



## A model for tubificid worm (*Tubifex tubifex*) production and its effect on growth of three selected ornamental fish

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**Abstract.** The demand for sludge worms, *Tubifex tubifex* is increasing with the growth of aquaculture as low-cost live feed in hatcheries and ornamental fish nurseries. The study was conducted to determine the optimal culture medium for *T. tubifex* worm's production and to find out the inclusion effects of worms as feed on ornamental fish rearing. Three media-based treatments (T-1: mustard oil cake 20%, wheat bran 10%, soybean meal 10%, rice bran 20%, cow-dung 20%, sand 10%, fish pond mud 10%; T-2: mustard oil cake 10%, wheat bran 10%, soybean meal 10%, rice bran 10%, straw 5%, fish scale, stomach and fin 25%, cow-dung 20%, fish pond mud 10% and T-3: cow-dung 75%, sand 15%, fish pond mud 10%) with three replications of each were used to culture the worms for 100 days in nine cemented culvert system (160×25×10 cm<sup>3</sup>). The growth performance of three popular ornamental fishes koi carp (*Cyprinus carpio*), comet goldfish (*Carassius auratus auratus*) and goldfish (*Carassius auratus*) were determined by replacing the commercial feed at different level with tubificid rearing in fiberglass aquarium (45×30×30 cm<sup>3</sup>) for 32 days duration. Maximum yield (959.23 44.34 mg·cm<sup>-2</sup>) of tubificid biomass was recorded in T-2 (mustard oil cake-10%, wheat bran-10%, soybean meal-10%, rice bran-10%, straw-5%, fish scale, intestine, stomach and fin-25%, cow-dung-20%, mud collected from fish pond-10%) with a peak at day 70th. The fish fed with 50% commercial feed + 50% *T. tubifex* in TF-2 showed significantly ( $p < 0.05$ ) higher growth and survival performance than other treatments. The mean value of water quality parameters (i.e. temperature, pH and dissolved oxygen) were statistically insignificant ( $p > 0.05$ ) among the three treatments. Approximately 1.83 kg media were needed to produce 1.0 kg tubificid worm in treatment T-2. T-2 media is recommended as suitable media for commercial tubificid worm production and the inclusion of 50 % tubificid worms in the feed can be suggested for the ornamental fish rearing.

**Keywords:** Live feed, Growth parameters, FCR.

### Introduction

Bangladesh is ranked the third position among the fish producing countries in the world after China and India (FAO 2018). However, fish farming have been hindered due to shortage of necessary ingredients of fish feeds in Bangladesh. In aquaculture, fish feed is an essential component for fish production and one of the most indispensable items that covered almost 60% of the total fish production cost (Jewel *et al.* 2016). Although considerable variation exists among the fish feed ingredients, the most common sources of carbohydrates in fish feed is cereal grains which cannot be commercially replaced with the other sources. At present, the current requirement of fish feed is about 0.7-0.8 million metric tons (MT), whereas local feed manufacturers manufactured semi-industrial feed using simple mixing machine projected 0.5-0.6 million MT of feed per month. The demand for both sinking and floating fish feed an estimated is to have a Compound Annual Growth Rate (CAGR) of 5-7 % (DoF 2018).

Tubificid worms are aquatic invertebrates in Tubificidae family belonging to the Oligochaeta class usually known as sewage or sludge worms. *Tubifex tubifex* grows robustly in the sludge particularly in sewage-fed water canals and in open drains that formed reddish color colonies in the mud containing rich organic debris (Mandall *et al.* 2016, Haque *et al.* 2020). These sludge worms are capable of acquired nutrition by ingesting sediment, bacteria, and molecules by individual's body wall (Rodriguez *et al.* 2001). As a low-cost live fish feed for juveniles, the use of sludge worm has been long practiced in fish farms for its highly nutritious value (5.57 kcal·g<sup>-1</sup> in dry condition) (Mandall *et al.* 2016). Compared to artificial feed the cultured larvae performed very well in the live feed (Immanuel *et al.* 2001, Mahfuj *et al.* 2012, Mandall *et al.* 2016). Tubificid worms have been identified as a high-protein, high-amino-acid-profile source for fish growth (Jhingran 1982). This worm has a large market in fish hatcheries particularly in spawn production of catfishes and rearing of ornamental fishes (Mandal *et al.* 2016). The existing supply of tubificid worms in Bangladesh come from wild sources that are inconsistent and unreliable compared to fulfill demand. Besides, in summer, most of the drain become dry that create the scarcity of tubificid worm's collection. Therefore, the collection of wild tubificid worms from the sludge has a lack of purity and chance to the transmission of the pathogen to fish (Mandal *et al.* 2016). Subsequently, the culture of *T. tubifex* in the captive condition is necessary to meet up the requirement and offer a reliable delivery source. Moreover, the demand for ornamental fishes is increasing day by day (Ansari *et al.* 2014) and internationally, is considered as an industry due to its high market demand and profitability. The growth rate and survival rate of ornamental fishes depend on the types of feed ingested (Yuli *et al.* 2020). Sludge worm are one of the most important live foods that can play an important role as supplementary food to rearing the ornamental fishes. In this regard, the live feed is one of the essential factors to increase the growth performance of larvae due to its excellent supply source of carbohydrate, lipid, and protein (Conceicao *et al.* 2010). The high protein contained feed is required for farming ornamental fish that enhanced the production cost (Mente *et al.* 2017).

