Research article

Exploring the nutritional potential of *Monoraphidium littorale* and enriched copepods as first feeds for rearing Nile tilapia (*Oreochromis niloticus*) larvae

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A R T I C L E   I N F O

A B S T R A C T

One of the challenges in the aquaculture industry is providing nutritionally balanced and environmentally sustainable live food for fish larvae. Therefore, the rearing techniques of fish larvae with preferred starter food should be given importance for obtaining optimal hatchery production. Nile tilapia, *Oreochromis niloticus* larvae just after yolk absorption (body length 0.950 ± 0.004 mm; body weight 6.00 ± 0.02 mg) were reared in laboratory conditions for 16 days, feeding with 6 different diets to know their effect on survival and growth. The diets were live *Monoraphidium littorale* (T_1), live enriched copepods with *M. littorale* (T_2), powdered *M. littorale* (T_3), powdered enriched copepods (T_4), live *M. littorale* + live enriched copepods (T_5), and powdered *M. littorale* + powdered enriched copepods (T_6). The proximate composition, amino acid profile and fatty acid content of both *M. littorale* and copepods were analyzed. The biochemical analysis of the dried powder of enriched copepods and *M. littorale* revealed that both of them are excellent sources of protein, amino acids, and lipids, especially with monounsaturated and polyunsaturated fatty acids. The *O. niloticus* larvae fed the T_2 diet exhibited the most favourable outcomes, with significantly higher larval gain in weight and percent weight gain, in comparison to the larvae fed other diets ($p < 0.001$ for all comparisons). The LG% and SGR of the larvae were also significantly higher in T_2 in comparison to the T_1, T_3, T_4, and T_6 ($p < 0.001$ for all comparisons) except T_5. In addition, the highest percent survival rate of the larvae was observed in T_2 (95 %) followed by T_4 (93 %), T_6 (93 %), T_3 (82 %), T_5 (73 %) and then T_1 (43 %). Based on the present findings, it is recommended that live copepods enriched with *M. littorale* can be utilized as a starter food for the rearing of Nile tilapia, *O. niloticus* larvae in hatcheries because of its enriched nutritional profile.

1. Introduction

The aquaculture sector plays a vital role in global food security. One of the major challenges for the sustainability of this sector is to produce high-quality fish fry. Hatchery operators are facing problems in producing the best-quality fish fry and experiencing large-

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scale mortality due to inadequate nutrients in larval diets [1,2]. The mass production of suitable live food for the first feeding of fish larvae is the prerequisite of successful aquaculture hatchery operations. In these circumstances, exploring appropriate live foods and improved rearing techniques have been considered a priority area of research for obtaining the best growth and survival rate of fish larvae.

Microalgae, often referred to as "green gold", are increasingly recognized as beneficial live food sources for both larval and adult fishes due to their rich content of polyunsaturated fatty acids (PUFAs), carotenoids and vitamins [3–5]. As a result, larval rearing using green water techniques often enhances hatchery production significantly [6]. In this approach, microalgae are fed directly to the fish larvae, providing all essential nutrients necessary for their development [7]. Globally, there is innumerable advantageous evidence of adding microalgae in larval fish-rearing systems [8,9]. Despite challenges such as high production costs, susceptibility to contamination, and variability in proximate composition, microalgae are esteemed for their efficient photosynthetic capabilities and potential as a sustainable food and feed source [10–15]. Microalgae are also an enriched source of astaxanthin that has great prospects in aquaculture [16]. It is well documented that the food value of microalgae largely relies on their biochemical components including proteins, lipids, and carbohydrates [17]. *Monoraphidium* spp. are a great source of total lipids (12–35 %), proteins (28%–45 %) [18,19], higher fatty acids [20–24] and astaxanthin [25]. Recent studies demonstrate that *M. littorale* thrives in a low-cost medium such as 25 % digested rotten potato supernatant, producing enriched lipid (26.62 %) and protein (39.08 %), further enhancing its suitability as larval feed [19,26]. In addition, the utilization of preserved dried powder of microalgae might also be a cogent step for evaluating the growth of larvae in a cost-effective manner.

