

Optimizing Chicken Blood Quantity for Sustainable Tubificid Worm Cultivation

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ABSTRACT

Tubificids are widely used in aquaculture and valued as a key live food organism for both aquarium enthusiasts and commercial fish breeders. As the abundance of tubificids in nature is declining rapidly, it is essential to culture these worms using low-cost media ingredients to meet their increasing demand. Hence, this study focused on determining the optimal quantity of chicken blood as a soaking medium for fermenting the media ingredients (30% soybean meal, 20% wheat bran, 20% mustard oil cake, 20% cow dung, and 10% sand) for cultivating tubificid worms. The worms were cultured for 90 days under four treatments in flow-through culverts ($160 \times 25 \times 10 \text{ cm}^3$) with identical media, varying only in the soaking agents: Treatment I ($2 \text{ L} \cdot \text{kg}^{-1}$ chicken blood), Treatment II ($4 \text{ L} \cdot \text{kg}^{-1}$ chicken blood), Treatment III ($6 \text{ L} \cdot \text{kg}^{-1}$ chicken blood), and Treatment IV (control, rice gruel). The highest yield ($578.97 \pm 17.03 \text{ mg} \cdot \text{cm}^{-2}$) ($p < 0.05$) was recorded on the 70th day of culture in Treatment II, indicating that this medium is well-suited for large-scale production of tubificid worms. Furthermore, the water quality parameters such as temperature ($26.93\text{--}30.37^\circ\text{C}$), dissolved oxygen (6.10–7.07 ppm), and pH (7.2–7.6) were found to be within favorable limits, which confirms their appropriateness for culturing this live food. Using $4 \text{ L} \cdot \text{kg}^{-1}$ chicken blood as the soaking medium significantly enhanced tubificid yield, offering a cost-effective solution to meet the growing demand for live food in large-scale aquaculture.

Keywords: Aquatic oligochaete, Culture media, Live food, Soaking agent

INTRODUCTION

The aquaculture industry is advancing alongside global trends in Bangladesh (Samanta Chandan and Roy, 2024; Mariom *et al.*, 2026). The nation has emerged as a significant fish producer, achieving an output of 4.915 MMT, with aquaculture contributing 58.03% of the total production (DOF, 2023). This sector plays a crucial role in Bangladesh's agricultural and socioeconomic development, accounting for 2.43% of the national GDP, 22.14% of the agricultural GDP, and contributing 0.80% to the country's export revenues. Investing in its advancement is essential for the country's long-term growth (DOF, 2023).

Feed plays a pivotal role in the aquaculture industry by providing essential nutrients like proteins, lipids, carbohydrates, vitamins, and minerals necessary for fish. However, the industry is confronted with numerous obstacles, including limited availability and the high cost of feed ingredients necessary to produce adequate feed (Sulaiman *et al.*, 2025). Moreover, feed represents the largest expense in aquaculture, accounting for almost 70% of total production costs (Zebua *et al.*, 2025). The rising price of fish feed has recently raised serious concerns and has threatened the aquaculture industry's long-term viability.

Tubificid worms, belonging to the Tubificidae family (class Oligochaeta), are slender

aquatic invertebrates often referred to as sewage or sludge worms, ranging from 2 mm at hatching to 4–15 cm as adults (Aston, 1973; Alam *et al.*, 2021). Sludge worms have long been used in aquaculture as an affordable live food for juvenile fish (Alam *et al.*, 2021). They are also highly regarded in aquaculture for their excellent nutritional value, which makes them especially advantageous as a live food source for fish (Marian, 1982). With a nutritional breakdown of 63.32% protein, 28.84% lipid, and 7.95% ash, as well as an impressive caloric value of 5,575 cal·g⁻¹ (dry weight basis), tubificid worms rank among the best live foods for fish (Giere and Pfannkuche, 1982; Mollah and Ahamed, 1989). Additionally, the protein in tubificid worms has an amino acid profile well-suited for fish nutrition (Jhingran, 1982). Numerous studies have demonstrated the superiority of tubificid worms over formulated feeds in promoting growth and survival rates in fish larvae and fry (Phillips and Buhler, 1979; Mollah and Tan, 1982; Buddington and Doroshov, 1984; Alam and Mollah, 1988; Mollah, 1991; Mollah *et al.*, 2009; Mahfuj *et al.*, 2012; Alam *et al.*, 2021). Therefore, the demand for tubificid worms in the aquaculture industry in Bangladesh remains significantly high.

