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Research Article

Growth performance and survival rates of Nile tilapia (*Oreochromis niloticus* L.) reared on diets containing Black soldier fly (*Hermetia illucens* L.) larvae meal

Wachstumsleistung und Überlebensrate von Nil-Tilapia (*Oreochromis niloticus* L.) bei der Fütterung von Rationen mit Larvenmehl der schwarzen Soldatenfliege (*Hermetia illucens* L.)

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Summary

A 72-day feeding trial was conducted at Sagana fish farm to assess the effects of substitution of fish meal (FM) with black soldier fly larvae meal (BSFLM) on the growth performance and survival rates of Nile tilapia. Four test diets were formulated and substitution of FM by BSFLM made at 0%, 33%, 67% and 100%. 240 male Nile tilapia (52.3 ± 0.29 g) were divided into 4 groups (4 replicates) each and were placed in 16 hapa nets (15 fish/hapa), mounted in an 800 m^2 earthen pond. Fish were fed twice per day at 5% of the body weight. Sampling of the fish was done biweekly; mortalities were recorded daily while the physico-chemical parameters were monitored weekly. The growth performance and survival rates of the Nile tilapia were not significantly different ($p > 0.05$) between treatments. In conclusion, the present study indicates that full fat BSFLM can replace up to 100% of the FM without negative effects on the growth performance and survival rates of Nile tilapia, although the slight trend towards reduced growth for the 100% substitution diet points at the need for future studies on increasing the nutritional value of BSFLM, for example, by further processing.

Keywords: Fish meal, insect meal, alternative protein, defatting, nutrient

Zusammenfassung

Es wurde ein 72-tägiger Fütterungsversuch durchgeführt, um die Auswirkungen der Substitution von Fischmehl (FM) durch Larvenmehl der Schwarzen Soldatenfliege (*Hermetia illucens* L.; BSFLM) auf die Wachstumsleistung und die Überlebensraten von Tilapia (*Oreochromis niloticus* L.) zu erheben. Es wurden vier Testrationen formuliert bei welchen FM durch BSFLM in Raten von 0% (Kontrolle), 33%, 67% und 100% substituiert wurde. 240 männliche Tilapia (52,3 ± 0,29 g) wurden auf 4 Gruppen (jeweils 4 Wiederholungen) verteilt: 16 Hapa-Netze (15 Fische/Hapa) wurden in einem 800 m² großen Teich eingesetzt. Die Fische wurden zweimal täglich mit einer Menge von 5% der Lebendmasse gefüttert. Die Wiegung der Fische erfolgte alle zwei Wochen; die Mortalität wurde täglich aufgezeichnet, die physikalisch-chemischen Wasser-Parameter wurden wöchentlich kontrolliert. Die Wachstumsleistung und die Überlebensraten der Tilapia unterschieden sich zwischen den Behandlungen nicht signifikant ($p > 0,05$). Zusammenfassend zeigt die vorliegende Studie, dass vollfettes BSFLM bis zu 100% des FM ersetzen kann, ohne negative Auswirkungen auf die Wachstumsleistung und die Überlebensraten von Tilapia zu haben. Der Trend zu einem verringerten Wachstum bei der Ration mit 100% Substitution deutet auf die Notwendigkeit weiterer Studien zur Steigerung des Nährwerts von BSFLM, z.B. durch Weiterverarbeitung, hin.

Schlagwörter: Fischmehl, Insektenmehl, alternative Proteinquelle, entfetten, Nährstoffe

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1. Introduction

The global human population is on the rise, and projections have shown that by 2050, approximately 9.7 billion people will be inhabiting the globe (Béné et al., 2015; Shumo et al., 2019). This increase in population is directly impacting pressure on the global food basket. To meet the food demands of the increasing population, 70% more food needs to be produced globally (FAO, 2009; Schiavone et al., 2017; Shumo et al., 2019). Both capture fisheries and aquaculture sectors have been geared into providing fish and other aquatic products to the increasing population (Tidwell and Allan, 2001). However, the capture fisheries have been over exploited, leading to massive reduction in the fish yields. FAO (2018) and Barroso et al. (2014) reported a decline in the capture fisheries yields from 81.2 million tons in 2015 to 79.3 million tons in 2016. The main reason attributed to this decline was the El Niño effects.