Zooplankton are recognized for their high-quality protein and lipid profiles essential for optimal growth and survival of fish larvae [27]. Certain species of zooplankton possess elevated levels of digestive enzymes that stimulate larval appetite, making them preferred as starter feeds in aquaculture [28]. As a result, certain commercially important fishes, especially larvae, and fry, get their nutrition mostly from zooplankton. Hence, the nutritional profile of zooplankton still needs to be improved [29]. Enriching zooplankton with nutrient-rich microalgae offers a promising approach to enhance their nutritive value [30,31]. Copepods are superior in nutritional composition to other live food species and are being extensively utilized as starter feed for many fishes and shellfish in the hatchery [32–35]. Even, many researchers have reported that *Artemia* is inferior to copepods in terms of nutritive value when used as live feed [36–38]. In a bid to enhance the growth and survival rate of fish larvae, it has been reported that supplementation of solely live copepods can supplant the two traditionally employed live foods rotifer and *Artemia* [38]. Consequently, the balanced nutritiveness of copepods enhances the growth performance and the survival rate of fish larvae [32,39]. Among the copepods, *Cyclops* sp. and *Diaptomus* sp. from Cyclopoida and Calanoida order respectively are abundantly found in freshwater ponds that may have a great prospect in enhancing the growth of freshwater fish larvae, including *Oreochromis niloticus*.

Tilapia is widely distributed in the world and has emerged as a highly desirable fish due to its numerous advantages. These include its ability to adapt to a wide range of environmental conditions, ease of reproduction in captivity, resistance to environmental stress, diseases, and microbial pathogens, high-quality flesh, low trophic level feeding habits, excellent growth rate on both natural and artificial diets and higher acceptability to consumer with a good price [40,41]. The most common cultured genus of tilapia is *Oreochromis*, and around 89 % of the farmed fish of this genus is Nile tilapia (*Oreochromis niloticus*), and it is cultivated in at least 78 countries [42,43]. Despite its popularity, the rising costs and unpredictable availability of global fishmeal and fish oil supplies present significant challenges to tilapia farming [44]. The Food and Agriculture Organization (FAO) warns that excessive reliance on fishmeal threatens aquatic biodiversity and human food security [45], prompting a crucial need to identify cost-effective and sustainable protein sources for aquafeeds. Plant-based feedstuffs could be a viable alternative for replacing fishmeal in aquafeeds. As a result, several alternative ingredients, particularly those of plant origin, have been investigated, and some are currently used in tilapia feeds to reduce the amount of fishmeal [46]. Another hurdle in tilapia production is the limited availability of high-quality fingerlings of this fish has turned out to be a constraint on its production [44,47]. Live food including microalgae and zooplankton can be a potential alternative feed to replace fishmeal for sustainable aquaculture production. There is no information on the larval rearing of Nile tilapia

Fig. 1. Pure cells of the green microalga, *Monoraphidium littorale* [19]. Scale bar: 10 μm.

by using green microalga, *M. littorale,* and copepods enriched with *M. littorale*. With that in mind, Nile tilapia larvae were used as experimental fish for evaluating their feeding preferences to different diets containing *M. littorale* and enriched copepods in both live and dried powdered form in response to standardizing the appropriate first food of the fish.

2. Materials and methods

2.1. Microalgal strains and culture for larval rearing of O. niloticus

Pure microalgal strains of *M. littorale* were sourced from the Laboratory of Plankton Research, Department of Fisheries Management, Bangladesh Agricultural University, Bangladesh (Fig. 1). *M. littorale* was cultured in 25 % concentration of digested rotten potato supernatant (DRPS) at 26 ± 2 °C ambient temperature with a light intensity of 60 μmol m^{-2} s^{-1} at a photoperiod of 12:12 h, L:D with continuous aeration following the method used by Ritu et al. [19]. Cell density of *M. littorale* was determined using a Sedgewick-Rafter Cell under a microscope (B-510BT OPTIKA, Italy). Harvesting of *M. littorale* was performed during the exponential growth phase, followed by centrifugation to obtain a concentrated microalgal paste, which was then used to feed larvae of *O. niloticus*.

2.2. Collection and enrichment of copepods using M. littorale

Copepods were collected from the ponds adjacent to the Faculty of Fisheries, Bangladesh Agricultural University, Bangladesh, by a net with a mesh size of 50 μm, where *Diaptomus* sp. and *Cyclops* sp. were predominantly found (Fig. 2a and b). Immediately after collection, *Cyclops* sp. and *Diaptomus* sp. were rinsed with filtered freshwater to eliminate contaminants. The samples were then screened to exclude tiny fish larvae and prawns from the collected sample. Following multiple rinsing and screening processes, the remaining copepods (*Cyclops* sp. and *Diaptomus* sp.) were used to initiate the culture protocol. Subsequently, the clonal culture of copepods was established and sustained by providing *M. littorale* as an enriching food. During the culture period, copepods were harvested and transferred to a fresh culture container every 8 days [26].