Several studies have explored the selection of the suitable culture media for tubificid worms (Mariom and Mollah, 2012; Begum *et al.*, 2014; Jewel *et al.*, 2016). However, limited research has focused on the use of chicken blood as a soaking agent for the media ingredients. Our previous work (Islam *et al.*, 2015) showed that incorporating chicken blood to ferment the media ingredients enhanced the growth and yield of tubificid worms. Nevertheless, the study did not determine the optimal quantity of chicken blood required for soaking the media. Therefore, the present study was ultimately aimed at determining the optimal quantity of chicken blood for soaking the media ingredients such as wheat bran, soybean meal, mustard oil cake, cow dung, and sand for the sustainable production of tubificid worms.

MATERIALS AND METHODS

Study area

The research was conducted in the southern culvert system of the Faculty of Fisheries at Bangladesh Agricultural University (BAU) in Mymensingh (Latitude 24.72291° N, Longitude 90.43077° E).

Experimental design

Tubificid worms were maintained in a cement culvert system (160×25×10 cm³) under four treatments for 90 days to determine the appropriate quantity of chicken blood required to soak the media constituents. A total of 12 culverts were used in a completely randomized design, consisting of four treatments, each replicated three times, with the soaking agent serving as the only experimental variable. The culture medium suggested by Mariom and Mollah (2012) was used in all treatments, which contained 30% soybean meal, 20% wheat bran, 20% mustard oil cake, 20% cow dung, and 10% sand. Variable amounts of chicken blood were used in different treatments (2 L·kg⁻¹ in Treatment I, 4 L·kg⁻¹ in Treatment II, and 6 L·kg⁻¹ in Treatment III), whereas rice gruel was used in the control group (Treatment IV) for soaking the media components (Table 1). The system was placed under a tin shed to avoid influence from environmental factors such as rain and, sunlight.

Preparation of the culture unit

The worms were cultured following the standardized protocol established by Mariom and Mollah (2012). Briefly, the culvert system was prepared by being thoroughly washed and cleaned before the start of the experiment. To provide a steady supply of water from a deep well, each culvert was connected to a water storage tank. Each culvert was equipped with an intake (a porous PVC pipe 180 cm × 1 cm²) and an outlet to ensure constant water replenishment and removal.

Collection of media ingredients

Culture media constituents, including wheat bran, soybean meal, and mustard oil cake, were obtained commercially. Cow dung was acquired from BAU's dairy farm, and river sand was collected from the Old Brahmaputra River bordering the university premises. Fresh rice gruel was gathered from the kitchens of student dormitories at BAU, and chicken blood was procured from the K.R. Market within BAU, where chickens are sold and slaughtered.

Nutritional composition analysis of media components

The proximate composition of chicken blood was analyzed using standard procedures outlined by the Association of Official Analytical Chemists (AOAC, 1980) and presented in Table 2 along with the proximate composition of other media constituents.

Media preparation

All ingredients were accurately weighed in predetermined proportions using an analytical laboratory balance (TANITA, KD-160), thoroughly mixed with a stirring rod, and left to decompose for seven days (Hossain *et al.*, 2011). After seven days, $250 \text{ mg} \cdot \text{cm}^{-2}$ of the fermented media was introduced into the culverts so that each culvert received 1,000 g of the medium and left for 24 h to allow the medium to settle down.