With these declines, more focus is being put into aquaculture to increase the yields and hence bridge the gap. Currently, aquaculture is one of the fastest growing food sectors that is focused on providing quality, affordable and reliable protein sources to improve the livelihoods, curb malnutrition and reduce food insecurity (FAO, 2010; Béné et al., 2015). However, the industry's growth is slow, and this has been put into close context with the industry's high dependency on high quality protein feeds (Opiyo et al., 2018). Fish feeds contribute to over 60% of the total operational costs in a fish farm (Munguti et al., 2012) in which protein is the key nutrient needed in the feeds, and probably the most expensive one (El-Sayed, 1999; Munguti et al., 2012). Fish meal (FM) has been an important component in fish diets due to its high digestibility and acceptance alongside its high protein, essential amino acids and fatty acids profiles (Tacon and Metian, 2009). Recent studies have shown that fish meal stocks particularly in Kenya are declining due to over-exploitation alongside the seasonal closures of the Lake Victoria's Omena (*Rastrineobola argentea*) fishery (Munguti et al., 2009; Rana et al., 2009). This scarcity is leading to increased prices of the product, hence increasing the fish production costs and further deterring the industry's growth (Ayoola, 2010; Barroso et al., 2014; Muin et al., 2017). There is an urgent need to find a high quality, cost efficient and sustainable alternative protein source for FM (Schiavone et al., 2017) to be used in the formulation for Nile tilapia (*Oreochromis niloticus*) diets. Successful replacement of FM with black soldier fly larvae meal in the diets of tilapia without any negative effects on the growth performance and survival rates has been reported in previous studies (Cummins et al., 2017; Devic et al., 2018). More recently, researchers have documented black soldier fly as a viable and sustainable source of protein in tilapia feeds due to the larvae high quality protein, ability to utilize a wide range of cheap organic wastes alongside the low cost of production of the larvae. Nile tilapia is the most preferred culture species in the tropical and subtropical regions in the world (El-Sayed, 1999), accounting for about 75% of production in Kenya (Munguti et al., 2014). This is due to its fast growth, prolific breeding, resistance to diseases and ability to grow in a wide range of culture systems (Munguti, 2007). This study was motivated by the fact that the black soldier fly has an ability to transform low quality organic wastes into high quality protein that can be utilized in the production of feeds for *O. niloticus*, allowing for the aquaculture production to remain economically and environmentally viable (Ayoola, 2010). Hence, a feeding trial was conducted to evaluate the growth performance and survival rates of Nile tilapia when fed diets with gradually reduced proportions of FM, which were made up by increasing proportions of black soldier fly larvae meal. We hypothesized that locally produced black soldier fly larvae meal can basically substitute fish meal in diets for Nile tilapia; due to differences in protein content and essential amino acids, growth and survival rates of the *O. niloticus* could be negatively affected at higher inclusion rates of BSFLM.

2. Materials and methods

2.1. Ingredient collection, proximate analysis, diet formulation

The black soldier fly (*Hermetia illucens*) larvae, Fish meal (Omena, *Rastrineobola argentea*), Freshwater shrimp (*Macrobrachium rosenbergii*), cottonseed (*Gossypium spp*) cake, sunflower seed (*Helianthus annuus*) cake and maize (*Zea mays*) germ were sourced around local animal feed outlets in Thika, Kiambu county, Kenya, depending on the availability and cost effectiveness. Proximate composition of the individual ingredients and test diets was analyzed for dry matter (DM), crude protein (CP), crude fiber (CF), ether extracts (EE), and ash content. It was done in triplicate following the procedure by AOAC (1995) and presented as shown in Table 1 and 4, respectively. Nitrogen Free Extracts (NfE) were estimated by the difference method (DM-CP-EE-CF-Ash). Amino acids (AA) were analyzed

Table 1. Proximate composition of the analysed ingredients used in diet formulation (as fed basis)
Tabelle 1. Rohnährstoff- und Energie-Gehalt der Futterkomponenten (Angaben auf Basis Lufttrockenmasse)
DM = Dry matter, NfE = Nitrogen Free Extracts, ME = Metabolizable energy.