2.3. Dried powder preparation from M. littorale and copepods

For producing dried powder, *M. littorale* was cultured in a glass jar containing 25 % concentration of DRPS (Fig. 3a). Microalgal cells were harvested on the 12th day of the culture period using centrifugation, at which point cell concentration was at its peak (Fig. 3b). After centrifugation of *M. littorale*, the concentrated algal paste was collected and dried in an oven (SHARP, Japan) at 40 °C [26]. The dried algal paste was then powdered and stored in glass bottles with cork stoppers for use as feed for the larvae of *O. niloticus* during the experimental period (Fig. 4).
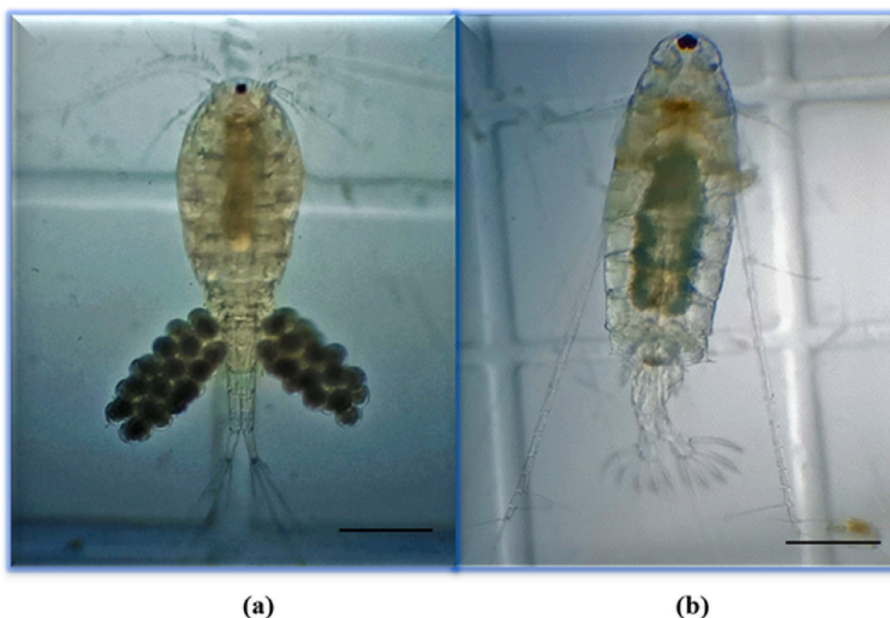

Fig. 2. Micrographs of the collected (a) *Cyclops* sp. and (b) *Diaptomus* sp. Scale bar: 200 μm.

Fig. 3. (a) Culture of *M. littorale* in 10 L glass jars, and (b) dried powder of *M. littorale*.

2.4. Proximate composition of M. littorale and enriched copepods

The proximate composition of enriched copepods and *M. littorale* including crude protein, crude lipids, carbohydrates and ash content was examined in triplicates followed by the method of Horwitz's [48]. The crude protein content was determined by analyzing the total nitrogen content of the samples using the Kjeldahl method with Behrotest® InKjel M digesting device and Behr S 1 steam distillation apparatus. The obtained nitrogen values were multiplied by the conversion factor (5.85 for *M. littorale* and 6.25 for enriched copepods) to obtain the crude protein content. For the analysis of the lipid content, samples were dried in the oven at 105 °C and then extracted the fat with acetone in a Soxhlet Extractor for about 4 h. The crude lipid content of the sample was estimated by using the following equation:

Percent (%) of crude lipid content = (D-B/A) × 100, where D is the weight of crude lipid with the beaker, B is the empty beaker weight and A is the weight of the sample. Ash content was determined by incinerating samples in a ceramic crucible in an electric muffle furnace at 600 °C for 6 h. Carbohydrate content was calculated by subtracting the sum of other components from 100.