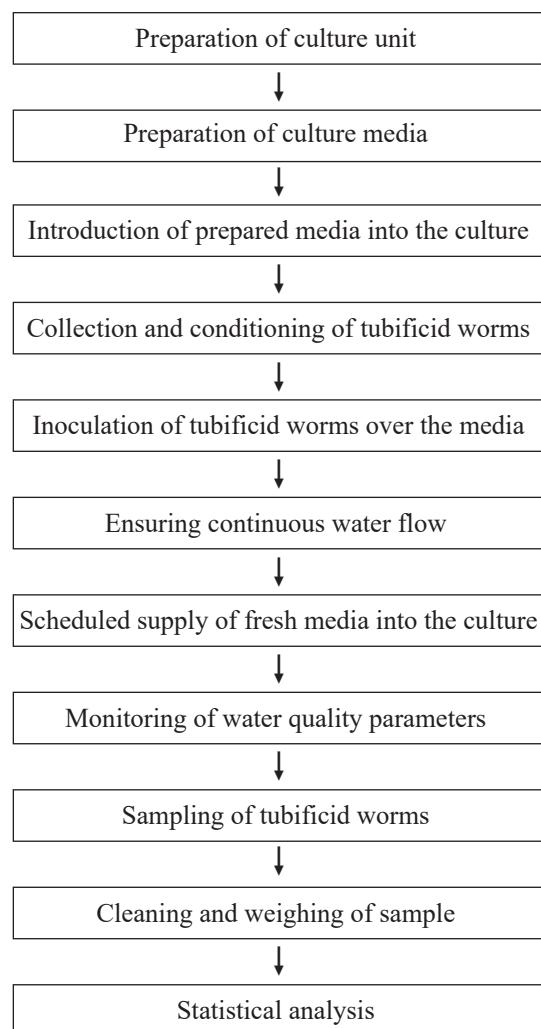


Table 1. Composition of media elements in experimental diets containing different treatments.

Media ingredient	Ingredients (%)			
	$2 \text{ L} \cdot \text{kg}^{-1}$ (Trt. I)	$4 \text{ L} \cdot \text{kg}^{-1}$ (Trt. II)	$6 \text{ L} \cdot \text{kg}^{-1}$ (Trt. III)	Rice gruel (Trt. IV)
Mustard oil cake	20	20	20	20
Wheat barn	20	20	20	20
Soybean meal	30	30	30	30
Cow-dung	20	10	10	10
Fine sand	10	10	10	10
Rice gruel	-	-	-	As required
Chicken blood	2	4	6	-

Table 2. The proximate composition analysis of media ingredients.

Ingredients	% Dry matter basis						Reference
	Dry matter	Protein	Lipid	Ash	Crude fiber	Nitrogen-free extract (NFE)	
Soybean meal	90.14	45.29	2.93	9.74	-	42.03	Sarowar and Mollah, 2009
Mustard oil cake	91.28	30.13	6.99	11.58	-	51.44	Sarowar and Mollah, 2009
Wheat bran	89.83	14.19	3.87	4.89	-	77.05	Sarowar and Mollah, 2009
Rice gruel	1.63	6.69	4.37	3.082	1.15	84.71	Mariom and Mollah, 2012
Chicken blood	15.09	80.85	0.85	2.23	-	0.98	Present study

Collection and inoculation of the wild tubificid worms

Naturally occurring tubificid populations were harvested from drains on the BAU campus, rinsed under running water, and inoculated onto the media at a rate of $1.25 \text{ mg} \cdot \text{cm}^{-2}$. Following worm inoculation, the culture media were replenished at 10-day intervals at a rate of $250 \text{ mg} \cdot \text{cm}^{-2}$ (Mariom and Mollah, 2012).

Sampling, cleaning, and weighing of tubificid worms

Approximately 40 days following inoculation, the growth and colonization of the worms were observed in the culture culverts.

Therefore, sampling (Figure 1) was initiated on the 40th day of culture and conducted at 10-day intervals prior to the media replenishment. The worms were collected and separated from the media and unwanted particles under running tap water, and the excess moisture was blotted using tissue paper. The worms were then weighed using a laboratory electronic balance (METTLER TOLEDO, PG503-SDR, Switzerland).

Calculation of production parameters

The production parameters, including standing biomass, harvested biomass, and total production, were determined using the following formulas.

