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Performance of Chicken Blood for the Production of Tubificid Worms as Live Food for Fish

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Abstract: Tubificid worms have been proved as one of the most important and cheap live foods for fish larvae and also for the laboratory animals particularly because of having high nutritional value. The present study was undertaken with a view to finding out the effects of chicken blood on the production of tubificid worms. The experiments was conducted to culture tubificid worms under running water in cemented culvert system of same size (160×25×10 cm^3) under two treatments namely treatment I (culture medium containing a mixture of 20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand soaked with chicken blood) and treatment II (culture medium containing a mixture of 20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand soaked with rice gruel). The experiment was continued for 90 days to detect the effect of chicken blood on the yield tubificid worms. The highest yield (1020.86±19.84 mg cm^{-2}) was found at 70th day culture duration. Only 0.71 kg media ingredients priced Tk. 18 about US$ 0.23 (where 1 US$= 78 Tk.) was needed to yield 1 kg worms indicated the suitability of this media for large scale production. Use of chicken blood to soak the media ingredients proved most promising in giving even 1.5 times higher yield than rice gruel.

Key words: Culture Media · Chicken Blood · Live Food · *Tubifex tubifex*

INTRODUCTION

Tubificid worms are aquatic invertebrates known as sludge or sewage worms under the class Oligochaeta and family Tubificidae. These reddish small (3-4 cm long) [1, 2] annelids are mainly found in the old canals having organic detritus, drains with flowing water etc. aquatic environment where rich in organic detritus. They take sediments to gain nutrition by selectively digesting bacteria therein and absorb nutrient molecules by using their body walls [3]. *Tubifex tubifex* (Tubificidae) is particularly interesting because it is an indicator of pollution [4, 5]. Therefore reduction of organic matter recede the density of *Tubifex tubifex* [6].

Tubificid worms have been proved as one of the most important live foods for freshwater aquaculture particularly because of having high food value (5575 cal g^{-1} on the basis of dry weight) [7] that makes them nutritious for fish [8]. Mollah and Ahamed [9] identified proximate composition of tubificid worms and found 63.32% crude protein, 28.84% crude lipid and 7.95% ash. Jhingran [10] also recommended tubificid worms as a good source of protein and amino acid profile for fish growth. Larvae and fry showed well response in terms of survival and growth rate because of using tubificid worms as their foods compared to others [11-16). Ecologically, it is an important source of food for leeches, crustaceans, insects and fishes.

Feed is the single most expensive item in aquaculture. Feed suitability in larvae nursing is a critical phenomenon. Tubificid worms have been reported to be an important live food for nursing of larvae of many commercially important fishes mostly catfishes. So, it is important to know some details the biology and life history [17] of

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these worms with an ultimate aim to develop a suitable culture technique. Several scientists [9, 18, 19] developed many techniques for mass tubificid worms but they have limitation in terms of production rate, cost, simplicity of culture technique and; suitability and availability of culture media. Mollah and Ahamed [9] demonstrated the suitable media for sustainable growth of the worms for the first time in Bangladesh. However, further study was necessary to resolve some more critical aspects for giving the fine tuning to make the technique much easier, economical and adaptable to the ultimate users In this study, growth performance of tubificid worms was compared between chicken blood and rice gruel medium along with 20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand mixture.

MATERIALS AND METHODS

To detect the effects of chicken blood on the yield of tubificid worms an experiment was conducted for 90 days with two treatments each have five replicates. Blood was used to observe whether this large volume of wastes from the urban areas can be useful in enhancing the production of these worms at commercial scale. The media composition found best by Mariom and Mollah [20] i.e. 20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand was used for culturing tubificid worms in both the treatments. Only difference was that in treatment-I blood was used to soak the media ingredients while in treatment-II rice gruel was used to soak the media ingredients (Table 1). Ten cemented culverts of size 160x25x10 cm^3 were used to conduct a 2x5 factorial design (two treatments each with five replications) where media were the only experimental variable. Each culvert was facilitated with inlet and outlet system. Continuous water flow at the rate of 1.22±0.28 L min^{-1} was maintained to keep the dissolved oxygen above 5 ppm.