Parameter	Unit	FWS[a]	FM[b]	BSFLM[c]	Cottonseed cake	Sunflower cake	Maize germ
DM	g/kg	906	937	958	905	920	894
Crude protein	g/kg	666	621	253	273	423	322
Crude fibre	g/kg	80	59	80	287	179	95
Ether extracts	g/kg	96	124	273	86	99	114
Ash	g/kg	287	300	147	53	53	32
NfE	g/kg	-	-	205	205	166	331
ME	MJ/kg	13.6	13.8	16.2	10.2	12.4	13.8

for FM and BSFLM, using high performance liquid chromatography according to EU standard methods (Commission regulation (EC) No 152/, 2009). The AA composition of FM and BSFLM is shown in Table 2.

2.2. Diet formulation and preparation

Four test diets were formulated by substituting FM protein with BSFLM at 0, 9.8, 19.5 and 29.3% of BSFLM, representing substitution rates of 0, 33, 67 and 100%, respectively. Starch was used as a filler material to top formulations to 100%. The formulation of the complete diet involved thoroughly mixing of the ingredients in the proportions given in Table 4. To attain a consistency for pelleting and make a soft dough of the powdered mixture, tap water was added. To obtain a homogenous diet, the feeds were minced several times, and pellets were made using a pelletizer. The pellets were sun-dried and stored at room temperature. The composition of test diets and their proximate nutrient content are shown in Table 4.

2.3. Feeding trial

The feeding trial was conducted from October 2019 to January 2020 at Sagana fish farm (0°19'S and 37°12'E) of the Kenya Marine and Fisheries Research Institute (KMFRI). Male Nile tilapia fish were sourced from KMFRI Sagana Centre. The fish were acclimatized for 1 week in a hapa net

Table 2. Analysed Amino acid composition (g/kg feed; g/kg protein) of fish meal and black soldier fly larvae meal
Tabelle 2. Aminosäurezusammensetzung (g/kg Futter; g/kg Protein) von Fischmehl und Larvenmehl der Schwarzen Soldatenfliege

Essential amino acids	FM[a]		BSFLM[b]	
	g/kg feed	g/kg protein	g/kg feed	g/kg protein
Histidine	13.6	21.8	6.5	25.7
Threonine	22.8	36.7	11.1	44.0
Arginine	33.4	53.8	13.7	54.2
Valine	28.8	46.4	16.3	64.4
Isoleucine	25.4	40.8	12.0	47.5
Phenylanine	23.8	38.3	11.7	46.2
Leucine	44.1	71.1	19.1	75.6
Lysine	42.6	68.6	16.7	66.2
Methionine	16.3	26.2	4.5	17.6
Tryptophan	5.5	8.8	3.4	13.5
Non-essential amino acids				
Alanine	41.5	66.8	18.7	73.9
Tyrosine	18.8	30.2	18.0	71.3
Glycine	37.6	60.5	13.9	55.1
Asparagine	51.5	83	25.8	101.8
Glutamate	92.6	149.1	34.5	136.2
Serine	20.1	32.4	11.8	46.8
Cysteine	3.5	5.6	2.5	9.8

[a]Fish meal - Omena (*Rastrineobola argentea*), [b]Black soldier fly larvae meal.