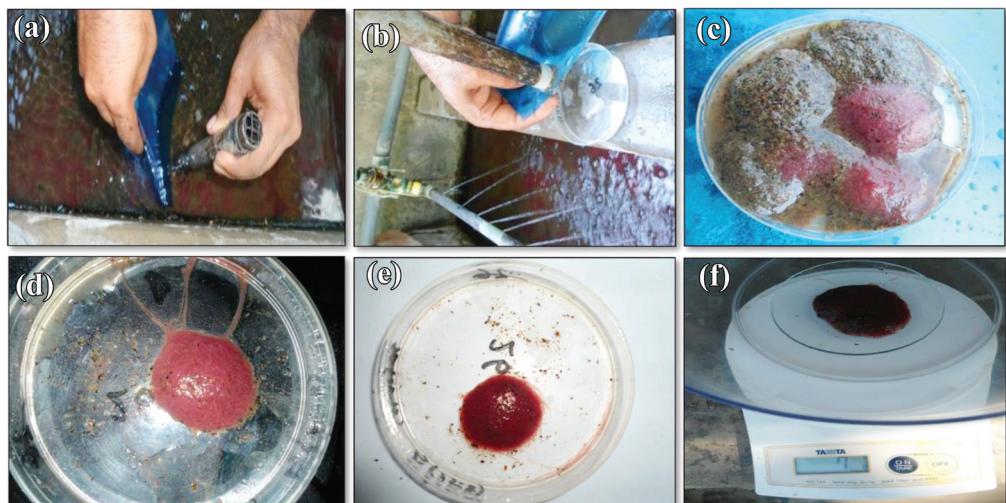


Figure 1. Procedure of sampling, cleaning, and weighing of tubificid worms: (a) collection of tubificid worms; (b) putting the worms on a petri dish; (c) collected worms before cleaning; (d) cleaned worms; (e) excess moisture wiped with tissue paper; and (f) weighing the sample.

Standing biomass

$$\text{Total live weight of worms present in the culture system at a given sampling time} \\ = \frac{\text{Culture area}}{\text{Culture area}}$$

Harvested biomass

$$= \frac{\text{Total harvested worm weight at day 90}}{\text{Culture area}}$$

$$\text{Total production} = (\text{Final standing biomass} \\ - \text{Initial standing biomass} \\ + \text{Total harvested biomass})$$

Water quality profiling

Key water quality parameters, including water flow rate (measuring cylinder), water temperature (digital thermometer), pH (pH meter Model: HI 98127), and dissolved oxygen (DO meter Model: DO-5509), were monitored once every 10 days before each sampling. The water flow rate was calculated using the following formula.

$$\text{Water flow rate} = \frac{\text{Water volume in litres}}{\text{Time in minutes}}$$

Statistical analysis

Statistical comparisons were made using one-way ANOVA followed by Tukey's HSD test to identify significant differences among treatment means. All computations were carried out with Microsoft Excel 2021, with a significance threshold of $p<0.05$.

RESULTS

Suitable amount of chicken blood for the production of tubificid worms

The average standing biomass of tubificid worms in Treatment I, Treatment II, Treatment III, and Treatment IV was $436.92 \pm 37.23 \text{ mg} \cdot \text{cm}^{-2}$, $578.97 \pm 17.03 \text{ mg} \cdot \text{cm}^{-2}$, $404.91 \pm 25.35 \text{ mg} \cdot \text{cm}^{-2}$, and $370.93 \pm 30.65 \text{ mg} \cdot \text{cm}^{-2}$, respectively, on the 70th day of the experiment (Figure 2). The results indicated a significant ($p<0.05$) difference in the standing biomass among the four different treatments (Figure 2). Additionally, the production in Treatment II ($578.97 \pm 17.03 \text{ mg} \cdot \text{cm}^{-2}$) was significantly higher ($p<0.05$) than in Treatment I, Treatment III, and

