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Comparative growth of Tubificid worms in culture media supplemented with different nutrients

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Abstract
Tubificid worms have been proved as one of the most important live foods for freshwater aquaculture particularly because of having high food value. The present study was undertaken with a view to find out the effects of mineral, yeast protein and vitamin premix on the production of tubificid worms with a control media (20% mustard oil cake, 35% wheat bran, 25% cow-dung and 20% fine sand). The experiments were conducted to culture tubificid worms under running water in a rack culture system of same size (100×15 cm^2). Five treatments namely control (20% mustard oil cake, 35% wheat bran, 25% cow-dung and 20% fine sand), treatment-I (culture medium containing a mixture of 1% mineral premix in control feed), treatment-II (culture medium containing a mixture of 1% yeast protein based supplement in control feed), treatment-III (culture medium containing a mixture of 1% vitamin premix in control feed), treatment-IV (1% mineral premix + yeast protein + vitamin premix in control feed) were evaluated for the production of tubificid worms. The experiment was conducted for 120 days continuously. The highest yield (67.17±2.50 g) was found at 120 day of culture duration in treatment-II. Use of yeast protein supplement (treatment-II) in media ingredients proved most promising in giving higher yield than control media and other treatments. Mineral (treatment-I) and vitamin premix (treatment-III) had also positive effects on growth of tubificid worms. The lowest yield was found in treatment-IV.

Keywords: Tubificid worms, live food, control media

1. Introduction
Tubificid worms are aquatic invertebrates, belonging to the class Oligochaeta and family Tubificidae, used as an important live food for fishes. *Limnodrilus, Branchiura, Tubifex* etc. are the major representatives of the tubificid group [9]. The freshwater oligochaete *Tubifex tubifex* (Muller, 1774) has been observed to have great variability in life cycle and habitat distribution. Tubificids, aquatic oligochaete, are reddish in colour found mostly in old canals and drains in towns where steady and continuous water flow exists and where large amounts of organic detritus are present. A number of species of oligochaete worms are able to feed on waste organic materials such as, sludge and cattle excrement. It is well known that tubificid worms congregate together and form a permanently tight packed ball when removed from the sediment and exposed to air saturated water. The clumping behavior is also observed in natural environments. Food availability is supposed to play a major role in the distribution and abundance of tubificids [3]. Many environmental variables including water temperature, oxygen concentration, presence of competitors and substrate properties influence habitat selection and reproduction. The most important factor is temperature and oxygenated water that is kept clean of solids and waste. Tubificid worms keep as much of their bodies in contact with flowing water. This is why it is vital that enough oxygen be supplied to the water of the habitat. Without enough oxygen few worms will be able to live and reproduce. And sudden change in temperature and oxygen level encounters a massive die off that ruins the whole process.
In aquaculture, feed is the single most important item since nearly 60% cost is associated with fish feed. Tubificid worms are very important to freshwater intensive aquaculture particularly because of their food values. These worms have already been used to support commercial fish farming. In Bangladesh, Tubificid worms are used mainly as food for aquarium fishes. Tubificid worms have been reported to be an important live food in rearing the larvae of many commercially important fishes particularly the catfishes. Significantly higher survival rates and 10 times additional growth rate was recorded in *C. batrachus* larvae fed tubificid worms over those formulated dry feed [2].

They grow in a place with steady and continuous water flow with high organic detritus. In Bangladesh, the current total supply of these worms comes from wild harvests which are unreliable and inadequate in terms of demand. Harvest from the wild is hazardous to collect for unhealthy conditions. Information related to culture of tubificid worms in Bangladesh is meager. Little success has been reported of several attempts taken to develop a technique to culture tubificid worms [9, 1].

Thus, there is a need to develop a technique suitable to get reliable supply meeting the growing demand. Therefore, the present study was undertaken with a view to determine the effects of mustard oil cake, wheat bran, cow dung and fine sand with addition some nutrients, vitamins and mineral premix for production of tubificid worms.

2. Materials and Methods

2.1 Collection of tubificid worm

Tubificid worms were collected from a small canal (drain) near the Bangladesh Council of Scientific and Industrial Research (BCSIR) area, Rajshahi. All samples were taken from the upper layer (about 5 cm) of sediment, where the worms were most abundant. Samples than were taken to the Fish Technology Laboratory, BCSIR where the worms were separated from dust materials, washed several times in tap water. The worm is negatively aerotactic and prefers anoxic conditions [4]. Worms were placed in vertically arranged plastic tray (30cm×20cm) with a gentle water flow of around 1.5 L per minute and oxygenation to separate worms on the basis of its aerotactic characteristics. After separation, worms were kept in a disinfected plastic tray with air stone for aeration. It can be preserved several days. Every day it needs to be washed out feces of worms.

