Growth Efficacy and Nutrient Assimilation of Black Soldierfly and Fish Meal in Diet of *Clarias gariepinus* (Buchell 1822) Fingerlings

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Received date: April 13, 2023, Manuscript No. IPJARN-23-16348; Editor assigned date: April 17, 2023, PreQC No. IPJARN-23-16348 (PQ); Reviewed date: May 01, 2023, QC No. IPJARN-23-16348; Revised date: May 08, 2023, Manuscript No. IPJARN-23-16348 (R); Published date: May 15, 2023, DOI: 10.36648/2572-5459.8.4.076

Citation: Hammed AM, Amosu AO, Sunnuvu TF, Joseph OO, Dalmeida LO, et al. (2023) Growth Efficacy and Nutrient Assimilation of Black Soldierfly and Fish Meal in Diet of *Clarias gariepinus* (Buchell 1822) Fingerlings. J Anim Res Nutr Vol. 8 No.4: 076

Abstract

Growth performance and nutrient assimilation of Clarias gariepinus fingerlings fed different levels of Black Soldier Fly (BSF), Hermitia illucens and Fishmeal (FM) for twelve (12) weeks was conducted to develop a low-cost balanced fish diet. Six isonitrogenous crude protein diets were formulated, where BSF replaced FM at 0%, 20%, 40%, 60%, 80% and 100% inclusions. Fingerlings were fed twice daily at 4% body weight and subjected to growth performance and nutrient utilization analysis using indices such as Weight Gain (WG), Percentage Weight Gain (%WG), Feed Conversion Ratio (FCR), Gross Feed Conversion Ratio (GFCR), Protein Intake (PI), Protein Efficiency Ratio (PER) and % survivability. Results revealed that the highest cumulative weight gain (6.28 ± 3.23) was recorded in fingerlings fed 40% BSF diet inclusion, while the lowest (3.94 ± 0.82) was observed in the 0% BSF diet inclusion. There were no significant differences (p>0.05) in protein intake and percentage survivability, while there were significant differences (p<0.05) in weight gain, percentage weight gain, feed conversion ratio, gross feed conversion ratio and protein efficiency ratio. This study indicates that 40% BSF inclusion appear to be suitable in the diet of African catfish, C. gariepinus.

Keywords: Black soldier fly; *Clarias gariepinus*; Diet; Fishmeal; Growth performance; Nutrient; Protein

Introduction

Aquaculture is an enterprise that is growing rapidly and improving the economy worldwide. Fish are sources of food and revenue for the growing human population, restocking of streams, lakes and rivers to curb the shortage due to the decline in the wild capture and for sport fishing [1-3]. Fish is easily digestible, has ability to prevent and manage heart disorders and neurological diseases [4]. Consumption of fish by humans has being recommended for its role in prevention of heart disease [5]. The protein content of fish is also of immense nutritional value to pregnant women for proper development of the fetus and prevention of abortion. Fish oil enhances the proper mental development and immunity against disease among growing children [5]. Reports shows that fish provide relief from malnutrition, especially in developing countries. Fish flesh is tasty and highly digestible and it increases life expectancy [6]. Aquaculture aims at production of fish to provide protein in the diet [7]. It is man's most important single source of high quality protein, providing approximately 16% of the animal protein consumed by the world's population. The internal organs of fish are also an excellent source of specialized enzyme [8]. Cook, et al., [9] reported that optimal physical, cognitive and emotional development and function in humans requires access to food of adequate quantity and quality at all stages of the lifespan.