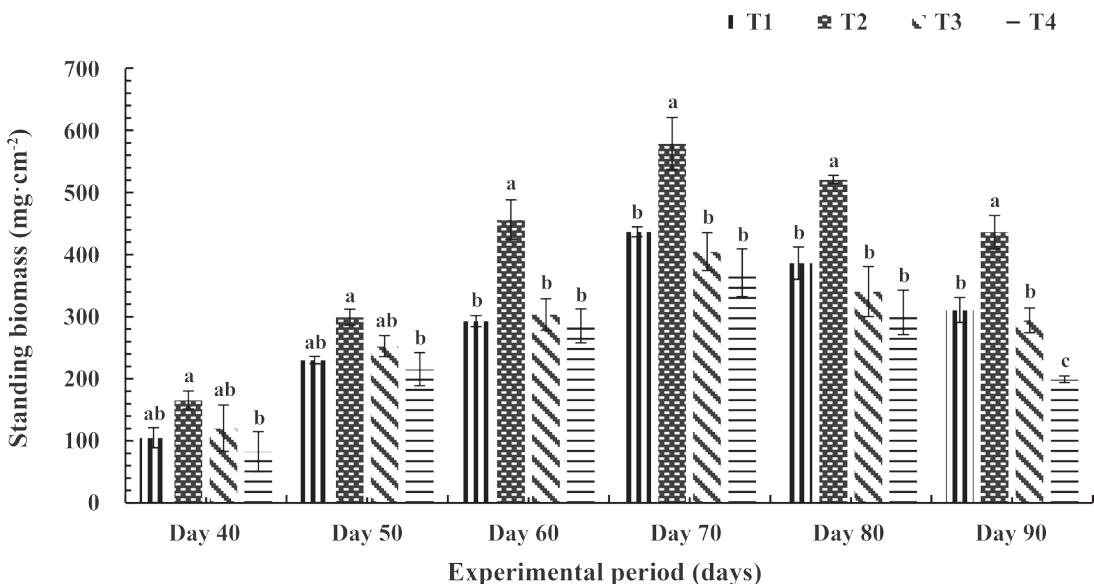


Figure 2. Standing biomass ($\text{mg} \cdot \text{cm}^{-2}$) of tubificid worms cultured for 90 days in media with varying chicken blood concentrations [T1: $2 \text{ L} \cdot \text{kg}^{-1}$; T2: $4 \text{ L} \cdot \text{kg}^{-1}$; T3: $6 \text{ L} \cdot \text{kg}^{-1}$; and T4: rice gruel (control)]. Bars represent means ($n = 3$) and error bars represent $\pm \text{SD}$. Different superscript letters above bars indicate significant differences ($p<0.05$) among treatments at each time point.

Treatment IV (Figure 2), while there were no significant differences ($p>0.05$) among Treatment I, Treatment III, and Treatment IV on the 70th day of culture (Figure 3). In all four treatments, a gradual increase in the standing biomass of tubificid worms was observed, reaching peak production on the 70th day (Figure 3) and then decreasing to the end of the experiment (day 90) (Figure 2).

Tubificid worms were harvested from all replicates at a rate of $40 \text{ mg} \cdot \text{cm}^{-2}$, and the results showed a significantly higher ($p<0.05$) total production in Treatment II ($676.26 \pm 38.40 \text{ mg} \cdot \text{cm}^{-2}$) than in Treatment I ($550.86 \pm 27.29 \text{ mg} \cdot \text{cm}^{-2}$), Treatment III ($534.20 \pm 35.69 \text{ mg} \cdot \text{cm}^{-2}$) and Treatment IV ($439.05 \pm 5.47 \text{ mg} \cdot \text{cm}^{-2}$) on the 90th day of the experimental (Figure 4).

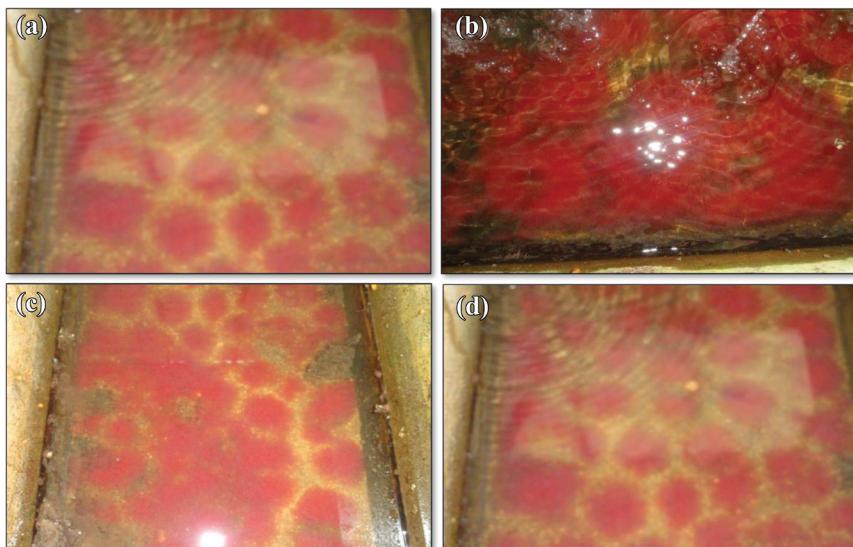


Figure 3. Colonies of tubificid worms cultured in media with varying chicken blood concentrations on day 70: (a) $2 \text{ L} \cdot \text{kg}^{-1}$; (b) $4 \text{ L} \cdot \text{kg}^{-1}$; (c) $6 \text{ L} \cdot \text{kg}^{-1}$; and (d) rice gruel (control).