2.2 Construction of stainless steel rack

Five stainless steel racks were made from a hardware shop having 100×15 cm^2 of each tray. Each rack contains four stainless steel tray made by 28-gauges stainless steel sheet.

2.3 Culture system

Four experiments were conducted from August to November, 2014. The worms were cultured indoor in a stain steel rack containing four trays to protect them from rain, sunlight and other natural hazards. Before starting the experiments, the culture racks were washed and cleaned thoroughly with fresh water. Each tray was facilitated with inlet and outlet system. Continuous water flow at the rate of 1.50±0.28 L min^{-1} was maintained to keep the dissolved oxygen above 6.00±21 ppm.

2.4 Feed formulation

To detect the effects of some specific nutrients, vitamins and mineral premix on the production of tubificid worms an experiment was conducted in stainless steel rack for 120 days with four treatments each have three replicates in different feed compositions. The media composition were 20% fine sand, 35% wheat bran, 20% mustard oil cake, 25% cow-dung according to Mollah and Ahamed [8] for culturing tubificid worms in every treatments. Only difference was that extra 1% mineral premix, 1% yeast protein supplement, 1% vitamin premix and 1% mineral + yeast protein + vitamin premix were mixed with the treatments-I, II, III, IV respectively. A laboratory electric balance (TANITA, KD-160) was used to measure the required amount of media ingredients and mixed thoroughly with a bamboo stick in separate bowl for every treatments. The mixture was kept in this form for seven days for decomposition before introducing into the culture unit as recommended by Hossain *et al,* [5]. Subsequent mixing was done twice a day for better mineralization. At the end of the seven days of mixing the required amount of well-mixed media was distributed to 5 each of the rack (including control) with the help of a small plastic bowl.

2.5 Inoculation of worms to the culture rack

After sometime of media introduction the conditioned tubificid worms were inoculated at the rate of 33 g per tray over the 10,000g media homogeneously as much as possible in each of the tray.

2.6 Water flow rate

The water flow rate was controlled by the adjustment of stop cork of the PVC pipes. Three centimeter water depth was maintained over the media by depth regulator**.** Water is loaded in an overhead tank by electric pump from outlet reservoir. Water flow rate was measured once in every 15 days by collecting water from the outlet for a certain period and subsequently measured with the help of measuring cylinder. The following formula was followed to measure the water flow rate:

$$\text{Water flow rate} = \frac{\text{Water quantity in litre}}{\text{Time in minute}}$$

2.7 Periodic supply of culture media: At 15th day of worm's inoculation, periodic supply of feed was calculated according to the harvesting biomass of each replica. It was maintained the ratio of media (g): worms (g) =10,000: 33**.** Feed was supplied by calculating the net growth (g) of worms to maintain the ratio.

2.8 Water quality parameters

Following water quality parameters were measured during the experimental period:

2.8.1 Water temperature: Water temperature of the culture trays were recorded with digital thermometer once in every 15 days before sampling.

2.8.2 Dissolved Oxygen: Dissolved oxygen (DO) was measured with the help of dissolved oxygen Meter (Model: DO 5509) once in every 15 days before sampling.

2.9 Sampling procedure of Tubificid worms

For the each treatment, sample was taken at 15, 30, 45, 60, 75, 90, 105 and 120 days after inoculation of worms. The worms were collected by a sampler (5×4 cm) with water and media from ten randomly selected places within each tray and cleaned by flowing water. Complete cleanliness was obtained by separating the unwanted particles with the help of forceps and dropper. Cleaned worms were weighed by electric balance. After that both harvested worms and media were replaced randomly in the culture tray.

2.10 Statistical analysis

Data were analyzed using one factor ANOVA through computer software package (SPSS 20 version) and the significant results were further tested to identify significant difference between means using Tukey's HSD post hoc at 5% probability level. Data were presented as mean±SD.

3. Results

The production and standing biomass of tubificid worms in control and four different treatments during the whole experimental period are presented in Table 1. The average standing biomass of treatment-I, treatment-II, treatment-III and treatment-IV were 60.33±0.29 g, 67.17±2.50 g, 62.25±1.15 g, 54.25±1.56 g respectively at the end of culture periods whereas the production of control media was 58.67±1.26 g (Table 1). Significant differences in standing/total biomass were observed among five treatments by ANOVA test (Table 3). The highest yield of 67.17±2.50 g was observed in treatment-II (the media with 1% yeast protein supplement) whereas lowest yield of 54.25±1.56 g was observed in treatment-IV (1% mineral + yeast + vitamin premix) at 120 day sampling (Table 1).

Statistical analysis revealed that the standing biomass of tubificid worms in treatment-II was significantly higher (P<0.05) than all other treatments throughout the culture period (Table 2).In every treatment, a gradual increase in the standing biomass of tubificid worms was observed up to the end of the experiment. It was observed that yeast protein (Treatment-II) and vitamin premix (Treatment-III) were the most positive effects on tubificid worms production.