The global aquaculture fish production rose from 7% in 1973 to more than 30% in 1997 [10]. In 2010, global aquaculture production reached 79 million tons, growing at an annual rate of 9.7% since 1998 [11]. However, aquaculture global fish production stood at 66.6 million tons in 2012 [12]. In 2014, world aquaculture production of fish accounted for 44.1% of total production from capture fisheries and aquaculture up from 42.1% in 2012 [8] and amounted to 73.8 million tons [13]. In 2015, global aquaculture production reached 106 million tons of live weight growing at an average annual rate of 6.6% since 1995 [14]. In sub Saharan Africa, aquaculture growth during 2001-2015 was averaged at 10.4%. In Nigeria, the last few years has witnessed spectacular growth in aquaculture due to spurred interests of many people in fish farming and this will continue to play an increasingly important role in meeting the demand for fish [15]. Most fish production in Nigeria is realized from pond based, tanks and water re-circulatory culture systems using polyculture farming techniques. However, the bulk of high-value fresh and brackish water finfish in these parts of the world are produced by intensive farming systems using inputs in the form of nutritionally complete formulated diets [16]. According to

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Heindl [17] about 8 million tonnes of fish are produced in China alone with the use of mixed or manufactured feed. While Fagbenro, et al., [18] reported about 0.46 million tonnes of fish was produced in Nigeria by using artificial diet.

One of the prominent species that have been successfully cultured in Nigeria is *C. gariepinus* [19]. It has a high growth rate, omnivorous feeding habit, high feed conversion rate and hardness, including its high resistance to handling and stress [20,21]. It is mostly used as an experimental fish since it possess necessary breathing organs which enables them to tolerate adverse aquatic and environmental conditions where other cultivated fish species cannot survive [22,23]. *C. gariepinus* exhibits considerable growth variation both under artificial culture and in the wild. The causes of such variation are seldomly not clear, although it has been suggested that inherent differences in feeding behaviour may contribute to this variation [24,25].

Amino acids play an important function as building blocks of proteins and are mainly obtained from proteins in diet and the quality of dietary protein is assessed from essential to nonessential amino acid ratio [26,27]. Fish feed is considered good if well accepted by fish, has high digestibility, easily available and is cost effective [28]. Good quality fish feed should contain all nutritional content supplied in their correct proportions [29]. The demand for alternative protein source in fish feed therefore, has become more prominent [30].

The aim of formulating good fish feeds is to attain utmost protein deposition and growth within least inputs of feed at a lowest cost. Experimental fish feeds can either be whole or supplemental. Whole diets provide all the nutrients essential for optimum growth and health of fish when reared in bulk in tanks and ponds. The main cost-effective source of protein for fish is plants and animals. Animal proteins are advanced compared to plant proteins since they have all essential amino acids and are readily digestible but costly. Traditional farmed ingredients have increased the processing of the feeds, hence has influencing success of aquaculture [31]. Supplemental diets are intended to sustain the ordinary food usually accessible to fish in ponds or outdoor cages. In fish farming nutrition is critical because feeds represent 50%-60% of production cost [32].

The development of natural and traditional aqua feeds like, termites, black soldier fly larvae, desert locusts, cockroaches and polychaetes have been used either as fish bait, wild fish food or supplementary diets in animal feeds [31,33] but without much research on their nutritional content. Fish farming management aims at attaining utmost fish biomass within a specific time frame [34]. Competition for protein for fish feeds in aquaculture with human and other livestock needs is a major challenge in the aquaculture industry. The high demand for protein based feeds points to the need for innovative ways of developing quality fish feeds for aquaculture to become tenable [23]. Studies have shown that insects such as termites, desert locusts, cockroaches, black soldier fly larvae (Figure 1) and worms such as polychaetes have high nutritional value in terms of proteins, fatty acids and mineral salts.

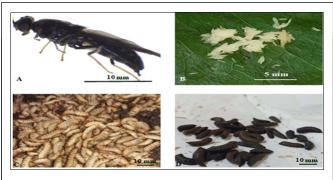


Figure 1: Life stages of black soldier fly *Hermetiaillucens*, A: Adult; B: Eggs; C: Larvae; D: Pupae.

Materials and Methods

Study area

The study was carried out on Lagos State University, (LASU). The LASU Fish Farm situated in Faculty of Science, Lagos State University, Ojo, Lagos State, Nigeria lying between $6^{\circ}26''N$ and $3^{\circ}12'E$.

