Production of Freshwater Infusoria and Blacksoldier Fly Larvae using Various Organic Substrates as Starter Feeds for Fish Larvae

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Abstract

Freshwater infusoria were raised on banana peels, potato peels, cabbage leaves and water lettuce for three weeks while Blacksoldier fry larvae (BSFL) raised on goat, pig and cow dung manures for four weeks was later fed to juvenile Nile tilapia as started feeds. Infusoria and BSFL were cultured in twelve plastic containers each 40 liter and nine plastic plates each 0.135 m^2 respectively. The density and biomass of infusoria; and water quality parameters were measured. The biomass and abundance of BSFL were determined. The growth performance and survival rates of Nile Tilapia (Oreochromis niloticus) were studied at three inclusion levels of BSFL (25, 75 and 100%) and fed twice a day in twelve polyethene tanks 20 litre each for two months. The mean density and biomass of infusoria were higher in banana peels and lowest in water lettuce leaves, at water temperature ranging from 21.5 ± 0.3 to 22.5 ± 0.5°C. Turbidity was generally highest in Lettuce leaves and lowest in Banana peels. Freshwater infusoria were active within 15 days of the experiment and became inactive with increasing turbidity. The biomass and abundance of BSFL were higher in pig manure and lowest in goat manure, and showed significant differences among inclusion levels ($p<0.05$). The growth rate of juvenile tilapia was numerically higher in treatment receiving 100% of BSFL (6.63±1.67 g) and lowest at 25% (1.92±31.11g) of BSFL. Results suggest potential of banana peels and pig manure as good substrate and higher inclusion level of BSFL to grow Nile tilapia.

Keywords: Organic substrates, Live feed, Infusoria, Protozoa, Starter diet, Nile tilapia.

Introduction

Feed cost is one of the bottleneck in aquaculture development globally. The use of fish meal as a major protein source to farmed fish is associated with high cost of almost 60 percent of the total production cost. As such, several researches have been looking at alternative fish feeds from various materials including plants, insects and some invertebrates to replace fish meal (Nagano *et al.,* 2000, Figueiredo *et al.,* 2005). Most hatcheries depend on manufactured feeds as source of food for fingerling, but these feeds are usually very expensive, and most of them are in large particle sizes which may be difficult to be consumed by fish fry and fingerlings. Production of fish seeds both in hatchery and at farmyard is still a challenge in most of the developing countries because of poor survival of fry and juveniles partly due to lack of early stage fish feeds.

Generally, both fry and fingerling need live food such as artemia and rotifers since fries are small in size, have poor locomotion and olfactory organs and their digestive system are not well developed to utilize manufactured feeds. However, there is limited research on the use of invertebrates such as freshwater infusoria and Black soldier fly larvae that can be raised on relatively cheaper organic substrates to serve as protein sources to farmed fish especially at juvenile stages (Nagano *et al.,* 2000, Montagnes *et al.,* 2010).

Infusoria are microscopic single celled animals in the class ciliate and phylum protozoa found and cultured in freshwater. They can inhabit ponds and tanks of freshwater, brackish water and marine habitats having decaying weeds, organic matter and debris. Also need warmth condition of 25-28°C, aeration, alkaline environment and pH of above 7, conditions away from direct sunlight and water free from chlorine (Cortes *et al.,* 2013). They are much smaller in size than artemia, soft bodied and nutritionally very rich therefore, can be used as starter diet for fish early stages (Marc, 2009).