Culture Unit Preparation: Tin shaded culture unit was prepared by cleaning cemented culverts for culturing tubificid worms. Culverts were connected with a water reservoir tank by stop cork where the water was constantly supplied from the deep well. In order to provide optimum environment for tubificid worms, constant water renewal and removal facilities were provided through inlet and outlet system with each culvert. As inlet, a porous PVC pipe (180 cm long and 1 cm^2 diameter) was set up longitudinally over each culvert with the help of bamboo sticks. Each culvert was connected with an outlet for draining out the water from the culvert.

Table 1: Combination of different media ingredients in two treatments

	% of ingredients	
Media Ingredients	Treatment-I	Treatment-II
Mustard oil cake	20	20
Wheat bran	20	20
Soybean meal	30	30
Cow-dung	20	20
Fine sand	10	10
Rice gruel	-	As required
Chicken Blood	As required	-

To soak the media ingredients with sufficient amount of chicken blood and rice gruel was used in treatment-I and treatment-II respectively.

Collection of Required Ingredients for Media Preparation: Chicken blood was collected from the local market named K.R market located in Bangladesh Agricultural University (BAU) where broiler chickens are slaughtered. Other ingredients like mustard oil cake, wheat bran and soybean meal were purchased from the downtown of Mymensingh, Bangladesh. About seven days decomposed Cow-dung was collected from the dairy farm of BAU and sand was collected from the Old Brahmaputra River flowing to the eastern side of BAU campus. Rice gruel was collected from the kitchen of Shaheed Shamsul Haque Hall of BAU.

Media Preparation: A laboratory electric balance (TANITA, KD-160) was used to measure the required amount of media ingredients and mixed thoroughly with a bamboo stick with sufficient amount of rice gruel and blood as mentioned previously in separate bowl. The mixture was kept in this form for seven days for decomposition before introducing into the culture unit as recommended by Hossain *et al.* [21]. Subsequent mixing was done twice a day for better mineralization. At the end of the seven days of mixing the required amount (250 mg cm^{-2}) of the well-mixed media was distributed to each of the culvert with the help of a small plastic bowl.

Collection of Tubificid Worms: Wild tubificid worms were collected from different drains of Mymensingh town and Bangladesh Agricultural University campus, Mymensingh. The collected worms were brought to the Mini Hatchery and Breeding Complex of Department of Fisheries Biology and Genetics in order to clean by using flowing water and held in a flow-through system for conditioning over 24 h prior to inoculation into the culverts for culture.

Inoculation of Tubificid Worms: After 24 h of media introduction into the culverts the conditioned tubificid worms were inoculated at the rate of 1.25 mg cm^{-2} (5 g $culvert^{-1}$) [22] over the media homogeneously as much as possible in each of the culvert.

Maintenance of Water Flow: Regular and optimum water flow (1.22±0.28 L min^{-1}) was maintained to keep optimum dissolved oxygen level (above 5 ppm) of culvert water. The water flow rate was controlled by the adjustment of stop cork of the PVC pipes. Four centimeter water depth was maintained over the media by depth regulator.

Periodic Supply of Culture Media: At 10th day of worm's inoculation, periodic supply of culture media was commenced at the rate of 250 mg cm^{-2} in respective culverts and the supply was maintained once in every 10 days intervals up to 90th day [22]. Culture media was distributed homogenously throughout the culverts. Water flow was stopped prior to addition of media.

Water Quality Parameters: Following water quality parameters were measured during the experimental period:

Water Flow Rate: Water flow rate was measured once in every 10 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:

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

Water Temperature: Water temperature of the culture culverts were recorded with digital thermometer once in every 10 days before sampling.

Dissolved Oxygen: Dissolved oxygen (DO) was measured with the help of dissolved oxygen Meter (Model: DO 5509) once in every 10 days before sampling.

pH: Water pH was measured by using portable digital pH Meter (Model: HI 98127) once in every 10 days before sampling.