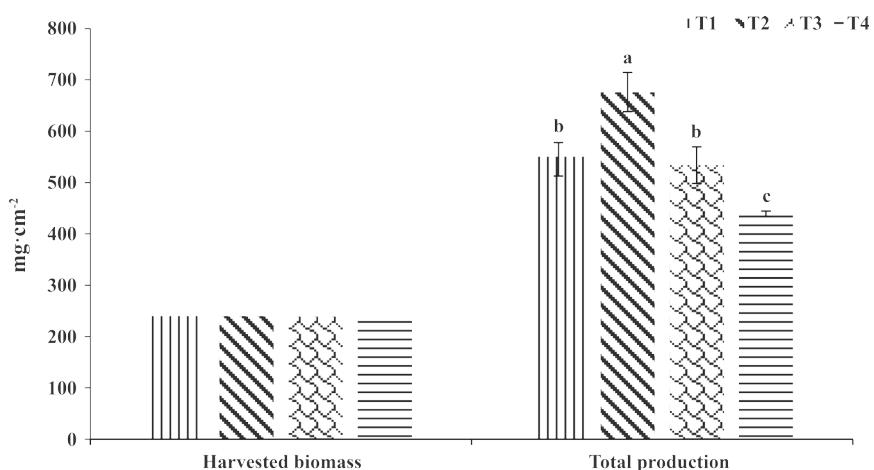


Figure 4. Harvested biomass ($\text{mg} \cdot \text{cm}^{-2}$) and total production ($\text{mg} \cdot \text{cm}^{-2}$) of tubificid worms cultured for 90 days in media with varying chicken blood concentrations [T1: $2 \text{ L} \cdot \text{kg}^{-1}$; T2: $4 \text{ L} \cdot \text{kg}^{-1}$; T3: $6 \text{ L} \cdot \text{kg}^{-1}$; and T4: rice gruel (control)]. Bars represent means ($n = 3$) and error bars represent $\pm \text{SD}$. Different superscript letters above bars indicate significant differences ($p<0.05$) among treatments at each time point.

Water quality parameters

Water quality parameters (temperature, dissolved oxygen, and pH) (Table 3) were monitored throughout the experiment at 10-day

intervals. In this study, the recorded values ranged from 26.93 °C to 30.37 °C for temperature, 6.10 to 7.07 ppm for dissolved oxygen, and 7.2 to 7.6 for pH, which was found suitable for culturing tubificid worm.

Table 3. Water quality parameters monitored during the 90-day experimental period.

Days	Treatment	Temperature (°C)	Dissolved oxygen (ppm)	pH
40	I	26.93±0.153	7.07±0.208	7.40±0.10
	II	27.10±0.100	6.73±0.321	7.30±0.100
	III	27.10±0.100	7.00±0.100	7.27±0.058
	IV	27.13±0.115	7.03±0.058	7.37±0.058
50	I	30.37±0.173	6.60±0.100	7.43±0.058
	II	30.23±0.208	6.30±0.100	7.23±0.058
	III	30.13±0.153	6.67±0.058	7.37±0.058
	IV	30.13±0.058	6.63±0.153	7.50±0.100
60	I	29.03±0.58	6.53±0.153	7.33±0.115
	II	29.20±0.100	6.37±0.058	7.20±0.100
	III	29.23±0.153	6.43±0.208	7.30±0.100
	IV	29.13±0.058	6.63±0.208	7.60±0.100
70	I	28.27±0.058	6.37±0.153	7.27±0.153
	II	28.03±0.058	6.43±0.058	7.30±0.100
	III	28.17±0.153	6.23±0.058	7.33±0.153
	IV	28.27±0.058	6.30±0.100	7.57±0.058
80	I	28.27±0.058	6.33±0.115	7.23±0.252
	II	28.07±0.154	6.80±0.100	7.20±0.100
	III	28.17±0.208	6.30±0.000	7.37±0.153
	IV	28.23±0.208	6.50±0.173	7.23±0.058
90	I	29.23±0.058	5.90±0.100	7.43±0.208
	II	29.13±0.058	6.10±0.100	7.50±0.100
	III	29.30±0.100	6.30±0.100	7.33±0.153
	IV	29.17±0.153	6.30±0.100	7.23±0.058