On the other hand, Black soldiers fly (BSF) is a common and wide spread fly of the family Stratiomyidae. They are voracious consumers of various organic wastes including decaying fruits, vegetable wastes and animal manure. Life cycle of the BSF can be divided into four phases; eggs, larvae, pupa and adult stages. Adult range from 15 to 20 mm in length, larvae can reach 27 mm in length and 6 mm in width (Sheppard *et al.,* 2002). Black soldier fly frequently visit agriculture settings because the large amount of organic waste left by livestock offers abundant sites that meet their reproductive needs. The female deposit as many as 500 eggs in cracks and crevices near or in decaying matter and other organic wastes. The eggs hatch into larvae in about four to fifteen days by storing them in moisture and shaded environment for the growth of larvae. Black soldier fly larvae can eat up organic manures twice their body weight daily and soon convert organic manures into little fat bodies containing up to 43% fat. The BSFL are reported to have 40% protein on dry matter basis, while the fat content and fatty acid may depend on the substrate (Lalander *et al.,* 2020). They contain more zinc and iron than lean meat and their calcium contents is higher than milk which make them an ideal source of food for fish and chicken.

Rapid expansion of insectariums is considered to be one of the response to the global increase in demand of high quality protein for human consumption (Gorrens *et al.,* 2021), fish and animals feed (Belghit *et al.,* 2019; Oppong, 2017). Black Soldier Fly may offer alternative source of protein for feed formulations and source of biomaterials (Muchdar *et al.,* 2021; Nairuti *et al.,* 2022). Their ability to convert and decompose variety of organic wastes and human excreta (Lalander *et al.,* 2020) into valuable protein make them potential materials for the growth of fish (Karapanagiotidis *et al.,* 2014) and other animals like pig and poultry (Diener *et al.,* 2011, Zhou *et al.,* 2013). The residual from the organic wastes decomposed by the Black Soldier Fly Larvae can be used as inorganic fertilizers (Nyakeri *et al.,* 2017). Conversion of organic waste to valuable protein reduces or eliminates harmful bacteria such as *Salmonella entirica, Staphylococcus aureus, Streptococci, Clostridium whelchii, Eberthella typhii* and *E.coli* through ingestion and digestion or regeneration of antimicrobial compound (Erickson *et al.,* 2004).

Successful fish seed production in aqua hatcheries mainly depends on the supply of abundant quantity of proper live food organisms at appropriate time. Fish larva have small mouth size (less than 1mm), primitive digestive system (both anatomically and physiological), poorly developed sensory organ (visual, chemoreceptor and olfactory organ) very susceptible for stress, and slow locomotion (Mukai *et al,* 2016). Therefore, freshwater infusoria may serve as "Living Capsules of Nutrition". Providing appropriate freshwater infusoria and Blacksoldier fly larvae at appropriate time may play major role in achieving optimum growth and survival of the young finfish and shellfish (Nagano *et al.,* 2000). The present study intended to produce freshwater infusoria and Blacksoldier fly larvae using various organic substrates and subsequently use Blacksoldier fly larvae as starter feed for rearing fingerling of Nile tilapia in order to enhance growth performance and survival rate. The study provides basic knowledge on using goat manure, pig manure and cow dung manure as growth media (substrates) for Black soldier fly to lay their eggs, followed by development of larvae that were used as the source of feed to fish production.

Materials and Methods

Study area for Freshwater infusoria

The experiment was carried out at SUGECO unit of Sokoine University of Agriculture for three weeks. Twelve (12) buckets 40 liters each were used for holding the organic substrates namely, banana peels, potato peels and cabbage leaves which were purchased and collected from

Mawenzi Market in Morogoro municipality. The Lettuce leaves were collected from Magadu fish farm at Sokoine University of Agriculture. All organic materials were cut into small pieces to increase the surface areas. The experiment adopted complete randomized design (CRD). The experimental treatments were Banana peels (T1), Potato peels (T2), Cabbage leaves (T3) and Lettuce leaves (T4) each weighing 0.25 kg and replicated three times. The organic materials were fresh and left to undergo semi-decomposition for four days before were used. Each treatment received equal amount of freshwater at the beginning of the experiment and mid-way. Water quality parameters such as temperature, pH, turbidity and salinity were monitored by using Multiparameter equipment.