Collection of fish samples

A total of Three hundred and sixty *C. gariepinus* fingerlings of average body weight 5.95 ± 0.5 , were purchased from Sej. Farm ventures in Badagry, Lagos, Nigeria, transported to LASU fish farm, kept in a concrete tank for acclimatization and starved for 24 hrs to empty their guts in preparation for the experiment.

Experimental design

The experiment was carried out for twelve weeks. The fish were stocked into 18 labelled bowls and stocked with 20 pieces of fingerlings of known weight. The experiment was designed for six isonitrogenous diets, each with three replicates. Each treatment was fed different diet composition. The differences in feeds formulated lied in the percentage composition of fishmeal and black soldier fly. The feed was administered twice daily ad libitumly at 4% body weight, while weight changes were noticed and measured weekly.

Determination of growth performance and nutrient utilization

The weight data recorded were used to calculate Mean Weight Gain (MWG), Percentage Weight Gain (PWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Gross Feed Conversion Ratio (GFCR), Protein Intake (PI), Protein Efficient Ratio (PER) and percentage survivability following the method of Fashina-Bombata, et al.,; Hammed, et al.,; Hammed, et al., [35-37].

Weight Gain (WG)

The Weight Gain (WG) was calculated as: WG (g)=W_f-W_i;

Where W_f is the final fish body average weight (g), W_i is the initial fish body average weight (g).

Percentage Weight Gain (%WG)

The Percentage Weight Gain (%WG) was determined as:

$$\% WG = \frac{W_f - W_i}{W_i} x100 ;$$

Where W_f =final weight of fish, W_i =initial weight of fish.

Specific Growth Rate (SGR)

Specific Growth Rate (SGR) was calculated as;

$$SGR(\% day^{-1}) = \frac{I_n(W_f) - I_n(W_i)}{D} x100$$

where $I_n(W_f)$ is the natural logarithm of final fish body average weight, $I_n(W_i)$ is the natural logarithm of initial fish body average weight and D is the duration of the experiment (days).

Feed Conversion Ratio (FCR)

Feed Conversion Ratio
$$(FCR) = \frac{Total Amount of Feed Consumed(g)}{Weight gain(g)}$$

Gross Food Conversion Efficiency (GFCE)

The Gross Food Conversion Efficiency was calculated according to Stickney, as a percentage of the reciprocal of feed conversion ratio.

$$GFCE = \frac{1}{FCR} x 100$$

Table 1: Diets composition (g).

Protein Intake (PI)

The Protein Intake was calculated according to Garling and Wilson formula:

Protein Intake (g)=Feed intake (g) x %Protein in the diet.

Protein Efficiency Ratio (PER)

Protein Efficiency Ratio
$$(PER) = \frac{Weight \, Gain}{Protein \, Intake}$$

Percentage survivability

%Survivability =
$$\frac{No \ of \ fish \ stocked - Mortality}{No \ of \ fish \ stocked} x100$$

Statistical analysis

Experimental data were presented inform of mean \pm SD, with alpha level (p<0.05) Analysis of Variance (ANOVA). Levels of significance were determined at 5% confidence limit using Least Significant Difference (LSD) and the DNMR test [38].

Results

Table 1 below shows the percentage composition of each feed ingredients used in the diets formulation. The diets varied with increase in the percentage inclusion of BSF *i.e.* Diet¹ (0% BSF and 100% FM), Diet² (20% BSF and 80% FM), Diet³ (40% BSF 60% FM), Diet⁴ (60% BSF and 40% FM), Diet⁵ (80% BSF and 20% FM) and Diet⁶ (100% BSF and 0% FM) respectively. On the other hand, soybean meal, maize, oil, vitamin, CaCO₃ and premix had constant percentage diets' inclusions.