The ornamental fish culture is increasingly popular around the world due to its simple operating system as well as lower operating costs. For developing countries, the reproduction and nursing of ornamental fish represent a great opportunity for income growth in rural communities (Olivier 2001). Among 34 varieties of available ornamental fishes in Bangladesh, koi carp (*Cyprinus carpio*), comet goldfish (*Carassius auratus auratus*) and goldfish (*Carassius auratus*) were very popular in Bangladesh (Kabir and Hawkeswood 2021). Although the demand of tubificid worms is high in the ornamental fish rearing, but the significant progress has not been reported to improve the culture strategies of *T. tubifex* worms in Bangladesh yet (Ahamed and Mollah 1992, Mosharaf 2009, Mariom and Mollah 2012, Mandal *et al.* 2016). Hence, it is essential to develop a suitable culture medium for profitable production of tubificid worms as an alternative fish feed. Thus, the aim of this study was to identify the suitable culture medium for the finest growth of *T. tubifex* and their effect on growth parameters of three koi carp (*Cyprinus carpio*), comet goldfish (*Carassius auratus auratus*) and goldfish (*Carassius auratus*) ornamental fishes in Bangladesh.

## Materials and Methods

***Selection of media and duration of the experiment:*** The experiment was conducted to determine suitable media for tubificid worm production in the fish hatchery complex of Rural

Development Academy (RDA), Bogura for 100 days where different proportion of culture media ingredients used as experimental variables only (Table I).

**Table I. Media ingredients for three treatments**

Ingredients	Treatment-1	Treatment-2	Treatment-3
Mustard oil cake	20%	10%	-
Wheat bran	10%	10%	-
Soybean meal	10%	10%	-
Rice bran	20%	10%	-
Straw	-	5%	-
Fish scale, stomach, and fin	-	25%	-
Cow-dung	20%	20%	75%
Sand	10%	-	15%
Mud collected from the fish pond	10%	10%	10%

**Experimental design:** Nine cemented culverts ( $160 \times 25 \times 10$  cm<sup>3</sup>) under a tin shad were used in experiment with ( $3 \times 3$  factorial designs) with inlet and outlet facilities including a regulatory tap for water flow control. Initially, the rate of water flow was low ( $0.75$  liter·min<sup>-1</sup>) to avoid the erosion of base media while later it was increased to  $1.2$  liter·min<sup>-1</sup> continuously for constant oxygen supply in culture culverts.

**Base medium, inoculation and periodic supply of food substances:** The mud collected from the fish pond was used as a base medium with a thickness of  $2.5$  cm in each culvert, sieved with fine-mesh nylon net to remove the unwanted rough materials. To make the medium, other ingredients were collected from the local market, weighing by an electric balance to maintain the ratios (Table 1) and thoroughly mixed by a bamboo stick. Before introducing in the culvert, the media were allowed for decomposition for seven days and subsequent mixing was required twice a day for better mineralization as recommended by Hossain *et al.* (2011). Wild tubificid worm was collected from different drains of Rural Development Academy (RDA) campus and cleaned by using fine mesh nylon net with running water and kept for  $24$  h in a flowing system for conditioning before inoculation into the medium in culverts for culture. *T. tubifex* was inoculated uniformly covering the entire culvert at  $1.25$  mg·cm<sup>-2</sup> rate. The medium was applied as food spreading in each culvert homogenously  $3$  h after the application of wild tubificid inoculums at the rate of  $250$  mg·cm<sup>-2</sup>. Supply of the culture media was started after 10<sup>th</sup> day of tubificid worm's inoculation at the same rate and continued 10<sup>th</sup> days interval up to the end of the experiment.