Study Areas for BSFL and Nile tilapia

The experiments were carried out at Magadu Aquaculture Unit of the Department of Animal, Aquaculture and Range Sciences Sokoine University of Agriculture (SUA), Morogoro Tanzania. The unit is located about 2.5 km South of Morogoro municipality at an altitude of 550 m above sea level. The temperature varies between 27°C and 31°C yearly with an average annual rainfall of approximately 880 mm and a relative humidity of about 60°C.

Figure 1: BSF rearing facility inside the nylon net serving as a cage to prevent the animal from escaping

Experiment setup for culturing blacksoldier fly larvae

The tested substrates were Goat manure (GM), Pig manure (PM) and Cow dung manure (CM) which were collected from Magadu farm and Pig house at the Department of Animal Aquaculture and Range Sciences (DAARS). Blacksoldier fly larvae were placed in nine plastic plates measuring 0.134 m^2 each containing the substrates. All containers were placed in the mosquito net cage for confining the BSFL and grow them to the adult fly. The experiment was conducted in the laboratory for a period of 4 weeks at 28 ± 2°C and 65 ± 5% relative humidity. Each treatment was replicated three times and placed randomly on a wooden frame in a room.

Experimental set up for culturing Nile tilapia juveniles

The experiment comprised of three treatments (T1, T2 and T3) and each treatment was replicated three times. The fingerlings of *O. Niloticus*, approximate 9 g obtained from SUA hatchery unit were randomly distributed into each treatment containing nine (9) Poly-ethane tanks of 20 liters each. Stocking density was 10 fingerlings per each tank. At the beginning of the experiment, all fish in each tank were counted and weighed for body weight and length using digital weighing balance and measuring board respectively.

For the three treatments (i.e. T1, T2 and T3), diet T1 contained 25% of black soldier fly powder, diet T2 contained 75% and diet T3 contained 100 % black soldier fly powder (Table 1). Feeding was done twice a day between 10:00 am and 17:00 pm at 5% fish body weight. Growth rate, survival rate and body length were monitored through intermediate samplings carried out every one week, by counting, measuring body length and bulk weight of three separate sub samples. Scoop net was used to obtain the fish sample from each container. The fish were starved for 24 hours prior to samplings and weighing in order to limit stress and mortalities related to handling during measurements. The experiment was conducted for 60 days from late April to June 2022.

Data Collection

Data collection for freshwater infusoria

Samples of Freshwater Infusoria were collected by using 0.12 mm mesh size net. The organisms appeared transparent to yellowish

Table 1: Percentage (%) inclusion of Blacksoldier fly larva in formulated feed

Ingredients	Diet 1 (T1)	Diet 2 (T2)	Diet 3 (T3)
Fish meal	60.00	60.00	0.00
Maize bran	15.00	10.00	0.00
Cassava flour	8.00	8.00	0.00
Sunflower oil	5.00	5.00	0.00
Blacksoldier fly larvae	10.00	15.00	100.00
Vitamin	1.00	1.00	0.00
Minerals	1.00	1.00	0.00
TOTAL	**100.00**	**100.00**	**100.00**

with slime layer formation due to floating spore of infusoria in the air within 4-5 day of experiment. Then after the slime of surface water disappeared and water became clear. The harvesting and collection of the samples was done for a duration of three weeks.

Determination of density of Freshwater infusoria

One ml of water containing freshwater infusoria from each experimental unit was used to determine the number of individuals. The water samples were evenly distributed on the counting chamber and the number were counted through a light microscope. Three replicates were analyzed per sample, then the average number of individuals for each treatment was used to calculate density (N) as:

$$N = \left(\frac{Number\ of\ individuals\,(cells)}{Volume\,(ml)} \right)$$

Determination of biomass of freshwater infusoria

The wet weight (biomass) of freshwater infusoria were determined by weighing the harvested samples by using digital weighing balance after every three days in batch form.