Sampling Procedure of Tubificid Worms: Tubificid worms formed colonies after around 40 days of the inoculation into the culture system. Colony of tubificid worms in two different treatments is shown in Fig. 1a & b. To determine the production rate, sampling was started from 40th day of worm's inoculation and it keeps continued every 10 days interval up to 90th day before the introduction of new media into culverts. During each sampling, harvesting was done at the rate of 40 mg cm^{-2} to maintain the sustainability of culture. Crude tubificid worms were collected by glass tube having diameter 2.2 cm from three randomly selected places of each culture unit. Then the media and other undesired materials were cleaned from worms by water flow, using forceps and dropper. Finally tubificid worms were dried with tissue paper and taken weight by Tanita electric balance graduated in 0.000 g.

Fig. 1a: Colony of tubificid worms in treatment-I

Fig. 1b: Colony of tubificid worms in treatment-II

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.

RESULTS

The production and standing biomass of tubificid worms in two different treatments during the whole experimental period are presented in Table 2. The average standing biomass of tubificid worms in treatment-I and treatment-II were 1020.86±19.84 mg cm^{-2} and 620.75±4.19 mg cm^{-2} respectively at 70th experimental day (Table 2). The highest yield of 1020.86±19.84 mg cm^{-2} was observed in treatment-I (the media where chicken blood was used) at 70th day sampling. Significant difference in standing biomass was observed between two treatments by ANOVA test. Statistical analysis revealed that the standing biomass of tubificid worms in treatment-I was significantly higher ($P<0.05$) than in treatment-II throughout the culture period (Tables 2 and 3).