**Sampling of tubificid worms:** A sampling of tubificid worm was started after 40 days of tubificid worm inoculation in the culture media when it is formed colony and continued up to the end of the study with 10 days interval. The sampling of tubificid worms was carried by using a sampler i.e. glass tube having  $2.2$  cm diameter randomly from three selected areas of each culture culvert. Then the whole content of sample was kept in a nylon mesh net and washed slowly with running water and unwanted materials were cleaned by using forceps. After that, the collected biomass was kept in a beaker contains fresh water. Finally, the collected biomass were kept on blotting paper for soaking the excess water and then weighted.

**Measurement of water quality parameters:** The water quality parameters i.e. water temperature (°C), dissolved oxygen (ppm), pH were recorded every 10 days interval before sampling following standard methods (APHA 2002). But dissolved oxygen was determined after 30 min of media application and then 10 h after media settled to check the suitability for the culture of tubificid worms.

**Determination of the effect of *Tubifex tubifex* as food on growth of three ornamental fishes:** To observe the effect on growth of inclusion of tubificid worms in fish feed for three ornamental fishes namely, koi carp (*Cyprinus carpio*), goldfish (*Cyprinus auratus*) and comet goldfish (*Cyprinus auratus auratus*) were used.

Three selected popular aquarium fishes, koi carp fish, comet goldfish and goldfish, rearing in fiber glass aquarium (45×30×30 cm<sup>3</sup>) were stocked with 45 larvae for 32 days. Three feed regimes (TF-Treatment of Feeding) i.e. TF-1= 100% Commercial feed; TF-2= 50% Commercial feed + 50% *T. tubifex* and TF-3= 75% Commercial feed+25% *T. tubifex* with three replications were used to determine the effect on the growth of three selected popular aquarium fishes. The initial average weight in TF-1, TF-2 and TF-3 for koi carp larvae were 0.28±0.02 g, 0.29±0.02 g, and 0.28±0.02 g, respectively; comet goldfish larvae were 0.25±0.03 g, 0.24±0.03 g, and 0.25±0.084 g, respectively and goldfish larvae were 0.24±0.05 g, 0.23±0.05 g, and 0.23±0.05 g, respectively. Following calculations were used to observe the growth parameter of the three selected ornamental fishes:

Weight gain (g) = Mean final weight (g) – Mean initial weight (g)

Specific growth rate (SGR) (% body weight/day) =  $\frac{\ln W_2 - \ln W_1}{T_2 - T_1} \times 100$

Where, W<sub>2</sub> = final weight, W<sub>1</sub> = initial weight, T<sub>2</sub> = time of final weight in days, T<sub>1</sub> = time of initial weight in days.

Feed conversion ratio (FCR) =  $\frac{\text{Feed given (dry weight)}}{\text{Bodyweight gain (wet weight)}}$

Survival rate (%) =  $\frac{\text{Number of surviving fish}}{\text{Total number of fish stocked}} \times 100$

**Statistical analysis:** All the statistical analyses were performed by using SPSS software (SPSS Inc., version 25.0). Descriptive statistics and one-way analysis of variance (one-way ANOVA) with the level of significance at  $p < 0.05$  were followed. To identify the significant differences among the treatments Tukey's HSD post hoc was carried out.