Ingredient	Diets composition					
	Diet ¹	Diet ²	Diet ³	Diet ⁴	Diet ⁵	Diet ⁶
Fishmeal	51.5	41.2	30.9	30.9	10.3	0
BSFM	0	10.3	20.6	20.6	41.2	51.5
SBM	25.2	25.2	25.2	25.2	25.2	25.2

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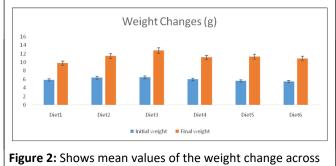
Maize	15.8	15.8	15.8	15.8	15.8	15.8
Vitamin C	1	1	1	1	1	1
Oil	5	5	5	5	5	5
CaCO ₃	0.5	0.5	0.5	0.5	0.5	0.5
Premix	1	1	1	1	1	1
Note: Values in the same row with the same superscript are not significantly different. (p>0.05).						

The cumulative growth data is presented in **Table 2.** The average body weight range between 5.47 ± 1.28 and 6.45 ± 0.26 g **(Table 2 and Figure 2)** between groups of *C. gariepinus* at the

beginning of the experiment and were not significantly different (P>0.05), indicating that groups were randomly divided and homogenous.

 Table 2: Mean cumulative growth rate and nutrient utilization parameters.

	Diet ¹	Diet ²	Diet ³	Diet ⁴	Diet ⁵	Diet ⁶
Initial weight (g)	5.83 ± 0.52 ^a	6.38 ± 0.30 ^a	6.45 ± 0.26 ^a	5.97 ± 0.93 ^a	5.60 ± 1.44ª	5.47 ± 1.28ª
Final weight (g)	9.78 ± 0.46 ^a	11.46 ± 1.82ª	12.73 ± 2.97ª	11.11 ± 4.00ª	11.27 ± 3.41ª	10.87 ± 4.67ª
Weight gain (g)	1.98 ± 1.68ª	2.76 ± 0.39 ^a	3.62 ± 2.71 ^b	2.32 ± 2.13 ^a	3.01 ± 2.17 ^b	2.92 ± 2.12 ^a
%Weight gain	34.55 ± 29.34ª	43.35 ± 34.56 ^a	56.70 ± 42.31 ^b	38.49 ± 35.02 ^a	53.92 ± 38.70 ^b	51.36 ± 37.26 ^b
SGR	0.37 ± 0.60 ^a	0.51 ± 0.57ª	0.72 ± 0.49 ^b	0.42 ± 0.52 ^a	0.71 ± 0.46 ^b	0.64 ± 0.57 ^b
FCR	5.72 ± 2.89 ^a	2.45 ± 1.98ª	1.09 ± 0.60 ^b	4.48 ± 0.02 ^a	1.92 ± 0.38 ^b	2.34 ± 0.21 ^a
GFCR	129.84 ± 112.87ª	187.21 ± 156.96 ^b	266.56 ± 214.18 ^c	158.72 ± 152.32 ^b	213.53 ± 166.28°	210.04 ± 167.40 ^c
PI	21.40 ± 21.29 ^a	21.59 ± 21.03 ^a	21.79 ± 20.58ª	21.38 ± 21.04ª	21.76 ± 20.65 ^a	21.74 ± 20.92 ^a
PER	0.19 ± 0.18ª	0.26 ± 0.23 ^b	0.32 ± 0.28 ^c	0.23 ± 0.23 ^b	0.27 ± 0.24 ^b	0.26 ± 0.23 ^b
%Survivability	92.91 ± 6.38ª	88.42 ± 15.2ª	92.25 ± 6.39 ^a	87.95 ± 14.92ª	91.71 ± 7.39 ^a	90.86 ± 6.16ª



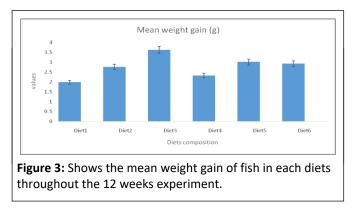
the diets throughout the experiment.

Note: The initial weights ranged from 5.47 \pm 1.28 to 6.45 \pm 0.26. And the final weights ranged from 9.78 \pm 0.46 to 12.73 \pm 2.97. The highest initial and final mean weight was recorded in

Diet³. Meanwhile, the lowest initial and final weights were recorded in Diet⁶ and Diet¹, respectively. However, there was statistically no significant difference in both initial and final weights across the diets.