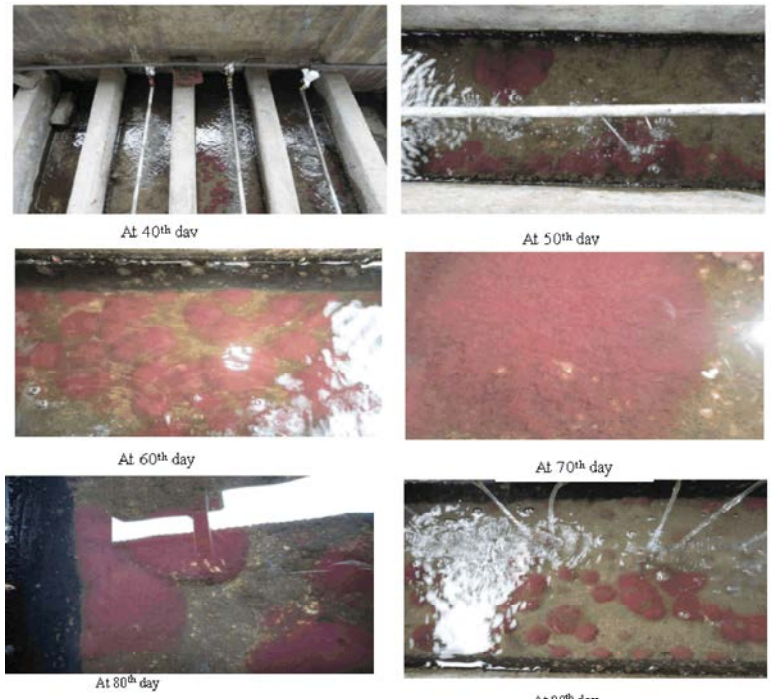

Fig. 2: Growth trends of tubificid worms in treatment-I

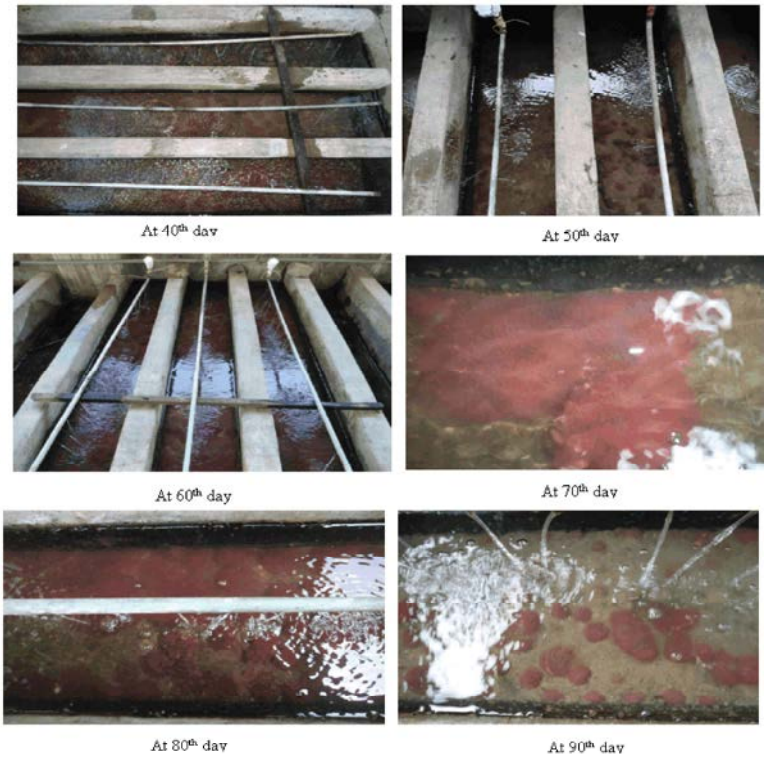

Fig. 3: Growth trends of tubificid worms in treatment-II

In both the treatments, a gradual increase in the standing biomass of tubificid worms was observed reaching a peak production at 70th experimental day (1020.86±19.84 mg cm^{-2} and 620.75±4.19 mg cm^{-2} in treatment-I and treatment-II respectively) and then showing a decrease in biomass up to the end of the experiment (Figs. 2 & 3).

Table 2: Standing biomass (mg cm^{-2}) of tubificid worms in two treatments at different days during 90 days experimental period (mean±SD)

	Experimental period in days					
Treatments	40	50	60	70	80	90
TR-I	256.05±7.09	432.23±6.79	651.47±7.98	1020.86±19.84	804.98±1.68	683.23±8.84
TR-II	243.53±6.64	390.81±9.19	470.66±5.10	620.75±4.19	581.72±3.05	415.60±12.18

Table 3: ANOVA table for mean total calculated production (mg cm^{-2}) of tubificid worms at 90 days experimental period

Sources of variation	Sum of Squares	Degrees of freedom	Mean Square	F value
Between Groups	533002.600	1	533002.600	13.465*
Within Groups	2295819.761	58	39583.099	
Total	2828822.361	59		

*Significant at 5% level of probability

Table 4: Total calculated production (mg cm^{-2}) of tubificid worms over 90 days (mean±SD) for two treatments

Treatments	Standing biomassat 90^{th} day (S)mg cm^{-2}	Harvested biomassover 90 days (H)mg cm^{-2}	Total calculated production(S+H) mg cm^{-2}
TR-I	683.23±8.84	240	923.23±8.84
TR-II	415.60±12.18	240	655.60±12.18

Table 5: Water quality parameters (water temperature, dissolved oxygen and pH) of two treatments (mean±SD) during 90 days experimental period

Experimental period in days	Treatments	Temperature (°C)	Dissolved oxygen (DO)(ppm)	pH
40	I	25.96±0.15	6.68±0.08	7.12±0.11
	II	25.98±0.18	6.78±0.11	7.20±0.18
50	I	26.80±0.21	6.98±0.13	6.94±0.11
	II	26.72±0.41	6.92±0.13	7.04±0.15
60	I	25.84±0.11	6.92±0.08	7.02±0.16
	II	25.92±0.19	6.92±0.13	7.03±0.22
70	I	26.86±0.11	7.02±0.16	6.98±0.16
	II	26.86±0.15	6.82±0.13	7.02±0.17
80	I	26.72±0.19	6.84±0.18	7.14±0.11
	II	25.90±0.16	6.84±0.27	7.11±0.16
90	I	26.76±0.21	6.88±0.16	6.86±0.23
	II	26.84±0.11	6.84±0.24	6.90±0.21

From 40^{th} experimental day tubificid worms were harvested at the rate of 40 mg cm^{-2} from both the treatments in order to sustain the culture [23]. This harvest rate showed higher standing biomass in treatment-I (683.23±8.84 mg cm^{-2}) than in treatment-II (415.60±12.18 mg cm^{-2}) (Table 4).