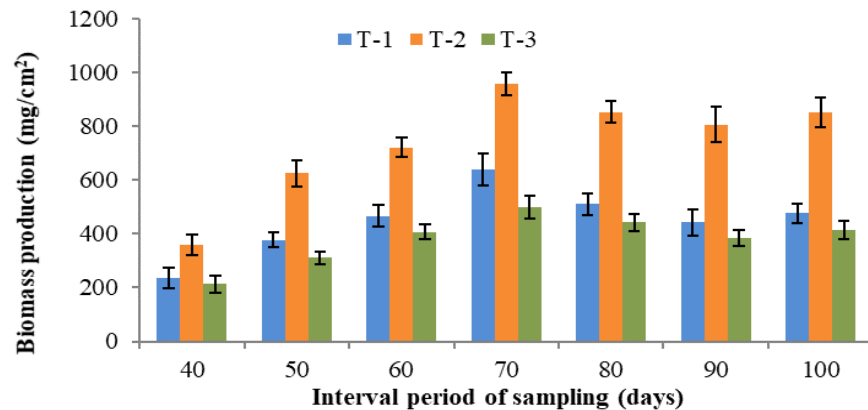
## Results

**Production of *Tubifex* worm in different culture media:** The highest mean standing biomass of *T. tubifex* was 640.3±61.22 mg·cm<sup>-2</sup>, 959.23±44.34 mg·cm<sup>-2</sup>, 500.53±41.27 mg·cm<sup>-2</sup> in T-1, T-2 and T-3, respectively at 70<sup>th</sup> experimental day (Table II, Fig. 1). An increasing trend of total standing biomass of *T. tubifex* was observed in all treatments until 70<sup>th</sup> experimental periods and then gradual decline of standing biomass up to the end of the study (100<sup>th</sup> day) (Fig. 1).

**Table II. Standing biomass ( $\text{mg}\cdot\text{cm}^{-2}$ ) of *Tubifex tubifex* in three treatments during 100 days study periods (Mean $\pm$ SD, n= 3)**

Treatments	Periods of the experiment (days)						
	40	50	60	70	80	90	100
T-1	235.99 $\pm 37.08^a$	378.11 $\pm 29.13^a$	467.37 $\pm 41.67^a$	640.3 $\pm 61.22^b$	511.39 $\pm 39.9^a$	444.49 $\pm 48.87^a$	476.59 $\pm 36.49^a$
T-2	359.23 $\pm 38.05^b$	625.4 $\pm 48.31^b$	723.03 $\pm 37.37^b$	959.23 $\pm 44.34^c$	854.67 $\pm 42.07^b$	806.87 $\pm 65.99^b$	853.27 $\pm 56.29^b$
T-3	213.89 $\pm 31.26^a$	311.22 $\pm 23.16^a$	407.52 $\pm 26.91^a$	500.53 $\pm 41.27^a$	443.18 $\pm 31.32^a$	386.57 $\pm 29.28^a$	415.58 $\pm 33.44^a$
F-value	13.11	66.29	65.52	67.07	100.79	61.44	90.02
Significance	*	*	*	*	*	*	*

Values with different superscripts in a vertical column are significantly different ( $p < 0.05$ )



**Fig. 1.** Standing biomass ( $\text{mg}\cdot\text{cm}^{-2}$ ) of *Tubifex tubifex* in three different treatments during 100 days of experimental periods. Vertical bars are represented as Mean $\pm$ SD.

A one-way ANOVA test revealed significant differences ( $p < 0.05$ ) in standing biomass among the three treatments. Results showed that the standing biomass of *T. tubifex* in T-2 was greater ( $p < 0.05$ ) than in T-1 and T-3 throughout the culture periods (Table II).

**Water quality parameters:** In every treatment like T-1, T-2 and T-3, the mean temperature values were  $30.84 \pm 1.72$  °C,  $31.71 \pm 2.45$  °C, and  $32.06 \pm 2.07$  °C, respectively. Similarly the mean pH values were  $7.15 \pm 0.44$ ,  $7.63 \pm 0.63$  and  $7.64 \pm 0.56$ , respectively in T-1, T-2 and T-3 treatments. Moreover, the mean dissolved oxygen values were  $6.35 \pm 0.34$  ppm,  $6.44 \pm 0.92$  ppm and  $6.11 \pm 0.37$  ppm, respectively in the three treatments (i.e. T-1, T-2 and T-3). Statistical analysis revealed non-significant differences ( $p > 0.05$ ) among the three treatments (Table III).

**Table III. Water quality parameters of three different treatments (Mean±SD) in culverts**

Treatments	Water quality parameters		
	Temperature (°C)	pH	Dissolved Oxygen (ppm)
T-1	30.84 ± 1.72 <sup>a</sup>	7.15 ± 0.44 <sup>a</sup>	6.35 ± 0.34 <sup>a</sup>
T-2	31.71 ± 2.45 <sup>a</sup>	7.63 ± 0.63 <sup>a</sup>	6.44 ± 0.92 <sup>a</sup>
T-3	32.06 ± 2.07 <sup>a</sup>	7.64 ± 0.56 <sup>a</sup>	6.11 ± 0.37 <sup>a</sup>
F value	0.27	0.77	0.12
Significance	NS	NS	NS

Means with same superscripts in the same column are non-significant ( $p > 0.05$ )

The present study focused that about 1.83 kg media (10 % mustard oil cake, 10 % wheat bran, 10 % soybean meal, 10 % rice bran, 5 % straw, 25 % stomach, intestine, fish scale, fin, 20 % cow-dung and 10 % mud) was required to produced 1 kg of tubificid worms in T-2 whereas 2.73 kg media in T-1 and 3.50 kg media in T-3.