2.5. Analysis of amino acids and fatty acids

The amino acid composition of *M. littorale* and enriched copepods was assessed using an automated amino acid analyzer (Model: S4300, Sykam GmbH, Germany). Initially, the powdered sample was treated with 25 mL of 7 N HCl, thoroughly homogenized, and then filtered to eliminate insoluble components. The mixture underwent a 24-h hydrolysis process in a hydrolyzer. Upon complete hydrolysis, excess HCl was neutralized with 7.5 N NaOH and verified using a pH meter (Model: sensionTM 156, HACH, USA). The

Fig. 4. Dried powder of copepods prepared for rearing of *O. niloticus* larvae.

sample volume was adjusted to 250 mL in a calibrated volumetric flask with a buffer solution maintaining pH at 3.4. After filtration with a 0.45 μm microfilter, a 1 mL sample, prepared by mixing 100 μL of the sample solution with 900 μL of a pH 3.4 dilution buffer (10x dilution), was sequentially analyzed for amino acid content. Simultaneously, a standard amino acid solution was analyzed for accurate quantification, quality assurance, and data comparison. Fatty acids were extracted from the samples using a chloroform and methanol mixture (2:1 ratio, v/v; containing 0.1 mg/100 g butylated hydroxytoluene) [49]. Following extraction, fatty acid methyl esters were prepared and analyzed via Gas Chromatography (Model: 14B, Shimadzu, Japan). Identification and quantification were performed by comparing retention times with authentic standards.

2.6. Larval rearing facility and feeding regime of O. niloticus

O. niloticus larvae used in this study were collected from the fish hatchery of the Sharnalata Agro Fisheries Ltd., Radhakanai, Mymensingh, Bangladesh. The three-day-old larvae were transported to the laboratory in oxygen-inflated plastic bags and acclimated to the laboratory environment for 18 h before starting the experiment. A feeding experiment with 4 days-old tilapia larvae (0.950 ± 0.004 mm; 6.00 ± 0.02 mg) was conducted using 6 different diets (treatments), each with three replications, over a period of 16 days. The diets were live *M. littorale* (T_1), live copepods enriched with *M. littorale* (T_2), powdered *M. littorale* (T_3), powdered enriched copepods (T_4), live *M. littorale* + live enriched copepods (T_5), and powdered *M. littorale* + powdered enriched copepods (T_6) where T_1 diet was used as a control. Eighteen equal-sized (5 L capacity) bowls were used for the experiment, with each bowl containing 3.5 L of tap water at a depth of 7.7 cm. Before use, the tap water was aerated overnight in a 50 L bucket to ensure the desired dissolved oxygen level and to adjust to the ambient room temperature. Each bowl was stocked with 20 larvae per liter of water. A broken stone was placed at the center of each bowl to provide shelter for the larvae. Continuous gentle aeration was provided in each bowl using air stone to maintain the desired dissolved oxygen level and to evenly disperse the feed throughout the water column. Feed was administered to the larvae three times a day, at 7:00 a.m., 2:00 p.m., and 9:00 p.m. Before each feeding, cultured copepods were collected by filtering through a 50 μm screen and rinsed quickly with freshwater. The required amount of dried powder of *M. littorale* and zooplankton was weighed and added to fresh water in a glass beaker. After repeated stirring with a glass rod, the resultant suspension was given to the fish larvae. Table 1 displays the feeding schedule for larvae raised on the six different diets. Every morning before feeding, approximately 30 % of the water was changed, and leftover feed and fecal matter were removed from each bowl by siphoning.

2.7. Estimation of growth parameters and survival rate of O. niloticus larvae

At the end of the experiment, the final length and weight of the larvae in different replicates of each treatment were measured. Five larvae were randomly selected from each replication to estimate the total length (mm), resulting in a total of 15 larvae measured per treatment and all surviving larvae were sampled for weight (mg). The growth performance in terms of gain in length (GL), gain in weight (GW), percent weight gain (WG%), percent length gain (LG%), and specific growth rate (SGR) of the body weight (% day $^{-1}$) as well as the survival rate were calculated using the following equations:

Gain in weight (GW) = (Final weight - Initial weight)

Gain in length (GL) = (Final length – Initial length)

Percent weight gain (WG%) = {(Final weight - Initial weight)/(Initial weight)} × 100 [50]

Percent length gain (LG%) = {(Final length – Initial length)/(Initial length)} × 100 [51]

Specific growth rate (SGR) = {(Ln Final weight - Ln Initial weight)/(Number of days)} × 100 [52]

Larval survival rate (S) (%) = {(Number of remaining healthy larvae)/(Total number of stocked larvae)} × 100 [53]

2.8. Measurement of water quality parameters

The water quality parameters, including pH, temperature, and dissolved oxygen were monitored daily during the culture period to

Table 1
Feeding regime of *O. niloticus* larvae in different treatments during the experimental period.

