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Ratio Optimisation of Media Ingredients for Mass Culture of Tubificid Worms (Oligochaeta, Tubificidae) in Bangladesh

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Abstract

Two experiments were undertaken in a small cement raceway culture system to determine the best media ingredients and their ratios suitable for the mass culture of tubificid worms. The first experiment focused to determine the best medium suitable for maximum yield, while the effect of rice gruel was measured in the second. At the 70th day of culture, a significantly higher ($p< 0.05$) yield of 678.62±11.50 $mg\cdot cm^{-2}$ worms was found in the culture medium containing the mixture of 20% wheat bran (WB), 30% soybean meal (SM), 20% mustard oil cake (MOC), 20% cow dung (CD) and 10% sand. The highest yield of worms (1,126.03±31.08 $mg\cdot cm^{-2}$) was obtained when rice gruel was used to wet the culture media ingredients (20% WB, 30% SM, 20% MOC, 20% CD and 10% sand) instead of water. The cost of producing 1 kg worms was reduced by half (US$0.53 to 0.31) as a result of using less media ingredients (1.46 kg instead of 2.43 kg). Results of this study suggest that the combination of 20% WB, 30% SM, 20% MOC, 20% CD and 10% sand is the best culture medium for a 70-day culture duration to obtain the maximum yield of tubificid worms.

Introduction

Aquaculture contributes nearly 40% of the total fish yield of Bangladesh, with the seed supply being derived mostly from hatchery production (DoF 2011). Of the total inland fish production, catfishes contribute nearly 2.4% (FRSS 2008). The farming of fish like stinging catfish, *Heteropneustes fossilis* (Bloch 1794), walking catfish, *Clarias batrachus* (Linnaeus 1758), pabda (*Ompok* spp.) and striped catfish, *Pangasianodon hypophthalmus* (Sauvage 1878) is becoming increasingly popular. However, a reliable and adequate supply of good quality seed of the desired species is essential to sustain the aquaculture industry, which in turn depends on the successful production and rearing of fish larvae. Live feed, particularly tubificid worms (Oligochaeta, Tubificidae), play the most important role in the rearing process of catfish larvae.

Use of live feed in aquaculture depends mainly on their availability, ease of use, preference by the cultured species and price. Live feed increase growth and survival of juvenile catfishes and crustaceans, and help in the raising of ornamental fishes (Proulx and Noüe 1985). Because of their high food value (5,575 $cal\cdot g^{-1}$ on a dry weight basis), tubificid worms are one of the best quality live feed used in intensive aquaculture (Giere and Pfannkuche 1982). These worms have already been tested in commercial fish culture in the former Union of the Soviet Socialist Republic (Lietz 1987), and they are being used across the world, including Bangladesh,

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as feed for ornamental aquarium fishes. The tubificid worms grow in sewerage drains and canals where organic load is very high. However, in Bangladesh, harvest from the wild is unreliable and inadequate to meet the growing demand. In addition, it is hazardous due to the unhealthy conditions prevailing in the natural habitats. In culture conditions, profitability and suitability of the media are determined by production cost and the yield of worms. Thus the development of a suitable medium and technique for large-scale commercial mass culture of these organisms is of immense importance. The ingredients used in preparing the culture media of these worms play a significant role in increasing fish yield.

Typically, water is used in wetting the ingredients and inducing their decomposition over a period of 7 days. Rice gruel is a by-product of boiled rice that is found in every household and restaurant across the country. In urban areas, there is no use of this large quantity of gruel, which can be an alternative medium for wetting the ingredients, for tubificid worm culture, instead of water. Rice gruel may have positive effects in enhancing the yield of tubificid worms, as it has a high starch (83%) and protein (8%) content (Mollah and Nurullah 1988).

Rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792) after being fed *Tubifex* sp. were observed to have significantly higher growth rate compared to those fed Oregon moist pellets (OMP) over a similar range of rations (Phillips and Buhler 1979). Buddington and Doroshove (1984) have demonstrated that white sturgeon, *Acipenser transmontanus* Richardson 1836 showed a 40% increased growth rate when fed tubificid worms as compared to inanimate pellets. Alam and Mollah (1988) found significantly higher survival and 10-times higher growth rates in walking catfish larvae which were fed tubificid worms as compared to those fed formulated dry feed. Despite several attempts, there has been little success in developing a suitable technique for the culture of tubificid worms in Bangladesh (Marian et al. 1989; Mollah and Ahamed 1989; Ahamed and Mollah 1992). Thus there is a need to develop an economically viable culture technique suitable for large-scale commercial production of tubificid worms to provide a reliable supply to satisfy the growing market demand.

The current study, therefore, was designed to determine the proper ratio of wheat bran and soybean meal suitable for mass culture of tubificids, and to measure the effects of the use of rice gruel as a wetting agent on the yield of tubificid worms. The culture medium requirement for producing per unit weight of worms was determined, along with the production cost.

Materials and Methods

Experimental worms and culture system

Tubificid worms were collected from Mymensingh town, Bangladesh. After collection the worms were cleaned by using continuous flow of water and held in a flow-through system in plastic bowls for conditioning over a period of 24 h before inoculation. Two experiments (the first for 80 days and the second for 70 days) were conducted from February to June 2009.The worms were cultured indoor in a small cement raceway culture system (160×25×10 cm) to protect them from rain, sunlight and other natural hazards (Fig. 1). Before beginning the experiment, the raceway was washed and cleaned with fresh water. A continuous subsurface well water was sprayed through horizontal perforated polyvinyl chloride (PVC) pipe (180 cm long and 1 cm diameter) over the raceway. The experiments were conducted in the Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Small raceway supplied with constant water flowing from the perforated polyvinyl chloride pipe

Fig. 1. Small raceway with horizontal perforated pipes to provide continuous flow of sprayed water.

Experiment 1: Combined effect of wheat bran (WB) and soybean meal (SM) with five culture durations on the yield of tubificid worms

This was a 3×3×5 factorial experiment in triplicate following the completely randomised design (CRD) method. The experimental variables were, three levels of wheat bran (WB, dry weight basis; 10, 20 and 30%) and soybean meal (SM, 10, 20 and 30%) and five culture durations (40, 50, 60, 70 and 80 days). In addition, mustard oil cake (MOC) (20%) and cowdung (CD) (20%) were also used in fixed ratios in all treatments (Table 1). In this experiment, dry quartz white sand collected from the district of Comilla, Bangladesh was used as substrate to settle the culture media into the raceways and to make the mixture of media into 100% dry weight (w/w). Before use, the sand was passed through a 1 mm mesh sieve.

Table 1. Media ingredients used in the experimental diets.

Ingredients	Levels of media ingredients (%)								
Wheat bran		10			20			30	
Soybean meal	10	20	30	10	20	30	10	20	30
Mustard oil cake	20	20	20	20	20	20	20	20	20
Cowdung	20	20	20	20	20	20	20	20	20
Sand	40	30	20	30	20	10	20	10	0
Total (%)	100	100	100	100	100	100	100	100	100

Experiment 2: Effects of rice gruel used to wet the media ingredients instead of water and culture durations on the yield of tubificid worms

A 3×4 factorial design was used in triplicate to evaluate the effect of rice gruel on the yield of tubificid worms. The experimental variables were water, water and rice gruel (1:1) and rice gruel and four culture durations (40, 50, 60 and 70 days).The ratio of the best media ingredients used in this experiment was obtained from the first experiment (20% WB, 30% SM, 20% MOC, 20% CD and 10% sand).The media from the first experiment were used to culture tubificid worms in all treatments for a culture period of 70 days.

Media supply

Each raceway was supplied with 1 kg medium. Before placing the media into the culture systems, the ingredients were mixed thoroughly by hand in a plastic bowl with water for the first experiment and water, water and rice gruel (1:1) and rice gruel for the second. In both experiments, the wet mixture of the media ingredients was held in this form for 7 days before placing it into the raceways for decomposition. Subsequent mixing was done twice a day for better decomposition. After 7 days, the well-prepared media were put into the raceways. Water flow rate was adjusted 24 h before inoculation of worms into the raceway.

