0% and 33% BSFLM inclusion performed significantly better than the fish fed on the 66% BSFLM inclusion which concurs with results presented by Nairuti *et al.* (2021) and Maranga *et al.* (2023). Previous studies have shown the protein quality in BSFLM to be high and also to possess almost similar amino acid profile as that of fish meal (Henry *et al.,* 2015). This may have been the reason for the excellent performance of fish fed on diets containing the insect meal. Lipid level in diet D1 and D2 are 8.93 % and 6.2 % respectively which are the levels optimum for the growth of *C. gariepinus*.

Replacing of FM by BSFLM at 66% and 100% showed reduced growth. The reason for the decrease in growth with increasing BSFLM inclusion could be due to increased fat content at inclusion levels of BSFL-66% and BSFL-100% in the diets, which might inhibit feed intake or diet digestibility (Watanabe, 1982, 2002; Ali and Jauncey 2004). For example, Fasakin *et al.* (2003) observed a decline in the growth of *C. gariepinus* fed full-fat maggot (*Musca domestica*) meal compared to the fish meal. The authors attributed this growth reduction to poor palatability and digestibility as well as poor amino acid content. In support of this, Talamuk (2016) found that the fat contents in the test diets increased with increasing BSFLM inclusion. In other studies, increasing dietary lipids in the diet of Nile perch juveniles produced poor growth, and poor FCR was achieved with fish fed on high lipid diet of 15%.

Furthermore, BSF has a naturally occurring polymer called chitin especially in its unprocessed form. Therefore, increased inclusion levels of BSFLM contributes to high chitin levels in the respective diets. Diets at substitution levels beyond 33% BSFLM could have contributed to reduced final weight, BWG and SGR. Chitin has been reported to inhibit lipid digestibility and lower nutrient absorption from the gastrointestinal tract, further lowering proper food absorption as confirmed in a study carried out by Kroeckel *et al.* (2012) who reported a reduction in growth rate in the juvenile turbot when fed on diets with substitution of FM by BSFLM above 33%, due to increased chitin which may have contributed to the slower growth of the fish. Gopalakannan *et al.* (2006) and Olsen *et al.* (2006) noted that any slight increase in the inclusion rate of chitin (>1%) in the diets of tilapia (*O. niloticus* × *O. aureus*) compromises the feed intake leading to poor growth.

Conclusion and recommendations

The findings from this study shows that black soldier fly larvae diet has the potential of replacing fishmeal and providing affordable quality protein in the diets of the African catfish. Feeding *C.*

gariepinus with a 33% BSFLM has the capacity to improve weight gain and other growth parameters the same way as the diet containing fish meal i.e. control diet containing no black soldier fly diet. With proper processing and value addition methods associated with the removal of chitin content, BSF can be used in formulations of the African catfish diet to improve growth performance. This can decrease the aquaculture production costs and improve sustainability and alleviate food shortage around the world.

Acknowledgement

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