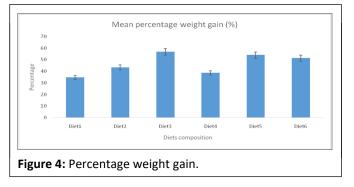
After 12 weeks of commencement of the experiment, the average body weight gain shows significant difference (P<0.05), affected by the increasing dietary levels. The weight gain of fish fed different protein level fell into two groups. The group of fish fed on diet 1, 2, 4 and 6 which contain (0% BSF and 100% FM), (20% BSF and 80% FM), (60% BSF and 40% FM) and (100% BSF and 0% FM) respectively had a significantly (P<0.05) lower body weight than the other treatments. Groups of fish fed on diets 3 and 5 which contain (40% BSF, 60% FM) and (80% BSF and 20% FM) had higher significant values.

After the experimental period, diets 3 and 5 resulted in a significantly (P<0.05) higher body weight **(Table 2 and Figure 3)** than the other treatments followed by diets 6 and 2.



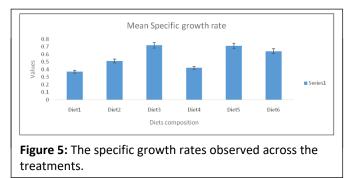
Note: In the first week, there was weight loss in all treatments; this is assumed to be as a result of the unfamiliarization of the fish to the new diets. However, the highest weight gain was recorded in Diet³ (3.62 ± 2.71), which had 40% BSF inclusions, followed by Diet⁵ (3.01 ± 2.17), which had 80% BSF inclusion. While the lowest weight gain was recorded in Diet¹ (1.98 ± 1.68), with 0% BSF inclusion.

The groups fed on diets 1 and 4 had a significantly (P<0.05) lower body weight. The data of weight as percent of the initial weight of fish are also presented in **Table 2 and Figure 4**.



Note: The percentage weight gain observed is shown in the above with varied diet composition. The values of the percentage weight gain observed in this study ranged from 56.70 \pm 42.31-34.55 \pm 29.34. Consequently, the highest percentage weight gain was recorded in Diet³, Diet⁵ and Diet⁶, 56.70 \pm 42.31, 53.92 \pm 38.70 and 34.55 \pm 29.34 respectively.

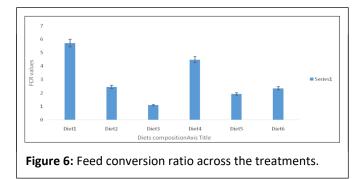
The differences in the final body weight were reflected by difference in the Specific Growth Rate (SGR) which is presented in **Table 2 and Figure 5**.



Note: The mean values of specific growth rate recorded in all the diets ranged from 0.37 \pm 0.60, 0.72 \pm 0.49. Diet³ and Diet⁵ were observed to have the highest specific growth rates, 0.72 \pm 0.49 and 0.71 \pm 0.46 respectively. On the other hand, Diet¹ had the lowest specific growth rate, 0.37 \pm 0.60.

At the end of the experiment, the specific growth rate was significantly different (P<0.05) in diets (1, 2 and 4) and diets (3, 5 and 6). The differences in the final body weight were reflected by difference in the specific growth rate.

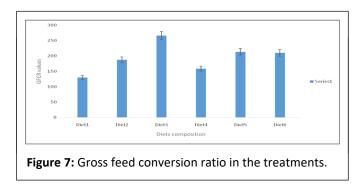
The group of fish fed on diet³ had a significant (P<0.05) better FCR **(Table 2 and Figure 6)** with value of 1:1 (1.09 \pm 0.60) followed by diet⁵ with FCR of 1.92 \pm 0.38 respectively.



Note: Figure 6 expressed feed conversion ratio across the treatments. Diet¹ was observed to have the highest feed conversion ratio, 5.72 ± 2.89 . Diet³, in which the highest weight gain was recorded, had the lowest FCR, 1.09 ± 0.60 , which can considered the best FCR value in this study. However, there was no statistical difference between Diet³ and Diet⁶.