**Effect of tubificid worms as feed on the growth of selected aquarium fishes:** The 32 days trial was carried out to observe the effect of *Tubifex tubifex* as feed (Treatment of feed-TF), on growth performance and survival rate of three selected aquarium species viz. koi carp (*Cyprinus carpio*), comet goldfish (*Cyprinus auratus auratus*) and goldfish (*C. auratus*). The growth parameters and survival rate of fish species are given in Table IV.

The highest average final weight of koi carp was  $3.04 \pm 0.13$  g in TF-2 (50% *T. Tubifex* incorporated diet). In the case of comet goldfish and goldfish, the highest average final weights in TF-2 were  $2.21 \pm 0.14$  g,  $1.97 \pm 0.27$  g, respectively (Table IV). The highest weight gain of koi carp was observed in TF-2 which is considerably higher ( $p < 0.05$ ) than those of the two treatments, followed by  $1.71 \pm 0.16$  g in TF-1 and  $1.76 \pm 0.23$  g in TF-3 (Table IV). Similarly, the highest weight gain of comet goldfish and goldfish were found  $1.97 \pm 0.11$  g  $1.84 \pm 0.09$  g in TF-2, which is significantly higher ( $p < 0.05$ ) than the other treatments (Table IV). At the end of the experiment, the maximum specific growth rate (SGR %) in koi carp fish, comet goldfish and goldfish were  $7.53 \pm 0.05$  %,  $6.81 \pm 0.12$  % and  $6.94 \pm 0.129$  % in TF-2, respectively, which were significantly higher ( $p < 0.05$ ) (Table IV). Significant difference ( $p < 0.05$ ) feed conversion ratio (FCR) was observed among the treatments. The FCR of koi carp fish was found lowest ( $1.78 \pm 0.06$ ) in TF-2. Similarly, in comet goldfish and goldfish, the FCR values were  $2.07 \pm 0.04$  and  $2.11 \pm 0.04$  in TF-2, which were significantly lower compared to other treatments. The survival rate of koi carp fish, comet goldfish and goldfish did not vary significantly ( $p > 0.05$ ) in all treatments (Table IV).

## Discussion

The highest mean stocking biomass of *Tubifex tubifex*  $959.23 \pm 44.34$  mg·cm<sup>-2</sup> was observed at the 70<sup>th</sup> day in T-2, thus indicating the suitability of this culture medium to increase biomass than other two treatments. Ahmed and Mollah (1992) and Mosharaf (2009) observed the highest production of tubificids were 419.4 mg·cm<sup>-2</sup> and 503.39 mg·cm<sup>-2</sup> in a culture medium consisting mustard oil cake, wheat bran, cow dung, and sand. Hasan *et al.* (2019) found the highest production  $683.98 \pm 3.86$  mg·cm<sup>-2</sup> in the medium of soybean meal, mustard oil cake and mud wetted in cattle blood. The presence of dietary protein in cattle blood might be the reason for the

maximum yield of *T. tubifex* (Hasan *et al.* 2019). Culture media having raw fishes was also responsible for the maximum number of the new cohort of tubificid worms (Begum *et al.* 2014).

**Table IV. Effect of replacement levels of commercial feed with *Tubifex tubifex* on growth performance in three selected aquarium fishes after 32 days rearing period (Mean  $\pm$  SD, n = 20)**