Table 1: Total biomass/standing biomass (g) of tubificid worms (mean ± SD) per tray in four treatments at different days during 120 days experimental period

Treatments / Days	Control	Treatment-I	Treatment-II	Treatment-III	Treatment-IV
15	35.58±0.38	37.25±0.95	42.00±1.38	39.75±0.93	35.25±0.50
30	37.5±1.13	39.75±0.28	43.75±1.48	43.00±0.53	38.25±0.63
45	39.35±1.73	40.50±0.65	44.75±1.70	47.00±0.9	41.25±0.65
60	42.75±0.80	46.00±0.80	48.75±1.23	50.0±0.88	44.50±0.95
75	47.00±1.33	49.00±1.55	52.50±1.45	52.5±0.78	47.75±1.18
90	49.75±1.00	52.25±1.28	54.50±1.53	55.75±1.00	50.00±0.38
105	53.25±0.63	55.25±2.25	59.00±1.48	59.50±0.65	52.25±0.90
120	58.75±1.26	60.33±0.29	67.17±2.50	62.25±1.15	54.25±1.56

3.1 Water quality parameters

Water quality parameters (temperature and dissolved oxygen) were observed throughout the experiment at every 15 days interval. Temperature and dissolved oxygen were between 28.45 and 32.76 °C and 6.0 and 7.2 ppm respectively (Table 4).

Table 2: Total calculated production (g) of tubificid worms over 120 days (mean±SD) for four treatments

Treatments	Initial Biomass (g)	Standing biomass (g)	Net growth (g)
Control	33.0	58.67±1.26^{b}	25.67±1.26^{b}
Treatment-I	33.0	60.33±0.29^{b}	27.33±0.29^{b}
Treatment-II	33.0	67.17±2.50^{a}	34.17±2.50^{a}
Treatment-III	33.0	62.25±1.15^{b}	29.25±1.15^{b}
Treatment-IV	33.0	54.25±1.56^{c}	21.25±1.56^{c}

Mean values in the same column with different superscript latter differ significantly (p <0.05).

Table 3: ANOVA table for mean total calculated production (g/tray) of tubificid worms at 120 days experimental period

Sources of variation	Sum of Squares	Degrees of freedom	Mean Square	F-value	p-value
Between Groups	269.86	4	67.47	28.862*	0.000
Within Groups	23.38	10	2.34		
Total	293.23	14			

*Significant at 5% level of probability

Table 4: Water quality parameters (water temperature, dissolved oxygen) of two treatments (mean±SD) during 120 days experimental period

Experimental period in days	Treatments	Temperature (°C)	Dissolved oxygen (DO)(ppm)
15	Control	30.08±0.13	6.70±0.08
	Treatment-I	31.65±0.18	6.78±0.13
	Treatment-II	31.96±0.17	7.18±0.18
	Treatment-III	30.29±0.22	6.58±0.13
	Treatment-IV	30.34±0.18	6.98±0.12
30	Control	29.78±0.12	6.68±0.11
	Treatment-I	29.75±0.18	7.10±0.20
	Treatment-II	30.32±0.15	6.75±0.08
	Treatment-III	30.45±0.10	6.21±0.10
	Treatment-IV	31.21±0.14	6.88±0.15
45	Control	30.76±0.16	6.00±0.13
	Treatment-I	29.98±0.21	6.34±0.22
	Treatment-II	28.45±0.18	6.71±0.14
	Treatment-III	29.34±0.19	6.48±0.09
	Treatment-IV	30.09±0.27	6.88±0.08
	Control	30.19±0.23	6.42±0.28
	Treatment-I	31.20±0.17	6.53±0.31

60	Treatment-II	32.65±0.11	7.05±0.21
	Treatment-III	30.91±0.24	6.98±0.13
	Treatment-IV	32.45±0.09	6.83±0.15
75	Control	29.42±0.29	6.76±0.18
	Treatment-I	30.30±0.07	6.72±0.10
	Treatment-II	28.83±22	6.95±0.08
	Treatment-III	31.60±18	6.88±0.19
	Treatment-IV	30.46±13	6.66±0.14
90	Control	30.90±15	6.08±0.12
	Treatment-I	32.24±17	6.98±0.11
	Treatment-II	30.40±11	6.78±0.16
	Treatment-III	29.34±09	6.76±0.14
	Treatment-IV	30.08±25	6.68±0.19
105	Control	29.17±10	6.66±0.26
	Treatment-I	30.44±14	6.56±0.22
	Treatment-II	32.35±12	6.49±0.13
	Treatment-III	30.89±18	6.89±0.18
	Treatment-IV	29.54±27	6.84±0.23
120	Control	30.32±13	6.88±0.27
	Treatment-I	31.12±10	7.20±0.19
	Treatment-II	30.35±15	6.95±0.13
	Treatment-III	32.76±12	7.08±0.16
	Treatment-IV	31.15±23	6.77±0.20