Water Quality Parameters: Water quality parameters (temperature, dissolved oxygen and pH) were observed throughout the experiment at every 10 days interval. Temperature, dissolved oxygen and pH of water were between 25.9 and 30.1°C, 6.0 and 7.1 ppm and 7.2 and 7.4 respectively (Table 5).

Production Cost: About 0.71 kg culture media (20% mustard oil cake, 20% wheat bran, 30% soybean meal, 20% cow-dung and 10% sand soaked with chicken blood) valued 18 Tk. (US$ 0.23) (where 1 US$ =78 Tk.) was required to produce 1.0 kg worms.

DISCUSSION

In the experiment, the highest yield of 1020.86±19.84 mg cm^{-2} was recorded at 70^{th} day sampling in treatment-I where chicken blood was used as a soaking agent. The observed exceptional and profound yield (1020.86±19.84 mg cm^{2}) recorded in treatment-I clearly indicate the positive effects of chicken blood on the production of these worms. It also demonstrated the beneficial effects of protein contained in the chicken blood on the growth of tubificid worms and suitability of blood to wet the media ingredients instead of rice gruel. Yield (620.75±4.19 mg cm^{-2} at 70^{th} day) in treatment-II i.e. media soaked with rice gruel denoted less suitability of it compared with that of treatment-I in terms of production. This finding indicates the suitability of this medium to enhance yield compared to the yield of 999.16±40.29 mg cm^{-2} and 659.35±16.88mg cm^{-2} as demonstrated by Mariom and Mollah [20] and Hossain *et al*. [21].

The experiment demonstrated that chicken blood is an important soaking agent and had a definite effect on the culture of tubificid worms. The media ingredients used in the study contained sufficient amount of organic carbon, minerals, proteins, vitamins etc. Organic carbon was the essential part for the reproduction of tubificid worms. Kaster [24] stated that 50% tubificids reached sexual maturity within 40 days at 15 °C temperature on 7% organic carbon content. He also stated that time requirement for reaching sexual maturity significantly decreased when temperature and organic carbon content in the culture media was increased. Chicken bloods contain 78.00% crude protein [25] on dry matter basis which is higher than rice gruel (6.69%) and; soybean meal (45.29%), mustard oil cake (30.13%) and wheat bran (14.19%) as reported by Mariom and Mollah [20] and Sarowar and Mollah [26] on dry matter basis. The production results proved the beneficial effects of excess protein on the growth of tubificid worms.

During the experimental period the water quality parameters (temperature, dissolved oxygen and pH of water in culverts ranged between 26.5 and 29.4°C, 6.2 and 7.3 ppm and 6.7 and 7.6 respectively) were in suitable and productive range for the production of these worms. Moreover, small amount of media ingredients and therefore low production cost was recorded during production of the worms compared to previous scientists report. Here only 0.71 kg culture media valued Tk. 18 (US$ 0.23) (where 1 US$ =78 Tk.) was required to yield 1 kg worms whereas Mariom and Mollah [20], Hossain *et al*. [21], Mosharaf [27], Ahamed and Mollah [22] Marian *et al.* [19] and Marian and Pandian [18] was used 1.01kg, 2.65kg, 1.99kg, 2.85kg, 25kg and 18kg media respectively to produce same amount of worms. So the above calculation indicated the suitability of this media for commercial production of tubificid worms.

CONCLUSION

Tubificid worms are important live food used for certain fish larvae in the hatcheries as well as in the rearing of aquarium/ornamental fishes. A number of studies have been conducted to develop a suitable culture technique of tubificid worms. The findings of the present study can be of significant importance to all fish hatcheries scattered all over world. The study revealed that as a soaking agent chicken blood was more suitable than rice gruel in terms of production. However, there remains some scope to further improve the culture technique on the basis of present study successfulness.

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