Table 3. Calculated essential amino acid (EAA) content (g/kg feed) of the test diets used for feeding Nile tilapia in the experimental ponds for 72 days. [a]D1 = Diet 1, [b]D2 = Diet 2, [c]D3 = Diet 3, [d]D4 = Diet 4.
Tabelle 3. Errechneter Gehalt an essentiellen Aminosäuren in den Rationen (g/kg Lufttrockenmasse), die über 72 Tage an Tilapia verfüttert wurden. [a]D1 = Ration 1, [b]D2 = Ration 2, [c]D3 = Ration 3, [d]D4 = Ration 4.

	Test Diets			
Essential Amino acids	**D1[a] (0%)**	**D2[b] (33%)**	**D3[c] (67%)**	**D4[d] (100%)**
Histidine	9.4	8.6	8.4	6.9
Threonine	16.5	15.4	14.9	13.2
Arginine	30.8	29.0	28.4	25.2
Valine	19.6	18.2	17.7	15.1
Isoleucine	16.3	15.1	14.5	12.7
Phenylanine	18.3	17.1	16.7	14.6
Leucine	29.2	27.0	26.0	22.1
Lysine	25.9	24.1	22.9	20.3
Methionine	10.5	9.7	9.2	8.1
Tryptophan	3.9	3.6	3.5	3.1

(4 × 3 m) that was mounted on an earthen pond, during which they were fed with commercial floating feeds. Thereafter, 240 male *O. niloticus* (52.3 ± 0.29 g, mean ± SE) were sorted from the acclimatized fish and were divided into 4 groups (treatments) with 4 replicates each and were distributed into 16 hapa nets (2 × 2 × 1 m) mounted in an 800 m^2 earthen pond with 15 fish per hapa. The four test diets (D1, D2, D3 and D4) were randomly assigned to the hapa nets by using a table of random numbers. Cover nets were placed over each hapa net to control predators. Fish were hand-fed twice a day at 10:00 and 16:00 hrs at 5% of the wet body weight. The quantity of feed was adjusted biweekly throughout the 72-day trial period. The fish were monitored daily for mortality. Dead fish were removed, counted and recorded. Sampling of the fish was done after every two weeks to monitor the growth, survival, feed efficiency and in addition to adjust the amount of feed to be offered to the fish. Physico-chemical water quality parameters (dissolved oxygen, water temperature, pH, conductivity, salinity and total dissolved solids) were monitored weekly at 10:00 hrs using multiparameter water quality meter (YSI industries, Yellow springs, OH, USA), while water samples were collected for analysis of ammonia, nitrites, nitrates and phosphorus in the laboratory following the procedures by APHA (1995).

2.4. Evaluation of dietary performance

To evaluate the growth and feed efficiency, the following standard formulas were used:

Body weight gain (BWG, g) = final weight (g) - initial weight (g).
Specific growth rate (SGR, %) = 100 × [(ln BW final (g) - ln BW initial (g)) / days of culture].
Feed conversion ratio (FCR) = feed provided/live weight gain (g).
Protein efficiency ratio (PER) = live weight gain (g)/total protein intake (g).
Survival rate (SR, %) = 100 × (final number of fish)/(initial number of fish).

2.5. Statistical analysis

The collected data was subjected to the Shapiro-Wilk test of normality followed by One-way ANOVA that tested differences in the growth response and survival rates of the stocked fish fed on different diets. To determine the pairwise differences among the diets, the Tukey-HSD post hoc test was employed. All the statistical analyses were performed using MS Excel and SPSS statistics (version 21). Results were interpreted to be significant at $p \leq 0.05$.

3. Results

3.1. Water quality

The physico-chemical parameters analyzed are presented in Table 5. The physico-chemical parameters showed significant differences ($p < 0.05$) throughout the culture period.