	Fish species	TF-1	TF-2	TF-3	F-value	Significance
Initial weight (g)	Koi carp	0.28 $\pm$ 0.02	0.29 $\pm$ 0.02	0.28 $\pm$ 0.02	-	-
	Comet goldfish	0.25 $\pm$ 0.03	0.24 $\pm$ 0.03	0.25 $\pm$ 0.04	-	-
	Gold fish	0.24 $\pm$ 0.05	0.23 $\pm$ 0.05	0.23 $\pm$ 0.05	-	-
Final weight (g)	Koi carp	1.98 $\pm$ 0.18	3.04 $\pm$ 0.13	2.04 $\pm$ 0.25	-	-
	Comet goldfish	1.81 $\pm$ 0.10	2.21 $\pm$ 0.14	2.04 $\pm$ 0.12	-	-
	Gold fish	1.56 $\pm$ 0.07	1.97 $\pm$ 0.11	1.79 $\pm$ 0.08	-	-
Weight gain (g)	Koi carp	1.71 $\pm$ 0.16 <sup>a</sup>	2.75 $\pm$ 0.11 <sup>b</sup>	1.76 $\pm$ 0.23 <sup>a</sup>	141.11	*
	Comet goldfish	1.56 $\pm$ 0.07 <sup>a</sup>	1.97 $\pm$ 0.11 <sup>b</sup>	1.79 $\pm$ 0.08 <sup>a</sup>	78.18	*
	Gold fish	1.58 $\pm$ 0.07 <sup>a</sup>	1.84 $\pm$ 0.09 <sup>b</sup>	1.68 $\pm$ 0.10 <sup>ab</sup>	8.75	*
SGR (%)	Koi carp	6.13 $\pm$ 0.05 <sup>a</sup>	7.53 $\pm$ 0.05 <sup>b</sup>	6.45 $\pm$ 0.10 <sup>a</sup>	355.09	*
	Comet goldfish	6.20 $\pm$ 0.17 <sup>a</sup>	6.81 $\pm$ 0.12 <sup>b</sup>	6.61 $\pm$ 0.14 <sup>b</sup>	13.06	*
	Gold fish	6.37 $\pm$ 0.44 <sup>a</sup>	6.94 $\pm$ 0.29 <sup>a</sup>	6.62 $\pm$ 0.29 <sup>a</sup>	2.07	NS
FCR	Koi carp	2.22 $\pm$ 0.05 <sup>b</sup>	1.78 $\pm$ 0.06 <sup>a</sup>	2.09 $\pm$ 0.03 <sup>b</sup>	59.03	*
	Comet goldfish	2.39 $\pm$ 0.04 <sup>b</sup>	2.07 $\pm$ 0.04 <sup>a</sup>	2.31 $\pm$ 0.06 <sup>b</sup>	31.84	*
	Gold fish	2.63 $\pm$ 0.04 <sup>c</sup>	2.11 $\pm$ 0.04 <sup>a</sup>	2.52 $\pm$ 0.04 <sup>b</sup>	135.18	*
Survival rate (%)	Koi carp	71.11 $\pm$ 4.45 <sup>a</sup>	72.59 $\pm$ 2.57 <sup>a</sup>	70.37 $\pm$ 1.28 <sup>a</sup>	0.412	NS
	Comet goldfish	70.37 $\pm$ 3.37 <sup>a</sup>	68.89 $\pm$ 2.22 <sup>a</sup>	69.63 $\pm$ 3.39 <sup>a</sup>	0.176	NS
	Gold fish	69.63 $\pm$ 4.10 <sup>a</sup>	70.37 $\pm$ 3.39 <sup>a</sup>	68.15 $\pm$ 4.62 <sup>a</sup>	0.180	NS

In a row, values with dissimilar superscripts are significantly different ( $p < 0.05$ )

n = number of each fish species for each treatment

Likewise, commercial fish flake food or other protein-rich marketable sinking aqua feed having *Spirulina* sp. showed better results in terms of growth and recruitment of *T. tubifex* (Randall *et al.* 2011). Culture medium consists of high protein has a significant role for replication and maturation of tubificid worms. In the present study, T-2 containing fish intestine, stomach, fin, scale which might be the source of protein produced the highest standing biomass of tubificid worm. In this study, the maximum production of tubificid worms was documented at 70<sup>th</sup> experimental day and followed by a decreasing trend in biomass until the end of experiment (100<sup>th</sup> day). Alike, the highest production of worms was also observed on same experimental day by Hossain *et al.* (2011) and Mariom and Mollah (2012). The observation showed 70<sup>th</sup> experimental period might be suitable for an ideal carrying capacity of worm's biomass