Inoculation of worms

Worms were inoculated at a density of 5 $mg{\cdot}cm^{-2}$ (i.e. 20 $g{\cdot}raceway^{-1}$). They were homogeneously spread over the media as much as possible in each raceway.

Periodic supply of culture media

Before sampling, each raceway was supplemented with 200 mg·cm^{-2} medium every 10 days at 9 am. The total quantity of each mediun was spread evenly throughout each raceway while the water flow was stopped.

Water quality

Continuous water flow was adjusted to maintain the dissolved oxygen in a suitable range (4-6 mg·L^{-1}) with the help of a PVC pipe fitted with a stop cork. Water flow rate was measured every 10 days by collecting water from the outlet for a period of 5 min and subsequently measured with the help of a graduated measuring cylinder. Water temperature (°C) in the culture raceway was measured with a thermometer at 10.00am once every 10 days before sampling. Dissolved oxygen (mg·L^{-1}) was determined by using a HACH DO meter (Model: HACH sension 6, USA) at the time of measuring water temperature.

Sampling

For the first experiment, samples were taken at 40, 50, 60, 70 and 80 days after inoculation of worms, and at 40, 50, 60 and 70 days for the second experiment. The worms were collected by a sampler (4.4×4.4 cm) with water and media from five randomly selected places within each raceway and cleaned by flowing water. Complete cleanliness was obtained by separating the unwanted particles with the help of forceps and dropper. Cleaned worms were dried with blotting paper and weighed (Mettler Electric Balance, Switzerland; graduated in 0.000 g).

Estimation of media and production cost

To produce 1kg worms, the cost of media ingredients used was determined in Bangladeshi Taka (BDT) for both experiments. The price of 1 kg SM, WB and MOC was 25, 18 and 20 BDT, respectively. The cost in BDT was then converted into US$ (1 US$=70 BDT). No cost was associated with rice gruel, cow dung and sand. Total media requirements per treatment up to 70 days was determined as follows; Initial media supply + periodic media supply (mg·cm^{-2})×area (cm^2)× frequency of media supply= 1.0 kg + (200×160×25×7 times)=1.0 kg + 5.6 kg=6.6 kg media.

Statistical analysis

Data were analysed by using ANOVA followed by Tukey's HSD post hoc for multiple comparisons. Data were presented as mean±SEM and analysed by using the statistical software SPSS version 11.5 with the level of significance at $p<0.05$.

Results

Experiment 1: combined effect of wheat bran (WB) and soybean meal (SM) and the yield of tubificid worms during five culture durations

The highest yield (678.62±11.50mg·cm^{-2}) of worms was found in the 20% WB and 30% SM treatment group while the lowest yield (73.16±5.61mg·cm^{-2}) was observed in the 10% WB and 10% SM treatment group. The highest yield was observed after 70 days of culture duration (Table 2). Maximum yield was observed after a period of 70 days from the initial stocking in all three treatments. The yield of the worms increased up to 70 days and then declined at 80 days, when the experiment was terminated.

Experiment 2: Effects of rice gruel on the yield of tubificid worms

Comparing all treatments of water, mixture of water and rice gruel (1:1) and rice gruel, sampling on the 70th day gave the highest yields (water: 353.22±19.04mg·cm^{-2}; mixture of water and rice gruel (1:1): 573.13±27.15mg·cm^{-2}; rice gruel: 1,126.03±31.08 mg·cm^{-2}; see Table 3). However, the highest yield of 1,126.03±31.08 mg·cm^{-2} worms were found in the treatment where rice gruel was used to wet the media ingredients. The yields of these culture media showed an increasing trend over duration.