Table 4. Composition and results from proximate analysis of test diets fed to Nile tilapia for 72 days containing different levels of black soldier fly larvae meal (BSFLM) as a replacement for fish meal (FM)
Tabelle 4. Zusammensetzung und Rohnährstoff-Gehalt von Rationen, die über 72 Tage an Tilapia verfüttert wurden und unterschiedliche Anteile an Larvenmehl der Schwarzen Soldatenfliege (BSFLM) als Ersatz für Fischmehl (FM) enthielten

Ingredient (g/kg)	**Test diets**			
	D1[a] (0%)	**D2[b] (33%)**	**D3[c] (67%)**	**D4[d] (100%)**
Freshwater shrimp	300	300	300	315
Fish meal	220	148	74	0
Black soldier fly larvae meal	0	98	195	293
Cotton seed cake	200	200	210	200
Sunflower seed cake	40	40	40	40
Maize germ	177	154	167	73
Starch	10	10	0	50
Fish oil	52	49	13	28
Premix	1	1	1	1
Proximate composition (g/kg)				
DM	915	911	912	927
Crude protein	309	313	303	303
Ether extracts	140	116	115	153
Crude fiber	98	98	81	164
Ash	143	113	93	255
NfE	225	271	320	52
ME (MJ/kg)	11.5	12.2	12.2	11.0

DM = Dry matter, NfE = Nitrogen free Extracts, ME = Metabolizable energy.
aD1 = Diet 1, bD2 = Diet 2, cD3 = Diet 3, dD4 = Diet 4.
**Metabolizable energy (ME) calculated according to Pauzenga (1985): (ME = 37 × % CP + 81 × % EE + 35.5 × % NfE)/ 0.0041868

3.2. Proximate analysis

The two protein sources of interest, FM and BSFLM, had different nutritional composition (Table 1). BSFLM showed higher contents of EE, crude fiber, NfE, DM and ME in comparison to FM, whereas FM had higher contents of ash and crude protein than BSFLM. BSFLM contained lower concentrations (g/kg feed) of amino acids (Table 2) than those of FM. On the other hand, concentrations of essential amino acids (Table 2) in g/kg protein were higher in BSFLM than in fish meal with an exception for lysine and methionine.

3.3. Growth parameters and feed utilization

Data on fish growth performance and survival rates are presented in Table 6. Mortality was very low and was only experienced in the early stages of the experiment.
No significant differences ($p > 0.05$) were found in the survival rates (SR). Further, the growth performance (BWG and SGR) and feed utilization efficiency (FCR and PER) were not compromised by the dietary treatments ($p > 0.05$). During the 72-day experimental period, the fish grew from an average initial weight of 52.3 ± 0.29 g to an average final weight of 119.1 ± 2.89 g. Growth trend curves for *O. niloticus* in hapas are presented in Figure 1.
Growth trend curves displayed similarities and overlaps between the treatments from week 1 all through to week 7. Nevertheless, by the 9th week, there was a separation of the curves between the diets till the end of the experimental period. By the end of the experiment, the growth curves for diet 2 indicated the highest weight followed by diet 1 and D3, while D4 (100% BSFLM inclusion) resulted in the lowest, though not significant, weight.

4. Discussion

4.1. Nutrient content of black soldier fly larvae meal

FM and BSFLM (Table 1) used in the present study had a different nutritional composition. The BSFLM contained more EE, crude fiber (CF), NfE and metabolizable energy

Table 5: Mean, minimum and maximum values of physico-chemical parameters of the water during the experimental period
Tabelle 5: Physikalisch-chemische Wasserparameter während des Versuchszeitraums (Mittelwert, Minima, Maxima)
DO = Dissolved oxygen, TDS = Total dissolved solids, PO_4 = Phosphates, NO_2 = Nitrites, NO_3 = Nitrates, NH_4 = Ammonium. Values represent mean ± SD, n = 3.

