Morphological identification and comparative growth of tubificid worms in culture media supplemented with different feed types

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1. Introduction

Aquaculture has been considered one of the main food-producing sectors to date, as it provides protein and other essential nutrients for people from low- to high-income countries worldwide. Despite its success, nutritional management, including balanced feeding, has been a significant challenge for sustainable aquaculture production (Dawood, 2021). Since the formulated feeds are species-specific, their conventional use in aquaculture might be less effective. Inappropriate formulation, nutrient leaching, less efficiency as a larval feed due to inefficient digestion, and static nature lead to finding more sustainable, cost-effective diets in aquaculture (Gamboa-Delgado, 2014). Microscopic plankton, micro, and macro aquatic invertebrates, i.e., Rotifers, *Artemia*, Moina, Daphnia, Chironomid larvae, are important live feed organisms in aquaculture. Live feeds contain high nutritional components that tend to be called "living nutritional capsules" (Kandathil Radhakrishnan et al., 2020). The oligochaete worm (*Tubifex tubifex*) is attributed as a cost-effective live feed (also as a processed diet) rich in polyunsaturated fatty acids (PUFA) and protein especially suitable for juvenile and brooder fish (Panikkar, 2002; Velasco- Santamaria, 2011; Saravana et al., 2015; Mandall et al., 2018). Despite the success as a potential live feed, numerous drawbacks, i.e., overexploitation, contamination due to pathogens, lack of knowledge, ineffective culture strategies, etc., in the Sri Lankan context, caused underutilization of the oligochaete worm. Thus, this study was mainly focused on identifying the available sites, species-level identification, and finding a cost-effective feed type for mass production of *T. tubifex*.

2. Materials and Methods

The *T. tubifex* samples were collected from definite sites covering Nuwara Eliya and Colombo districts. Firstly, GPS coordinates were taken and mapped using ArcGIS software. The species-level identification was done referring to external morphology, i.e., body length, number of segments, and the presence of setae using light and scanning electron microscopes. Subsequently, the identified species were used for the culture experiment providing three different feeds, i.e., Black soldier fly larvae leachate (T_1), coconut poonac meal (T_2), and poultry blood meal (T_3) based on their feeding behaviour and the nutrient requirements.

Nine (09) plastic trays (L x W x H; 40 cm x 30 cm x 7 cm) were used for the culture experiment, and every treatment was included of three culture trays as replicates (r=3). Initially, sixty grams (60 g) of live worm sample was placed in each tray together with soil sample collected from their natural environment as the substrate. The first feeding was 210 g on day 01, and subsequently, 42 g was added every week until the end of the experimental period (42 days). Gentle aeration was provided during the experimental period, and the water quality parameters, including temperature and pH, were measured. The weight of the worms was taken on the 28th, 35^{th,} and 42nd days respectively.

Table 01. Experimental design of the feeding experiment; Culture media as BSFL leachate (BSFLL), Coconut poonac meal (CPM), and Poultry blood meal (PBM)

Trt. (T) / Rep. (R)	T_1 (BSFLL)	T_2 (CPM)	T_3 (PBM)
R1	Tray 1	Tray 2	Tray 3
R2	Tray 4	Tray 5	Tray 6
R3	Tray 7	Tray 8	Tray 9

3. Results and Discussion

Tubificid worms are naturally found in sewage pits, drainage canals near slaughterhouses, hospitals, livestock farms, etc. Tubificid worms were commonly found in such areas in Colombo district; however, human-linked activities, including uncontrolled capture, have resulted in the rapid decline and even disappearance from their natural habitats. We found few locations in Colombo district where the worms can be seen, including Rathmalana, Mulleriyawa South, and Belagama areas; however, population decline has been noticed at an alarming rate. Currently, Nuwara Eliya district is the central location for supplying tubificid worms, yet the supply is not sufficient enough to meet the growing demand. However, most collection sites are highly contaminated with human effluents, mainly due to industrial wastes. Moreover, continuous disturbances for their natural habitats could also be a reason for the total disappearance of these worms from these locations.

Identifying *T. tubifex* using morphological characters often misleads for various reasons such as similarity that specimens bear with several other oligochaetes, phenotypic plasticity (i.e., key morphological characters) change due to environmental factors, age of the specimen, etc. Therefore, PCR based molecular approach (e.g., DNA barcoding/gene sequencing) can solve this identity crisis.