The group of fish fed diets 1, 2, 4 and 6 however had the lowest level of feed efficiency with FCR values of 5.72 \pm 2.89, 2.45 \pm 1.98, 4.48 \pm 0.02 and 2.34 \pm 0.21 respectively.

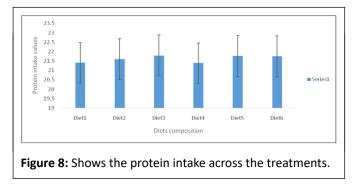
Table 2 and Figure 7 shows GFCR with significant difference (P<0.05) observed between diets (1), (2 and 4) and (3 and 5) respectively.



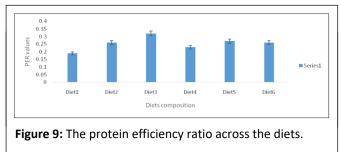
Note: Diet³ had the highest gross feed conversion ratio, followed by Diet⁵, 266.56 \pm 214.18 and 213.53 \pm 166.28, respectively. Again, Diet¹ had the least GFCR, 129.84 \pm 112.87.

No significant difference (P>0.05) was observed between fish fed diets (2 and 4) as well as diets (3 and 5) respectively when the means were compared.

The highest mean protein intake **(Table 2, Figure 8)** was observed in fish fed diet³, though no significant difference was observed across the six diets.

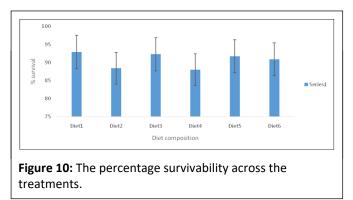


Protein efficiency ratio was calculated for each group and is presented in **Table 2 and Figure 9.**



The PER values were 0.19 ± 0.18 , 0.26 ± 0.23 , 0.32 ± 0.28 , 0.23 ± 0.23 , 0.27 ± 0.24 and 0.26 ± 0.23 respectively. No significant difference (P>0.05) was observed in fish fed diets 2, 3, 4 and 6. Statistical analysis revealed that fish fed diet¹ (0.19 \pm 0.18) had a significantly (P<0.05) lower PER value while diet³ (0.32 \pm 0.28) had a higher PER values than groups 2, 3, 4 and 6 respectively.

The percentage survivability across the treatments are expressed in **Table 2 and Figure 10**.



However, Diet¹ (92.91 \pm 6.38), with the least weight gain, appeared to have the highest percentage survivability, followed by Diet³ (92.25 \pm 6.39), respectively. Though, there was no significant difference in percentage survivability across the diets, except for diet⁴, in which the lowest (87.95 \pm 14.92) percentage survivability was recorded.

Discussion

The global production of fed fish tripled between 2000 and 2017 [39] while the annual catch of forage fish used to make