Determination of Water quality parameters

Water quality parameters which includes; pH, salinity, turbidity and temperature were monitored using DO-multiparameter equipment three times during every sampling day (i.e. morning, afternoon and evening)

Determination of Biomass of blacksoldier fly larvae

The larvae of black soldier fly were collected from one quota of each substrate in each replicate (i.e Goat manure, Pig manure and Cow dung manure) after 28 days. The collected individuals were sun dried at 38°C and air humidity of 47 for a period of 17 h according to the method by Nicholas *et al.* (2014). Thereafter, the dried samples were weighed to determine the dry weight by using digital weighing balance.

Determination of the abundance of black soldier fly larvae

Abundance was determined as the number of individual black soldier fly larvae per areas of the container sampled or collected from each substrate of each replicate. The mean values were calculated in order to find the total number of all black soldier fly larvae from each substrate. The mean abundance from each substrate were compared

Determination of fish weight and length

The wet weight (g) and length (cm) of the fingerlings were measured weekly by using a digital weighing balance and a ruler respectively. Initial values were measured before stocking, and final values at the end of the experiment, then the weight gain of fingerlings was calculated by using the following formula:

Average Weight Gain (AWG)

$$AWG = \frac{Final\ body\ weight(g) - initial\ body\ weight(g)}{time(days)}$$

The length gain was obtained by subtracting the initial length from the final length.

Determination of fish Survival Rate Percentage Survival (% S)

Tilapia survival rate (%) was calculated by using the formula below:

$$\%S = \left(\frac{Final\ number\ of\ fish\ at\ harvest}{initial\ number\ of\ fish\ at\ stocking} \right) * 100$$

Determination of Feed conversion ratio (FCR)

The feed conversion ratio (FCR) was calculated as;

$$FCR = \frac{Feed\ supplied\,(\mathrm{g})}{Body\ weight\ gain\,(g)}$$

Data analysis

The influence of organic substrates on density and biomass of freshwater infusoria and black solder fry on wet biomass of fish and water quality parameters were analyzed by using SPSS software through one way analysis of variance. The mean that were found to show significant differences at ($p<0.05$) were analyzed by using Tukey's post-hoc comparison.

Results

Density of freshwater infusoria

The density of infusoria was higher in banana peels, followed by potato peels and was lowest in water lettuce leaves (Fig. 2). The results showed significance difference in density among the treatments (p=0.002). The difference was observed between banana peels and water lettuce leaves treatments, between banana peels and cabbage leaves treatments. The number individuals/ml (density) decreased with increase in culture periods for all treatments (Fig. 3).

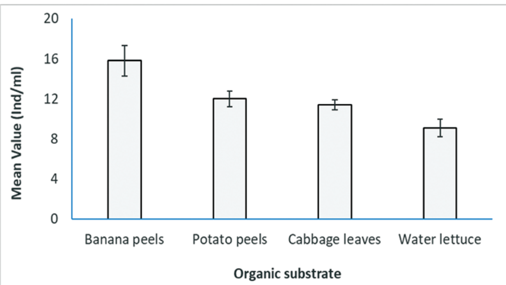

Figure 2: Density (Mean±SD) of infusoria cultured in different organic substrates

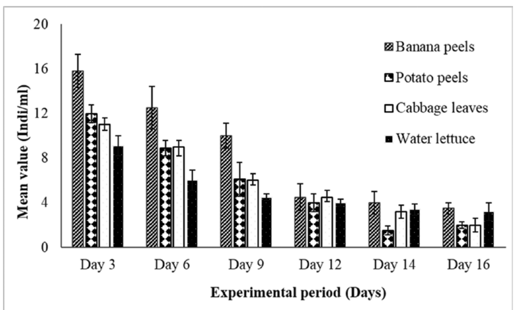

Figure 3: The density (Mean±SD) of freshwater infusoria over time (days) cultured in different organic substrates

Biomass of freshwater infusoria

The biomass of infusoria was higher in banana peels followed by potato peels and was lowest in water lettuce leaves (Fig. 4). There were significance differences in biomass among treatments (p=0.001). The differences was observed between banana peels and cabbage leaves treatments and between banana peels and water lettuce treatments.