Parameter	Unit	Mean	Minimum	Maximum
Temperature	°C	25.8 ± 1.1	23.5	27.8
DO	mg/L	6.8 ± 0.5	5.9	8.2
Conductivity	µS/cm	71.6 ± 16.9	51.1	102.6
TDS	mg/L	45.8 ± 9.8	33.8	63.1
Salinity	mg/L	0.03 ± 0.01	0.02	0.05
pH			7.4	9
PO_4	mg/L	0.002 ± 0.0004	0.002	0.003
NO_2	mg/L	0.001 ± 0.0002	0	0.001
NO_3	mg/L	0.001 ± 0.0002	0.001	0.002
NH_4	mg/L	0.01 ± 0.001	0.01	0.01

(ME) than FM, whereas FM showed a higher crude protein and ash content than BSFLM. FM had a negative value for NfE, and this may have been due to the fact that FM contains extremely low amounts of non-fibrous carbohydrates (Landau, 1992). The nutrient contents of FM used in the present study were similar to those reported by Cummins et al. (2017). The crude protein percentage of the BSFLM was similar to that obtained by Tschirner and Simon (2015), but lower than reported by Kroeckel et al. (2012) and Muin et al. (2017), who obtained levels exceeding 30%. On the other hand, EE of the BSFLM in the present study was similar to that reported by Devic et al. (2018) and Toriz-Roldan et al. (2019), but lower than that reported by St-Hilaire et al. (2017). BSFLM in the present study had lower contents of AA (g/kg feed) as compared to FM. However, when the amount of essential AA

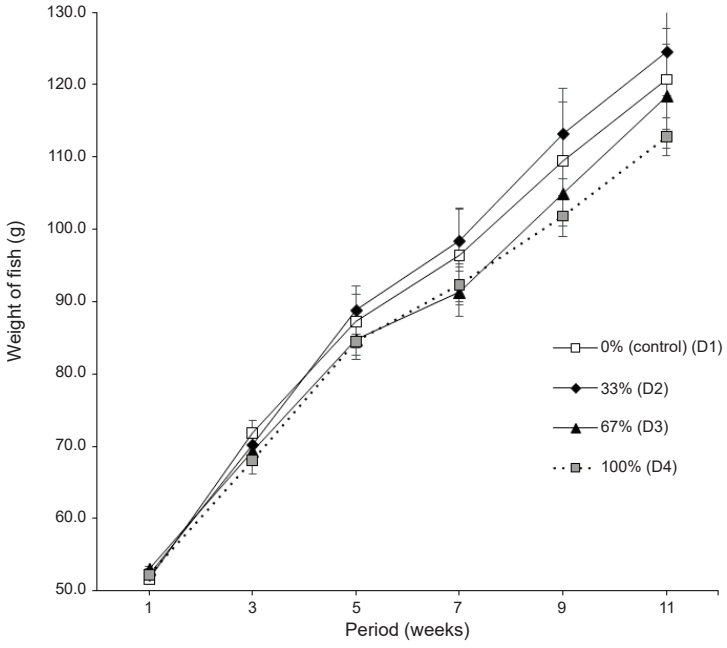


Figure 1. Growth curves for *O. niloticus* fed diets with varying levels of BSFLM during a 72-day culture period.
Abbildung 1. Lebendmasse-Entwicklung von *O. niloticus*, die Futter mit unterschiedlichem BSFLM-Anteil über 72 Tage erhielten.

Parameter	Diet 1 (0%)	Diet 2 (33%)	Diet 3 (67%)	Diet 4 (100%)	P-value
Initial BW (g)	51.6 ± 0.53	52.2 ± 0.60	53.0 ± 0.42	52.2 ± 0.74	0.445
Final BW (g)	120.7 ± 6.98	124.5 ± 6.00	118.4 ± 7.16	112.8 ± 2.62	0.580
BWG (g)	69.1 ± 7.33	72.3 ± 6.36	65.4 ± 7.45	60.5 ± 2.11	0.587
SR (%)	96.7 ± 1.92	100.0 ± 0.00	100.0 ± 0.00	98.3 ± 1.67	0.248
SGR	1.2 ± 0.09	1.2 ± 0.08	1.1 ± 0.09	1.1 ± 0.02	0.597
FCR	1.0 ± 0.06	1.0 ± 0.06	1.0 ± 0.07	1.1 ± 0.01	0.647
PER	3.3 ± 0.20	3.4 ± 0.18	3.3 ± 0.25	3.1 ± 0.03	0.826