Water Quality

Temperature of the water in the raceway ranged from 22 to 26 °C throughout the entire study period. Constant water flow rate (1.14-1.30 L·min^{-1}) was maintained and the dissolved oxygen ranged between 4 and 6 mg·L.$^{-1}$

Economics of yield and production

Total media ingredients, price, yield and production cost per treatment at 70th day of culture duration are given in Table 4.While in the first experiment, 2.43 kg (20% WB, 30% SM and 20% MOC) culture media at US$ 0.53was required to yield 1 kg worms, in the second experiment, only 1.46 kg (US$ 0.31) yielded the same quantity of worms when rice gruel was used to wet the medium instead of water.

Discussion

The first experiment showed that culture medium containing 20% WB, 30% SM, 20% MOC, 20% CD and 10% sand produced 678.62 mg·cm^{-2} tubificid worms at 70-day culture duration. Production in the present study was significantly higher than 200 mg·cm^{-2}and 419.4 mg·cm^{-2} reported in previous studies by Marian and Pandian (1984) and Ahamed and Mollah (1992). The observed 3.4 and 1.6 times higher yield in our study than in the previous findings indicates the suitability of this medium for large-scale commercial culture of tubificid worms.

Table 2. Yield (mg·cm^{-2}) of tubificid worms in different treatment groups over an experimental period of 80 days.

Level (%) of wheat bran	Level (%) of soybean meal	Culture duration (days)				
		40	50	60	70	80
10	10	73.16±5.61^{Be}	114.49±5.84^{Be}	205.89±4.30^{Ae}	290.74±12.48^{Af}	258.43±29.69^{Af}
	20	86.15±3.63^{Bde}	139.60±16.28^{Bde}	253.21±17.56^{Ade}	311.82±9.01^{Af}	291.17±14.94^{Aef}
	30	98.30±3.05^{Ccde}	168.73±7.85^{Bcde}	292.42±7.35^{Ad}	330.29±15.03^{Aef}	307.44±3.83^{Aef}
20	10	96.14±2.56^{Ccde}	179.82±9.40^{Bcde}	283.44±4.68^{Ad}	314.87±10.41^{Af}	320.58±14.96^{Adef}
	20	111.34±2.89^{Ccd}	225.78±19.94^{Bbc}	388.88±13.32^{Ab}	432.78±22.47^{Acd}	406.68±11.43^{Acd}
	30	260.51±11.61^{Ea}	378.81±10.63^{Da}	516.80±9.52^{Ca}	678.62±11.50^{Aa}	613.53±8.33^{Ba}
30	10	111.10±1.23^{Ccd}	187.65±11.11^{Ccd}	310.58±14.41^{Bcd}	406.84±18.40^{Ade}	385.71±21.38^{ABcde}
	20	156.47±4.30^{Db}	256.02±12.32^{Cb}	437.23±19.00^{Bb}	585.79±15.39^{Ab}	515.20±13.12^{ABb}
	30	132.51±12.30^{Cbc}	204.87±6.33^{Cbcd}	378.78±11.44^{Bbc}	500.04±12.76^{Ac}	425.61±22.64^{ABbc}

*Values are means (± SEM) in triplicate groups. Means followed by different superscript capital letters indicate significant differences within rows and small letters denote significant differences within columns (ANOVA, HSD; $p<0.05$).

Table 3. Yield (mg cm^{-2}) of tubificid worms in different treatment groups over an experimental period of 70 days.

Treatment	Culture duration (days)			
	40	50	60	70
Only water	105.69±9.21^{Cb}	218.84±14.72^{Bc}	295.75±14.78^{Ac}	353. 22±19.04^{Ac}
Water &rice gruel (1:1)	168.93±12.10^{Ca}	336.35±36.21^{Bb}	468.27±27.98^{Ab}	573.13±27.15^{Ab}
Only rice gruel	209.55±9.13^{Da}	519.96±12.89^{Ca}	783.78±30.38^{Ba}	1126.07±13.08^{Aa}

*Values are means (± SEM) in triplicate. Means followed by different superscript capital letters are significantly different within rows and small letters denote significant differences within columns (ANOVA, HSD; p<0.05).

Table 4. Total media ingredients, price, yield and production cost per treatment at 70th day of culture.