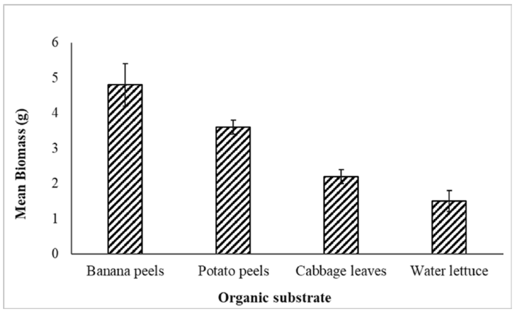

Figure 4: Biomass (Mean ± SD) of freshwater infusoria from different organic substrates

Water quality Parameters

Turbidity

The highest value of turbidity was observed in Lettuce leaves followed by Cabbage leaves and lowest in Banaba peels (Fig. 5).

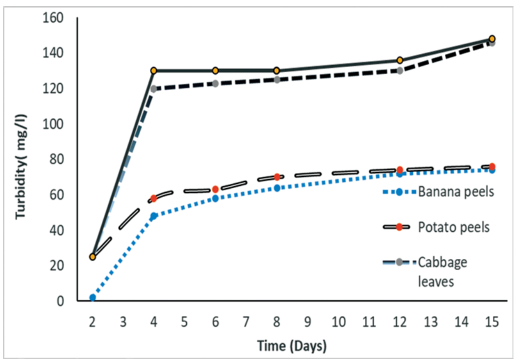

Figure 5: Turbidity level in culture systems of freshwater infusoria using organic substrates

Water quality parameters, showed significant differences among treatments (p=0.003). The difference was observed between banana peels and water lettuce leaves, banana peels and cabbage leaves. Also, the results revealed that turbidity level increased with increase in time in all treatments.

pH value

The mean pH values were slightly higher for water Lettuce leaves followed by cabbage leaves and lowest in banana peels (Fig. 6). Generally, the trend showed that pH value increased slightly over time (Fig. 6). At the end of the experiment, pH values almost in all experiments were near to neutral (7.0).

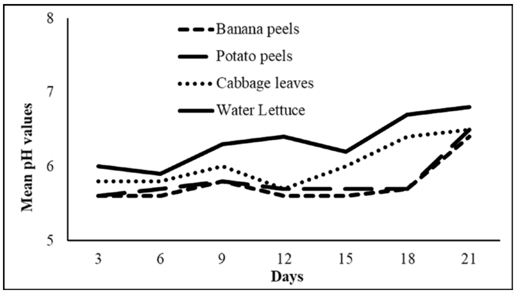

Figure 6: **pH value for different organic substrates used to culture freshwater infusoria**

Salinity and water temperature

Water salinity ranged from 0.02 psu to 0.08 psu showing a very slight variation from one treatment to another. This condition was suitable for culturing infusoria. Water temperature ranged from 21.5 ± 0.3 to 22.5 ± 0.5°C during the first week and became stable at 22.2 ± 0.4°C almost in all experiments.

The Biomass of Black soldier fly larvae

The biomass of black soldier fly larvae was higher in treatment receiving pig manure and lowest in treatment receiving goat manure as substrate (Fig. 7). The results showed significant differences among treatments (p<0.05). The differences were noted between pig manure and goat manure only, but no significant differences was observed between cow dung manure and either goat or pig manure.