The standing biomass (cumulative mean weight) of tubificid worms in three different culture media is presented in Figure 1. At the end of the experimental period, the highest standing biomass of 212.98 ± 1.17 g was observed in T_3, which poultry blood meal was given as the feed. The standing biomass of all the tested treatments was significantly different ($p<0.05$) at 35^{th} and $42^{nd,}$ days respectively. It also showed that there was a significant difference ($p<0.05$) between T_1 (BSFLL) and T_3 (PBM) on the 28^{th} day. Overall, the highest significant growth of tubificid worms was achieved in poultry blood meal throughout the feeding experiment.

In contrast, the treatment used black soldier fly leachate as the feeds had the least growth. The highest yield of worms fed with poultry blood meal could be due to the high dietary protein (crude protein) of 93.8 % (DM basis). A high level of worms inoculum 50 mg/cm^2 might have grown and propagated quickly in protein-rich media that seemingly plays a crucial role in maturing and reproduction, leading to increased biomass (Hasan et al., 2019). According to Agustinus (2016), the use of chicken manure as culture media significantly influences the population density of Tubifex worms (*Tubifex* sp.) than other media such as rotten lettuce media, chicken feed, etc. The earlier studies conducted by Brinkhurst and Kennedy (1965) and Kosiorek (1974) could culture *Tubifex* in captivity using mud enriched with organic matter. According to Marian and Pandian (1984), tubifex-fed cow dung showed higher growth and fecundity than that fed with lettuce (Lactuca sativa). Barman (1986), Mollah and Ahmed (1989; 1992), and Hossain et al. (2011; 2012) found that mustard oil cake (MOC) was the most suited feed for Tubifex than wheat bran (WB), soybean meal (SM), or cow dung (CD). Islam et al. (2015) used poultry blood as a wetting media and found a higher worm yield than rice gruel.

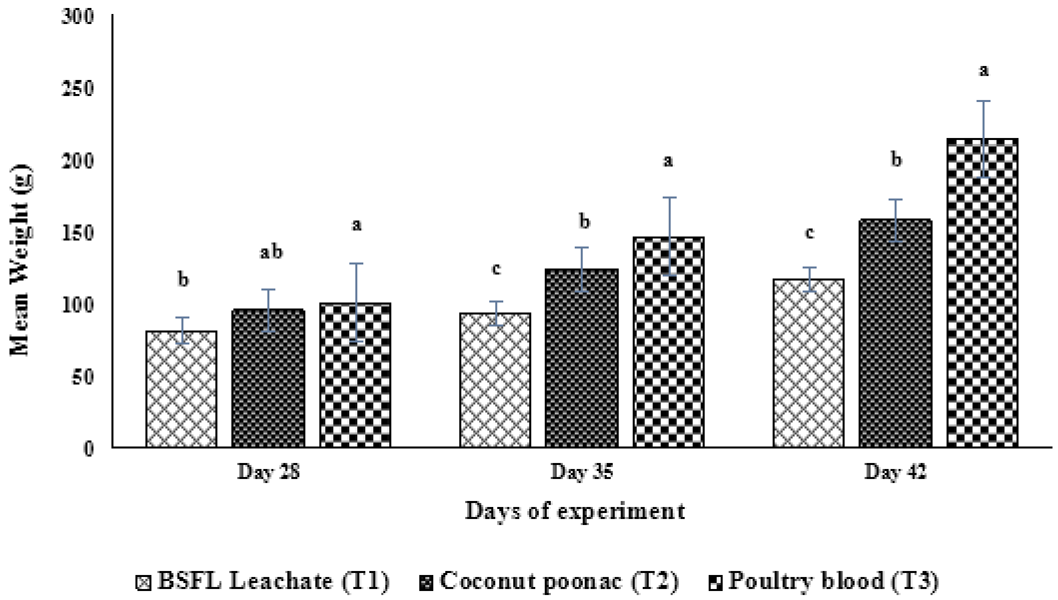

Figure 1. Tubifex worms mean weight (g) at 28th, 35th, and 42nd days after being fed with three feed types. The bars indicate standard errors. Significant ($p<0.05$) differences are indicated with different letters

4. Conclusions

This study found that identifying tubificid worms using morphological characters' might not be the accurate method. Hence, a molecular-based identification method should be performed for precise identification. We have noticed that overexploitation and other related issues have resulted in declining the existing populations. The feeding experiment revealed that poultry blood is the best feed for Tubifex. Captive breeding could be the best way to conserve this valuable live feed for continuous production to meet the increasing demand.

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