production of its short generation time (42 days) (Marian and Pandian 1984). The trend of decreased biomass after 70<sup>th</sup> experimental days may be due to the death of a huge number of tubificid worms by virtue of lacking of sufficient food, oxygen depletion, etc. (Mariom and Mollah 2012). Fecundity of *T. tubifex* depends on water quality parameters especially temperature, rate of water flow to increase oxygen supply and type of organic contents of the culture media (Marian and Pandian 1984). The mean temperature, pH and dissolved oxygen of the three culture media were ranged between  $30.84 \pm 1.7$  to  $32.06 \pm 2.07$  °C,  $7.15 \pm 0.44$  to  $7.64 \pm 0.56$  and  $6.11 \pm 0.37$  to  $6.44 \pm 0.92$  ppm. A similar observation was also documented by Islam *et al.* (2015) and Begum *et al.* (2014). In this study, the water quality parameters were feasible and in suitable range for the replication of tubificid worms. About 1.83 kg medium was needed for the production of 1.0 kg worms in the present study, whereas Marian and Pandian (1984) reported 18 kg and Marian *et al.* (1989) observed 25 kg cow-dung was needed to produce the same amount of worms. Besides, 2.85 kg and 1.99 kg media ingredients were required for the production of 1 kg worms found by Ahamed and Mollah (1992) and Mosharaf (2009), respectively. In contrast, 1.01 kg and 0.71 kg culture media (mustard oil cake - 20%, wheat bran - 20%, soybean meal - 30%, cow-dung - 20%, fine sand- 10%) were needed to produce 1.0 kg worm, which is reported by Mariom and Mollah (2012). Although the lower amount of media were required in their studies based on the present market rate the production cost of T-2 (10 % mustard oil cake, 10 % wheat bran, 10 % soybean meal, 10 % rice bran, 5 % straw, 25 % fish scale, stomach and fin, 20 % cow-dung and 10 % mud) in the present experiment is economical than two above mentioned studies.

The growth performances of three selected ornamental fishes were determined by replacing commercial feed with tubificid worms at a different level. The highest weight gain (g) in koi carp, comet goldfish and goldfish were  $2.77 \pm 0.04$ ,  $1.96 \pm 0.03$  and  $1.84 \pm 0.12$ , respectively in TF-2 (50 % commercial feed + 50 % *T. tubifex*). After finishing the experiment, statistically significant SGR % ( $p < 0.05$ ) were found in koi carp fish, comet goldfish and goldfish i.e.  $7.53 \pm 0.05$  %,  $6.81 \pm 0.12$  % and  $6.894 \pm 0.29$  %, respectively in TF-2. In the case of FCR, the best results were also found in TF-2. In the present study, 50 % replacement of *T. tubifex* with a commercial diet may increase its protein content that might be the reason for better growth performance. Similarly, the best growth performance was observed in koi carp (Mahfuj *et al.* 2012) and goldfish (Raseduzzaman *et al.* 2014) by feeding 50 % pellet feed with 50 % chopped tubificid worms. The highest absolute weight gain was also observed in comet goldfish reported by Husnan *et al.* (2014) by feeding tubificid worms. The highest growth rate in comet goldfish larvae were observed by Mellisa *et al.* (2018) by fed with live chopped tubificid worm. Husnan *et al.* (2014) demonstrated the greatest growth performance of comet goldfish larvae in terms of the highest absolute weight gain and survival rate by feeding *Tubifex* sp. The feed can be easily eaten by fish if that feed is suitable with their feeding habits which ultimately affect fish growth. Compared to the hardly digestible feed, fish simply prefer easily digestible food (Marian and Pandian 1984). The special smell, better bright color and merely visible characters fascinated fish to prey and easy capture that made *Tubifex* sp. as better live food (Marian and Pandian, 1984, Conceicao *et al.* 2010, Husnan *et al.* 2014). Tubificid worms showed better results as a low-cost live food for larvae rearing of omnivorous, carnivorous and ornamental fishes compared to other live food i.e. rotifers, *Brachionus* sp., *Artemia* sp., *Moina* sp. etc. (Bucher 1977). The use of tubificid worms as alternative feed over formulated diets, significant survival rates and better growth performance was observed in larvae and fry rearing of ornamental koi



carp (*Cyprinus carpio*) (Mahfuj *et al.* 2012), *Clarias batrachus* (Mollah and Nurullah 1988), *Channa striatus* (Sarowar *et al.* 2010).

From this study it has been suggested that in media-based tubificid worm's culture medium with 10% mustard oil cake, 10% wheat bran, 10% soybean meal, 10% rice bran, 5% straw, 25% fish scale, stomach and fin, 20% cow-dung and 10% mud showed highest production. *T. tubifex* also has a significant effect on the growth performance of koi carp fish (*C. carpio*), comet goldfish (*C. auratus auratus*) and goldfish (*C. auratus*) fry rearing.

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