DISCUSSION

This study quantified the optimal chicken blood concentration for media preparation while cultivating tubificid worms. The highest yield was recorded in the medium containing chicken blood at $4 \text{ L}\cdot\text{kg}^{-1}$ (Treatment II) throughout the experiment. This amount of chicken blood as a soaking agent likely promoted worm growth through enhanced nutrient bioavailability. The production of tubificid worms in Treatment I ($2 \text{ L}\cdot\text{kg}^{-1}$ chicken blood) and Treatment III ($6 \text{ L}\cdot\text{kg}^{-1}$ chicken blood) was less than that in Treatment II. Our results reveal a non-linear, “Goldilocks-type” response to chicken-blood loading, with $4 \text{ L}\cdot\text{kg}^{-1}$ maximizing biomass. We hypothesize that insufficient nitrogen and heme-bound micronutrients at $2 \text{ L}\cdot\text{kg}^{-1}$ limit microbial conditioning of the substrate, whereas excess organic loading at $6 \text{ L}\cdot\text{kg}^{-1}$ elevates oxygen demand and ammoniacal accumulation, constraining worm feeding and survival. On the other hand, the lowest production among all the treatments was observed in Treatment IV, where the soaking agent was rice gruel, indicating the inefficacy of rice gruel over chicken blood in terms of nutrient availability. Consistent with the current findings, Islam *et al.* (2015) reported peak biomass production ($1,020.86 \pm 19.84 \text{ mg}\cdot\text{cm}^{-2}$) on the 70th day sampling using chicken blood as a soaking agent, confirming its efficacy for tubificid cultivation. In another study, cattle blood-wetted media demonstrated higher production ($683.68 \pm 3.86 \text{ mg}\cdot\text{cm}^{-2}$) of tubificid worms over rice gruel and water-wetted media (Hasan *et al.*, 2019).

All treatments showed gradual biomass accumulation with a peak on day 70, after which it declined until the end of the experimental period on day 90. A similar growth trend was also reported previously (Mariom and Mollah, 2012; Mollah *et al.*, 2012; Mariom and Mollah, 2013; Islam *et al.*, 2015; Alam *et al.*, 2021). On the 70th day, the highest standing biomass was recorded in all treatments: $436.92 \pm 37.23 \text{ mg}\cdot\text{cm}^{-2}$ (Treatment I), $578.97 \pm 17.03 \text{ mg}\cdot\text{cm}^{-2}$ (Treatment II), $404.91 \pm 25.35 \text{ mg}\cdot\text{cm}^{-2}$ (Treatment III), and $370.93 \pm 30.65 \text{ mg}\cdot\text{cm}^{-2}$ (Treatment IV). Biomass in Treatment II was significantly higher ($p < 0.05$) than in other treatments, while no significant differences ($p > 0.05$)

were found among treatments I, III, and IV. Previous studies similarly reported peak tubificid biomass on the 70th day of culture (Mariom and Mollah, 2012; Mollah *et al.*, 2012; Mariom and Mollah, 2013; Islam *et al.*, 2015; Hasan *et al.*, 2019; Alam *et al.*, 2021). The highest total production was also obtained in Treatment II ($676.26 \pm 38.40 \text{ mg}\cdot\text{cm}^{-2}$) at the end of the experiment. The quadratic fit identified an optimal loading range of approximately $4 \pm 0.5 \text{ L}\cdot\text{kg}^{-1}$, which enhanced standing biomass by about 56 % compared with the control. This finding suggests that moderate chicken-blood enrichment provides a balanced supply of nutrients without inducing oxygen depletion or toxic metabolite accumulation. Operationally, media supplementation around day 60–70 and periodic removal of inhibitory compounds are recommended to sustain favorable conditions and extend peak productivity beyond day 70.