Ingredients	Levels of media ingredients (%)								
Wheat bran	10			20			30		
Soybean meal	10	20	30	10	20	30	10	20	30
Mustard oil cake	20	20	20	20	20	20	20	20	20
Total weight of media (kg)	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
Price (BDT)/kg medium	8.3	10.8	13.3	10.1	12.6	15.1	11.9	14.4	16.9
Total price of medium (BDT)	55	71	88	67	83	100	79	95	112
Total yield (kg)	1.16	1.25	1.32	1.26	1.73	2.71	1.63	2.34	2.0
Cost (BDT) of production·kg^{-1} worms	47	57	67	53	48	37	48	41	56
Cost (US$) of production·kg^{-1} worms	0.67	0.81	0.96	0.76	0.69	0.53	0.69	0.59	0.80

. This higher yield could be due to the suitability of the nutrient supply for better growth and proliferation of the worms. The lower yield reported in the previous study by Ahamed and Mollah (1992) in a medium of 35% WB, 25% CD, 20% MOC and 20% sand could be due to the absence of soybean meal rendering it as a less suitable culture medium. Use of 30% SM in the present study could have provided the required level of protein for growth and propagation. While the present study shows that 2.43 g media ingredients (20% WB, 30% SM, 20% MOC, 20% CD and 10% sand) produce 1 g of worms, other studies required 18 g, 25 g (75% CD and 25% sand) and 2.85 g of media to produce the same quantity (Marian and Pandian1984; Marian et al.1989; Ahamed and Mollah 1992).

The lower production of worms in treatment groups receiving 10% WB in combination with 10, 20 and 30% SM clearly indicates the effects of media ingredients on the production of these worms. This lower production could have resulted from a lower level of nutrients available in the culture medium. In contrast, the treatment groups receiving 30% WB yielded better production than those receiving 10% WB, but showed lower production than those containing 20% WB. This variable production of worms could be because of inadequate supply of nutrients and binder (sand) in the culture medium. Without binder (sand), the major part of the media ingredients may have been washed out with water flow, although the medium (30% WB mixed with 30% SM, 20% MOC and 20% CD) had high nutrient content.

The highest worm yield was found on day 70 of culture. This denotes the maximum utilisation of nutrients from these media ingredients for proper growth and also indicates the suitability of the culture duration in maximising the growth of tubificid worms. Culturing the worms for less than or more than 70 days is not optimal and commercially not viable because, earlier harvest will result in a smaller yield, while a longer culture period will increase the culture cost. Mariom and Mollah (2012) have also demonstrated that a culture duration of 70 days is optimum for the production of tubificid worms. The yield declined after 80 days of culture, which can be associated with the decreased carrying capacity of the culture medium.

In experiment 2 (effect of rice gruel), the observed exceptional yield (1,126.07 $mg{\cdot}cm^{-2}$) was found on the70th dayof culture in medium containing 20% WB, 30% SM, 20% MOC, 20% CD and 10% sand, which was determined from the first experiment as the better medium. This demonstrates the beneficial effects of excess carbohydrate on the growth of tubificid worms. In the present study, both experiments demonstrated that WB and SM are important media ingredients and have a definite effect on the production of tubificid worms. As reported by Ahamed and Mollah (1992), these ingredients contain valuable minerals and organic matter. Kaster (1980) stated that 50% of tubificid worms reached sexual maturity within 40 days at 15 °C on 7% organic carbon content in the media. Kaster also demonstrated that the duration required to reach sexual maturity significantly decreased when temperature and organic carbon content increased in the culture media.

The reduction of production cost and media requirement by half when rice gruel is used instead of water clearly indicates the positive effects of rice gruel in producing higher yields of tubificid worms. Carbohydrate in the gruel could have enhanced the growth and proliferation of the worms.

From an environmental perspective, water temperature and dissolved oxygen concentrations play an important role in the large-scale mass culture of tubificid worms (Li 2001).The water temperature (21 to 26°C) and dissolved oxygen (4 to 6 $mg{\cdot}L^{-1}$) indicate optimum limits, with water flow rate of 1.14 to1.30 $L{\cdot}min^{-1}$ (Poddubnaya 1980; Davis 1982; Marian and Pandian 1984).