4. Discussion

In the experiment, the highest yield of 67.17±2.50 g/tray (≈ 44.78 mg.cm^{-2}) was recorded at 120th day of experimental period in treatment-II where commercially formulated yeast protein based supplement was used to see its effect on production of Tubificid worms. It indicates the positive effects of yeast protein on the production of these worms. Use of yeast protein supplement in media ingredients proved most promising in giving higher yield in treatment-II than other treatments. The production of 67.17±2.50 g was almost two times higher than the inoculation rate of 33 g/tray worm. Intake of little amount of extra protein and amino acids may increase metabolism, health and wellness of the worms and this may contribute with their growth and development. Addition of supplements also increases the worm's response to their environment and attraction to the feed supplied. Treatment-I and treatment-III containing mineral and vitamin premix respectively also performed a moderate yield. The lowest production was found in treatment-IV where mineral, yeast and vitamin premix were used. The lower production in this treatment may be due to high nutrition load at that treatment. The higher the nutrient addition levels the longer the period of oxygen depletion became. During oxygen depletion the number of oligochaetes was strongly reduced or even became zero [11].

Mustard oil cake, wheat bran, cow-dung and sand are important media ingredients for culturing Tubificid worms. In this study the choice of media substrates containing a mixture of various ratio of mustard oil cake, wheat bran, cow-dung and fine sand for culture of Tubificid worms and the rate of media application was made on the basis of previous studies conducted by Marian and Pandian [6] and Ahamed and Mollah [1]. Fecundity of *Tubifex tubifex* depends on the temperature, rate of water flow and organic content of the culture media [6]. Sexual maturity is more rapid at a higher temperature and enough dissolved oxygen content when adequate organic carbon (especially mustard oil cake) is present in the culture media. During the experimental period the water quality parameters (temperature and dissolved oxygen were between 28.4 and 32.8 °C and 6.0 and 7.2 ppm respectively) were in suitable and productive range for the production of tubificid worms. Saiful *et al*. [10] found temperature and dissolved oxygen of water in culture media of tubificid worms ranged between 26.5 and 29.4 °C, 6.2 and 7.3 ppm respectively in their study.

In present experiments at BCSIR, we only found that addition of nutrients like vitamins, soluble proteins remarkably enhances growth of the worm. To find out the actual response of yeast protein, mineral and vitamin on growth performance of Tubificid worm, further analysis is required in molecular level. But the control of gene expression may be involved complex interactions of hormonal, neural and nutritional factors.

The standing biomass of tubificid worms (67.17±2.50 g/tray ≈ 44.78 mg.cm^{-2}) in treatment-II (yeast protein supplement) was significantly higher (P<0.05) than all other treatments throughout the culture period. But production in our present study is relatively lower (67.17±2.50 g/tray ≈ 44.78 mg.cm^{-2}) than the findings of Marian and Pandian [6] who found the production of tubificid worms was 200 mg.cm^{-2} in their study. The lower production in our study may be due to collection of sample (tubificid worms) and media ingredients from wild sources without any disinfection. Further study is needed to find out a suitable cost effective media for commercial production of tubificid worms.

5. Conclusion

Tubificid worms have been found to be one of the best qualities live foods in rearing the larvae of hatchery produced catfishes, prawn, eel and ornamental fishes. It has become an important component in commercial aquaculture because of its high food values. However, reliable supply of required quantity of tubificid worms is still a bottleneck in the rearing processes of catfish fry and fingerlings. In the present experiments at BCSIR, we found that addition of nutrients like vitamins and soluble proteins remarkably enhances growth of the worm. They are usually collected from wastewater flow rich in organic detritus. The current total supply of these worms comes from wild harvests which are unreliable and inadequate in terms of demand. Harvest from the wild is hazardous because aquarium fish and other aquatic animals could be infected by *Mycobacterium spp*. through the use of live feeds. Worms from sewage-contaminated waters may harbor human pathogens responsible for tetanus,

hepatitis, and other bacterial and viral diseases. In reality, little success has been reported of several attempts to develop a technique to culture tubificid worms which are non-hazardous and sustainable. In our recent works at BCSIR, after mixing feed ingredients we autoclaved it and it was a little step to hygienic tubificid worms production (most of the available scientific articles describe various culture techniques where decomposed cow dung, chicken manure, oil cakes etc are directly used but we found most of the culture media are unhygienic, self-decaying and were not sustainable and suitable for commercial production). So the need for pollution free culture technology comes to front. Further study is needed to make out a suitable cost effective media for commercial production of tubificid worms.

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