The proximate analysis of media ingredients revealed nutritionally adequate levels of organic carbon, proteins, and micronutrients essential for tubificid growth. Organic carbon content was particularly significant, serving as both an energy source and reproductive stimulant (Mariom and Mollah, 2012). Previous studies also reported that the media composition plays a crucial role in the growth and maturity of tubificid worms. Kaster (1980) stated that 50% of tubificids reached sexual maturity within 40 days at 15°C temperature on 7% organic carbon content. Marian and Pandian (1984) reported a yield of $200 \text{ mg}\cdot\text{cm}^{-2}$ on a substrate consisting of 75% cow dung and 25% sand, while Ahamed and Mollah (1992) achieved maximum production ($419.4 \text{ mg}\cdot\text{cm}^{-2}$) using a medium comprising 35% wheat bran, 20% mustard oil cake, 25% cow dung, and 20% sand. This was later surpassed by Mollah *et al.* (2012), who reported $503.39 \pm 22.98 \text{ mg}\cdot\text{cm}^{-2}$ yields with modified ratios (20% wheat bran, 35% mustard oil cake, 25% cow dung, and 20% sand), suggesting optimal nutrient balance requires higher oil cake content. Therefore, the current study explained that the beneficial effects of a sufficient amount of nutrients, especially protein contained in the chicken blood, significantly enhance tubificid worm growth, establishing its superiority over rice gruel as a media-conditioning agent.

The key water quality indicators, including temperature, pH, and dissolved oxygen, also play a significant role in the proper growth and sexual maturity of this worm (Kaster 1980; Li-RenXi, 2001). It has been observed that, when temperature and organic carbon content in the culture media increase, the time requirement for reaching sexual maturity significantly decreases (Kaster, 1980). In this study, the recorded physicochemical parameters, including temperature (26.93–30.37 °C), dissolved oxygen (6.10–7.07 ppm), and pH (7.2–7.6) remained within the optimal ranges previously reported for *Tubifex* culture (Mariom and Mollah, 2012; Mollah *et al.*, 2012; Mariom and Mollah, 2013), suggesting that substrate stoichiometry, rather than bulk water chemistry, primarily governs the observed optimum.

This study achieved markedly higher production efficiency, requiring only about 1.01 kg of culture medium (valued at BDT 20, ≈ US \$0.16) to produce 1 kg of worms. This corresponds to a 94–96% reduction in substrate use compared to conventional cow dung-based systems, which demand 18 kg (Marian and Pandian, 1984) and 25 kg (Marian *et al.*, 1989) of substrate per kg of worm biomass. Economically, the culture medium employed in this study proved substantially more efficient for tubificid worm production than those previously reported.

This study demonstrated that chicken blood-fermented media significantly outperforms both water (Mollah *et al.*, 2012) and rice gruel (Mariom and Mollah, 2012) as a culture substrate for tubificid worms, yielding greater biomass production over the 90-day trial period. The amount of chicken blood in Treatment II (4 kg·L⁻¹) was found to be the most suitable for mass production of tubificid worms, while Treatment I and Treatment III yielded less, suggesting that aquaculture farmers can adopt this optimized feeding strategy to efficiently produce this worm biomass. This could provide a cost-effective and sustainable source of protein-rich feed for fish and shrimp, enhancing farm productivity and reducing reliance on commercial feed. Safety and regulatory considerations are critical for practical application. Chicken blood, as an animal by-product, carries potential pathogen

risks; therefore, pasteurization or similar treatments are recommended to ensure microbiological safety. Additionally, odor and fly management should be implemented during culture, and all procedures should comply with local standards for handling animal by-products. Addressing these factors is essential to the transition of this method from controlled trials to safe, deployable practice.

CONCLUSIONS

Of the three different amounts of chicken blood used as a soaking agent, the one with 4 kg·L⁻¹ chicken blood was found to be the most effective, yielding the highest production (578.97±17.03 mg·cm⁻²) of tubificid worms. The success achieved in this study provides a valuable foundation for future research on this subject.

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