Conclusion

The study revealed that the culture medium containing 20% WB, 30% SM, 20% MOC, 20% CD and 10% sand is suitable for producing 678.62 $mg{\cdot}cm^{-2}$ tubificid worms after a culture period of 70 days. The use of rice gruel instead of water to wet the media ingredients may increase the yield by nearly two-fold (1,126.07 mg cm^{-2}) and reduce the production cost by half over a 70-day culture period.

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References

Ahamed, M.T. and M.F.A. Mollah. 1992. Effects of various levels of wheat bran and mustard oil cake in the culture media on tubificid production. Aquaculture 107:107-113.

Alam, M.S. and M.F.A. Mollah. 1988. Formulation of an artificial dry feed for primary nursing of catfish (*Clarias batrachus*) larvae. Bangladesh Journal of Fisheries 11:71-75.

Buddington, P.K. and S.J. Doroshove. 1984. Feeding trials with hatchery produced white sturgeon (*Acipenser transmontanus*). Aquaculture 36:237-243.

Davis, J.R. 1982. New records of aquatic Oligochaeta from Texas with observation on their ecolological characteristics. Hydrobiologia 96:15-29.

DoF (Department of Fisheries) 2011. National Fisheries Week (Jatio Matshya Shaptah in Bangali). 136 pp.

FRSS (Fisheries Resources Survey System). 2008. Fisheries Statistical Yearbook of Bangladesh 2007-2008. Department of Fisheries (DoF), Bangladesh. pp. 34-35.

Giere, O. and O. Pfannkuche. 1982. Biology and ecology of marine oligochaete, a review. In: Oceanography and Marine Biology (ed. M. Barnes), pp. 173-308. Aberdeen University Press.

Kaster, J.L. 1980. The reproductive biology of *Tubifex tubifex* Muller (Annelida: Tubificidae). American Midland Naturalist 104:364-366.

Lietz, D. M. 1987. Potential for aquatic oligochaetes as live food in commercial aquaculture. Hydrobiologia 155:309-310.

Li, R.-X.. 2001. Studies on the growth and reproduction of *Tubifex tubifex* Müller. Acta Hydrobiologica Sinica 25:14-20.

Marian, M.P., S. Chandran and T.J. Pandian. 1989. A rack culture system for *Tubifex tubifex.* Aquaculture Engineering 8:329-337.

Marian, M. P. and T. J. Pandian. 1984. Culture and harvesting techniques of *Tubifex tubifex*. Aquaculture 42:303-345.

Mariom and Mollah, M.F.A. 2012. Development of a suitable culture medium for the production of Tubificid worms. Asian Fisheries Science 25:40-51.

Mollah, M.F.A. and M.T. Ahamed. 1989. A note on preliminary study of culture of tubificid worms. Bangladesh Journal of Fisheries 12:91-95.

Mollah, M.F.A and M. Nurullah. 1988. Effects of feeding frequency on the growth and survival of catfish (*Clarias batrachus*) larvae. Bangladesh Journal of Fisheries 11:9-14.

Phillips, G.R. and D.R. Buhler. 1979. Influences of dieldrin on the growth and body composition of fingerling of rainbow trout (*Salmo gairdneri*) fed Oregon moist pellets or tubificid worms (*Tubifex* sp.). Journal of the Fisheries Research Board of Canada 36:77-80.

Poddubnaya, T.L. 1980. Life cycles of mass species of Tubificidae (Oligochaeta). In:Proceedings of the first international symposium on aquatic oligochaete biology. Sydney, British Colombia, Canada, May 1-4, 1979. Aquatic oligochaete biology (ed. R.O. Brinkhurst and D.G. Cook), pp. 175-184. New York: Plenum Press.

Proulx, D. and J. De La Noüe 1985. Growth of *Daphnia magna* on urban wastewaters tertiarily treated with *Scendesmus* sp. Aquaculture Engineering 4:93-111.

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