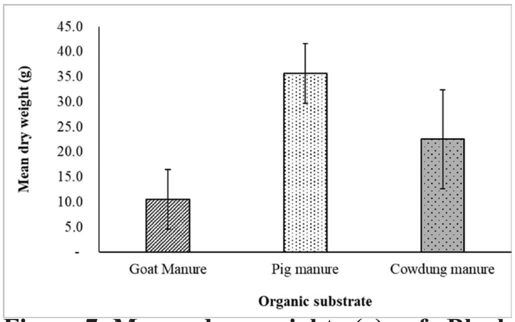

Figure 7: **Mean dry weight (g) of Black soldier fly larvae (BSFL) cultured from Goat, pig and Cow-dung manure substrates**

Abundance of black soldier fly larvae from each substrates

Black soldier fly larvae were more abundant in Pig manure substrate and lowest in cowdung manure substrate (Fig. 8). There were significance differences in BSFL abundance among treaments. The differences were between pig manure and goat manure and between pig manure and cowdung manure substrates.

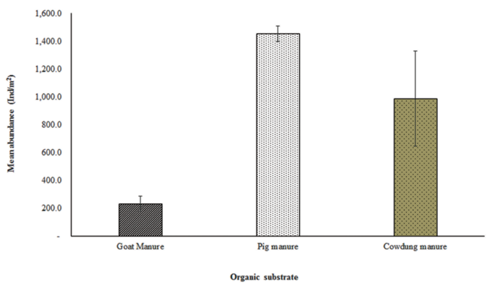

Figure 8: **Mean abundance (ind./m²) of Black soldier fly larvae (BSFL) recorded from Goat, Pig and Cow-dung manure substrates**

Nile tilapia juvenile weight (g), length (cm) gain and survival rate

The results showed that the weight, length and survival rates of Nile Tilapia were numerically higher in treatment received 100% inclusion level of BSFL followed by treatment received 75% BSFL and was lowest in 25% inclusion levels of BSFL (Table 2). There was no significant difference in fish weight, length and survival rates among treatments (p>0.05)

Table 2: Results of Weight (g), Length (cm), Survival rate (%) and FCR of juvenile Nile tilapia fed different inclusion levels of Blacksoldier fly larvae (BSFL) in formulated diet as a source of protein

Inclusion level of BSFL (%)	Weight (g)	Length (cm)	Survival Rate (%)	FCR
25	6.50 ± 0.20	4.10 ± 0.36	96.67	9.70 ± 0.00
75	7.67 ± 0.76	3.60 ± 0.31	100.00	8.27 ± 0.81
100	9.67 ± 1.89	5.43 ± 0.12	100.00	6.70 ±1.48

Feed Conversion Rate (FCR)

The results indicated that, Nile tilapia fed with 25% BSF had numerically higher FCR (9.7) followed by 75% BSF (8.27) and those receiving 100% BSFL (6.7) had the lowest FCR values (Table 2). There was no significant different in FCR among treatments ($p>0.05$).

Discussion

The observed differences in freshwater infusoria among the tested substrates with highest density and abundance being recorded in banana peels could be attributed by differences in organic matter contents in the studied substrates (Holmes *et al.,* 2013). Previous studies reported that the amount of organic matters in the media may influence the growth of infusoria and that as the culture duration increases the abundance and density of infusoria tends to decrease possibly due to exhaustion of organic matter contents in the substrates (Mukai *et al.,* 2016; Holmes *et al.,* 2013). Lowest density and abundance mean values recorded in water lettuce leaves as well as cabbage leaves which had the highest turbidity and pH values suggest that, the two environmental parameters were not suitable to support the growth and multiplication of freshwater infusoria. The freshwater infusoria were increasing and generally very active during the first two weeks of the culture period (i.e. 15 days) and started to be inactive especially in culture systems which were characterized by higher turbidity values probably due to depletion of oxygen in the systems. Although measurement of oxygen levels in the culture systems were not done during this study, but research revealed that oxygen depletion in a very turbid culture media may cause suffocation, hence, affects the growth performance and activeness of the farmed (Infusoria) organisms (Das *et al.,* 2012, Rhodes and Phelps, 2006). Therefore, the lowest mean values of freshwater infusoria in water lettuce leaves and cabbage leaves may be due to higher levels of suspended materials in the culture systems. Salinity range did not vary among treatments despite a slight increase with an increase in culture duration. The observed salinity was suitable for raising freshwater infusoria especially paramecium species (Mukai *et al.,* 2020).