fishmeal and fish oil decreased from 23 Mt 16 Mt [40]. In fish culture, feeding constitutes more than 50% of the operating costs, with protein being the most expensive dietary source. The majority of aqua-feed formulations are based mainly on fishmeal as the dietary protein source. The availability of fishmeal in the future to sustain the rapid expansion of aquaculture and aqua-feed production is questionable [41,42]. The formulations differences between FM and BSFL affected the level of BSFL, which may be incorporated into the diet. With over 40% share of BSFL, the potential environmental sustainability of the diet, as well as the extrusion process and feed properties may be influenced impaired. Different inclusion levels of BSFL have shown promising results in a variety of aquaculture species; where up to 100 % replacement for fishmeal is possible without serious adverse effects [33]. However, in many cases, when levels of inclusion are above 50%, negative effects on growth parameters could be seen. Privadarshana, et al., [43] reported that this is probably due to high chitin content, high crude fat content and inability of fish species and growth stage to utilise insect-based diets. The body composition of BSFL fed fish, in many cases, seem to be positively affected. Nutritional analysis on the use of BSFL for C. gariepinus is a novelty. There was a study describing the application of insect species in the diet of this aquatic faunas species [44,45]. The feed acceptability suggests that insectderived materials may be strong attractants for aquatic animals [46]. The gross composition of the experimental diets indicated that Diet¹ had no BSFL inclusion and fishmeal was completely replaced with BSF in Diet⁶, while Diet², Diet³, Diet⁴ and Diet⁵ had 20%, 40%, 60% and 80% BSFL inclusions. Fish fed Diet³, which had 40% BSFL inclusion, had higher weight gain than other levels of inclusion, while Diet¹ with 0% inclusion, had the lowest weight gain. However, this result was different from the report of Talamuk, et al., [47], who reported highest and lowest weight gain at 10% and 30%, respectively. This may be due to the mode of percentage inclusion or culture system. On the other hand, the result was similar to that of the study of Muin, et al., and Rana, et al., [48,49] who incorporated 25%, 50%, 75% and 100% BSFL meal in Oreochromis niloticus fingerlings diets. Highest values of WG and PER were reported at 50% inclusion rate. Fish fed with 50% BSFL meal showed the highest SGR and best FCR values. Figure 4 indicated the percentage weight gain of fish fed Diet³, Diet⁵, and Diet⁶, were significantly different from other treatments. The lowest percentage weight gain was observed in Diet¹. Meanwhile, statistically, there was no significant difference between the specific growth rate of Diet³, Diet⁵, and Diet⁶, as they were observed to have the highest specific growth rate, 0.72 ± 0.49, 0.71 ± 0.46, and 0.64 ± 0.57, respectively. Similar result was observed in FCR and GFCR. Diet³ and Diet⁵ had the best FCR while the highest value of FCR was observed in Diet¹. Similarly, the same study carried out by Muin, et al., (2017) on incorporation of BSF in the diet of Oreochromis niloticus, ideal value of FCR was observed in Diet³, which had 50% BSF inclusion. Consequently, Diet³ also had the highest gross feed conversion ratio.

The protein and amino profile of black soldier fly is similar to that of fish meal [50]. This study revealed that feed intake was remarkably reduced at the beginning and gradually increased with time. Statistically, there was no significant difference in protein intake across the treatments. However, Diet3 had the highest protein intake value, 21.79 ± 20.58. Conversely, the protein efficiency ratio varied across treatments. And the PER in Diet³ was observed to be significantly different from other treatments. Percentage survivability, as expressed in Figure 10, appeared to be highest in Diet¹ (92.91 \pm 6.38) with 0% BSF inclusion, whereas Diet⁴, with 60% BSF inclusion, had the least percentage survivability, 87.95 ± 14.92. In the present research, there were no significant differences among treatments in terms of feed intake, which reveals that all of the experimental feeds could be provided to fish as whole daily rations without consumption delay or unacceptability. However, all other growth performance and feed utilization parameters, including BW, BWG, SGR, PWG, FCR and PER an improvement was observed as a consequence of increasing BSFL incorporation into the diets. All the aforementioned findings are in agreement with the results of Mateusz, et al., [51].

Conclusion

This study revealed that growth and nutrient utilization varies ¹¹. with levels of black soldier fly can be utilized as a potential alternative to fishmeal in the diets of African catfish, *C*. ¹². *gariepinus*. Fishmeal can be completely replaced with black soldier fly in the diet of African catfish, without adverse effect on 13. growth. About 40% replacement of fishmeal with black soldier fly is the recommendable level of incorporation of black soldier fly in the diet of African catfish.

Authorship Contribution Statement

Ayofe M Hammed and Albert O Amosu: Conceptualization, methodology, software, validation, writing-original draft. Lucas O Dalmeida and Theophilus F Sunnuvu: Formal analysis, investigation, writing-review and editing, project 17. administration. Olufemi O Joseph and Oluwajuwon A Fasasi: Methodology, resources, supervision, review and editing. 18.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or conflict of interest.

Acknowledgement

We want to show appreciation to the following: Department of fisheries, faculty of science, Lagos State University; Department of Agricultural Science, fisheries unit of the Department of Agricultural Science, Lagos State University of Education and Sej. Farm Ventures in Badagry area of Lagos, Nigeria.

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