BW - Body weight; BWG - Body weight gain; SGR - Specific growth rate, SR - Survival rate; FCR - Feed conversion ratio; PER - Protein efficiency ratio. Diets represent: Diet 1- control (without black soldier fly larvae meal inclusion), Diet 2 (33% substitution rate), Diet 3 (67% substitution rate) and Diet 4 (100% substitution rate, i.e., maximum BSFLM inclusion).

in the protein (g/kg crude protein) was computed, values for BSFLM were found to be higher than for FM with an exception of lysine and methionine. This is an indication that the nutritional value of the BSFLM (in terms of AA profile of the protein) may have been slightly higher than that of FM. A study done by Keembiyehetty and Gatlin (1992) showed improved survival and growth rates in fish fed on diets rich in dietary lysine. The calculated values of EAA (Table 3) were similar between the diets and met the suggested dietary AA contents for growth of Nile tilapia (Santiago and Lovell, 1988). Henry et al. (2015) argued that the variability in the nutrient contents of BSFLM can be attributed to factors such as type of substrate used to rear the larvae, stage of harvesting, methods of processing and duration of drying. Defatting of the larvae has been argued to lead to an increase in CP and reduction in EE (Castell, 1986; Renna et al., 2017; Dumas et al., 2018). Defatting of the larvae resulted in the increase of CP and AA and a decrease in EE contents as compared to the present full fat larvae (Kroeckel et al., 2012). Further, when 3 types of substrates were used to rear the larvae (Shumo et al., 2019), different nutritional contents were realized whereby the larvae that was reared on kitchen waste yielded higher EE contents in comparison to those reared on brewers' waste and chicken manure. The 4 test diets (Table 4) met the nutritional requirements for tilapia commercial feeds (Munguti et al., 2014) except for diet 4 that exhibited unexplainably higher values of CF and ash than the recommended values.

4.2. Growth parameters and feed utilization

All the fish appeared healthy and with no disease outbreaks throughout the 72-day trial period. All the measured water quality parameters (Table 5) were within optimal ranges for Nile tilapia growth and health (Popma and Masser, 1988). The mortalities were not diet-related but may have been due to the stress caused during fish handling at the time of stocking. Further, all the diets were well accepted by the fish, paralleling reports by Adewolu et al. (2010). There was no significant difference ($p > 0.05$) in the SR percentage in the present study between all the test diets (Table 6) which ranged from 96.7% to 100%. The present study mean SR was higher than that reported by other authors (Liu et al., 2012; Ye et al., 2012; Bulbul et al., 2013; Cummins et al., 2017) under clear water culture systems, but was similar to studies done by Ye et al. (2011) under green water culture systems, where the fish may have had continuous access to supplemental nutrients from the natural food web, hence increasing the chances of survival. Additionally, feed acceptance by the fish and good water quality parameters, that were well within the recommended ranges (Popma and Masser, 1988) may have further contributed to the high mean SR. No significant differences ($p > 0.05$) between the test diets were found for BWG and SGR (Table 6) in the present study. These results are in agreement with those of Toriz-Roldan et al. (2019) and Devic et al. (2018), when *O. niloticus* were reared on diets containing varying proportions of BSFLM. The results of the present study suggest that it is possible to substitute up to 100% fish meal with black soldier fly larvae meal in diets for Nile tilapia without distinct negative effects on growth performance, particularly in feed formulations where the amino acid is fortified by other protein sources, such as freshwater shrimp meal, which has a high content of CP (Munguti et al., 2009) as seen in this study. However, it is important to note that although not significant, the growth performance showed a slight trend towards reduced growth of fish that were fed the diet without any fish meal, further suggesting that freshwater shrimp meal does not completely alleviate the substitution effect. An increased number of replicates in the trial would probably have led