Pig manures seemed to be more superior in supporting the growth of Blacksoldier fly larvae compared to goat manure and cow-dung manure. Pig manures are considered to be rich in nutrients due to the variety of feed staff including household leftovers which they feed on. Previous studies have reported that pig manure can easily decompose and are rich in terms of dry matter, carbon, nitrogen, energy as well as phosphorus and calcium contents compared to goat manure and cow-dung manure. The lowest mean values in cow-dung manure as well as goat manure could be highly influenced by the higher amount of cellulose, hemicellulose and lignin content than in pig manure (Rehman *et al.,* 2017). Similar observation has been reported when compared the effects of chicken manure and cowdung manure on growth performance of BSF (Rehman *et al.,* 2017; Erickson *et al.,* 2004; Xiao *et al.,* 2018).

History characteristics of BSFs, such as survival rate and development time are reported to be determined by a variety of factors such as temperature (May, 1961), relative humidity (Holmes *et al.,* 2012), genetic strain (Zhou *et al.,* 2013), feed availability (Diener, & Foundation, 2009) and feed composition of the substrates (Gobbi *et al.,* 2013). The results from this study showed that formulated diets containing BSFL can positively impact the growth performance of Nile tilapia. The higher numerical values in weight, length as well as survival rates of Nile tilapia recorded in treatment receiving 100% inclusion level suggest that BSFL could serve as an alternative protein sources to juvenile Nile tilapia. Lack of significant differences in weight and length among treatments receiving 100%, 75% and 25% inclusion levels of BSFL could be attributed by uneven growth rate among individual fish in the same treatment that led to within group differences as indicated by high values of standard deviation.

The growth rate of juvenile Nile tilapia in the present study was generally very low compared to the findings from studies conducted in earthen ponds probably due to lack of access to natural foods which supply important nutrients especially amino acid to farmed fish (Munguti *et al.,* 2009). Lack of statistically significant difference in survival rate and FCR among treatments are indication that all inclusion levels of BSFL were well accepted by the fish (Nairuti *et al.,* 2022). However, in the present study mean survival rate of juvenile Nile tilapia was higher than that reported by Cummins *et al.,* (2017) under clear water culture systems. Similarly, the low FCR in Nile tilapia fish fed 100% BSFL is in line with Xiao *et al.,* (2018) findings in yellow catfish (Pelteobagrus fulvidraco) when conventional protein sources were replaced at 100% with BSFL.

Conclusion and recommendation

Banana peels as well as potato peels seemed to be the best organic materials for raising freshwater infusoria under neutral to slightly alkaline pH and ambient temperature. It was also observed that the maximum density and biomass of freshwater infusoria can be reached within seven days of production period. The decrease in density and biomass of freshwater infusoria with increasing culture period was probably due to competition for space, oxygen and exhaustion of organic matter contents. Pig manure was the best substrate for the production of black soldier fly larvae compared to goat manure and cow dung manure. The present study successfully assessed the response of juvenile Nile tilapia fed dried BSFL. Lack of significant differences in growth arte, survival rate and FCR among treatments despite within variation suggest that BSFL can potentially replace the conversional feed types and serve as alternative protein sources.

Studying the influence of freshwater infusoria on the growth performance of juvenile fish is recommended. Black soldier fly larvae can be used to partially and fully replacement fish meal in formulated diets of juvenile Nile tilapia (*Oreochromis niloticus*). Mass production of blacksoldier fly larvae should be encouraged so as to ensure their availability and affordability. Understanding the chemical composition and safety of the BSFL produced using various organic materials is also important. Determination of cost of producing juvenile Nile tilapia fed with Black Soldier Fly pre-pupae meal is essential.

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