to differences in the growth parameters. Continuation of the experiment to fish of market weight (approximately 500 g) would also be expected to result in differences in the growth performance of the fish. With all the factors constant, the fish would record a probably higher BWG, but lower SGR were the fish reared up to market size. On the other hand, Muin et al. (2017) reported significant differences in the growth parameters; substitution of FM by BSFLM of > 50% led to a decrease in the BWG and SGR of *O. niloticus*. The differences between the results of this experiment and those from previous studies may be at least partially attributed to the culture conditions coupled with differences in the availability of nutrients in the diets. The previous study (Muin et al., 2017) was conducted in plastic tanks, while the present study was conducted in an earthen pond where access to natural food can be expected to have improved the nutrient and particularly amino acid supply of fish (Munguti et al., 2009). No significant differences ($p > 0.05$) were found in the FCR between the test diets containing varying proportions of BSFLM (Table 6). This may be seen as an indication that BSFLM can replace FM up to 100% without any obvious negative effect on growth parameters. The FCR found in this experiment, appears to be extremely low (El-Saidy and Gaber, 2005; Omasaki et al., 2017; Mengistu et al., 2020). The reason for this cannot be clearly defined, but the values for dissolved oxygen and water temperature found in this experiment have probably contributed to the low FCR (Mengistu et al., 2020). Additionally, the access to natural food can be expected to have improved the nutrient and particularly amino acid supply of fish (Munguti et al., 2009), further contributing to the low FCR. Similar to the present study, no significant differences were reported for FCR when Muin et al. (2017), Devic et al. (2018) and Toriz-Roldan et al. (2019) replaced FM with BSFLM in the diets of *O. niloticus*. However, significant differences were reported by Adewolu et al. (2010) and Kroeckel et al. (2012) in the FCR between the dietary treatments in their studies. The authors of the previous study speculate that chitin may have been the dietary factor behind the depressed and significant differences in the FCR among the test diets since it has been argued to inhibit the absorption of nutrients from the intestinal tract and lead to reduced feed utilization efficiencies (Razdan and Pettersson, 1994). The chitin content in insect-derived feed components will however strongly depend on the stage at which the insects are harvested (Van Huis, 2013); as the BSFLM used herein were harvested at a stage in which the exoskeleton was not developed, chitin uptake should not have played an important role in this experiment. Additionally, defatting of the larvae that has been argued to increase nutrient availability, digestibility and acceptability of the larvae (Sheppard, 2008) may have contributed to the better FCR values reported by Kroeckel et al. (2012) than those of the present study that utilized full fat larvae. There was no significant difference ($p > 0.05$) in the protein efficiency ratio (PER) between the treatments (Table 6). Akiyama et al. (1992) noted that the protein availability, digestibility and utilization can be influenced by the chemical composition of the ingredient, freshness of the raw material, method of processing and storage, and length of storage. The PER in the present study agrees with that reported by Adeniyi and Folorunsho (2015) and Devic et al. (2018) who used varying proportions of BSFLM to replace fish meal in the diets of *Clarias gariepinus* and *O. niloticus*. In the present study, the PER suggests that a replacement of FM by BSFLM up to 100% is possible, without any negative effect on this feed utilization parameter. Yigit et al. (2006) reported significantly lower PER between the control diet and the diets that had > 75% of FM substituted by poultry by-products. Increased inclusion of poultry by-product meal may have led to amino acid imbalances in the diets, consequentially reducing the protein retention. Similar amino acid imbalances were not present in the current study owing to the similar AA profile of FM protein and BSFLM protein (Table 2). In conclusion, the present study indicates that even full fat BSFLM can replace up to 100% of the FM without negative effects on the growth performance and survival rates of Nile tilapia in a green water culture system, particularly in feed formulations where the amino acid is fortified by other protein sources, such as freshwater shrimp meal in this experiment. Nevertheless, the slight trend towards reduced growth for the 100% substitution diet points at the need for future studies on strategies to optimize the production and utilization of BSFLM in aquaculture in general and the production of Nile tilapia in particular.

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