Treatments	Feeding schedule			Feeding frequency (Times/day)
	Day (1–5)	Day (6–10)	Day (11–16)	
T_1	75000 cells mL^{-1}	150000 cells mL^{-1}	300000 cells mL^{-1}	3
T_2	4 ind. mL^{-1}	8 ind. mL^{-1}	16 ind. mL^{-1}	3
T_3	0.03 g L^{-1}	0.06 g L^{-1}	0.12 g L^{-1}	3
T_4	0.04 g L^{-1}	0.08 g L^{-1}	0.16 g L^{-1}	3
T_5	37500 cells mL^{-1} + 2 ind. mL^{-1}	75000 cells mL^{-1} + 4 ind. mL^{-1}	150000 cells mL^{-1} + 8 ind. mL^{-1}	3
T_6	0.015 g L^{-1} + 0.02 g L^{-1}	0.03 g L^{-1} + 0.04 g L^{-1}	0.06 gL^{-1} + 0.08 g L^{-1}	3

[T_1: live *M. littorale*, T_2: live copepods enriched with *M. littorale*, T_3: powdered *M. littorale*, T_4: powdered enriched copepods, T_5: live *M. littorale* + live enriched copepods, and T_6: powdered *M. littorale* + powdered enriched copepods. Feed was given to the larvae three times a day, at 7:00 a.m., 2:00 p. m., and 9:00 p.m.].

maintain a healthy environment for larval development. A combined pH and temperature meter (HI98107, HANNA) was used to determine pH and temperature whereas DO was determined using a portable oxygen meter (DO-5509, LUTRON, Taiwan).

2.9. Statistical analysis

To normalize the percentage data, we performed an arcsin square root transformation on the proportion data. The data normality and equality of variance were checked by performing Kolmogorov–Smirnov one-sample test and Levene's test, respectively. When data was parametric, we performed an independent *t*-test to determine whether the enriched copepods and *M. littorale* differed in their amino acids and fatty acids content. In addition, we performed a one-way ANOVA test to determine whether the larvae fed with 6 diets differed in their GW, LG%, WG%, SGR, and survival rate, followed by pairwise post-hoc comparisons using a Tukey HSD correction. When data was non-parametric, the Kruskal-Wallis test was performed to test whether the GL of larvae differed in response to 6 diets. Then, to determine the direction of the difference between the 6 diets, we used pairwise post-hoc tests with a Bonferroni correction. All statistical analyses were conducted using SPSS software (version 25.1, IBM SPSS Inc.). All tests were 2-tailed unless stated otherwise and the α was set at 0.05.

3. Results

3.1. Proximate composition of the enriched copepods and M. littorale cells

The proximate composition of the enriched copepods and cells of *M. littorale* revealed distinct nutritional profiles (Figs. 5 and 6). Enriched copepods exhibited a composition predominantly composed of crude protein (65.45 %), crude lipid (17.71 %), carbohydrate (7.82 %) and ash content (9.02 %). In addition, green microalga *M. littorale* contained higher levels of crude protein (39.02 %) and lipid (26.62 %), accompanied by substantial carbohydrate (24.14 %) and ash content (10.22 %).

3.2. Analysis of amino acids and fatty acids

Essential and non-essential amino acids varied among enriched copepods and *M. littorale* (Total essential amino acids: Independent *t*-test, $t_4 = 32.21$, $p < 0.001$; Total non-essential amino acids: Independent *t*-test, $t_4 = -56.27$, $p < 0.001$) (Fig. 7). The analysis of amino acid content revealed that enriched copepods contained 46.81 ± 0.09 % essential amino acids, while *M. littorale* exhibited 43.26 ± 0.17 %. Non-essential amino acids comprised 53.2 ± 0.10 % in copepods and 56.74 ± 0.04 % in *M. littorale* (Table 2). Sixteen amino acids were identified, with 9 categorized as essential in both enriched copepods and *M. littorale*. Lysine, arginine, leucine, and histidine were prominently abundant among essential amino acids, with leucine being the highest in *M. littorale* and lysine in copepods. Alanine was the most abundant non-essential amino acid in *M. littorale*, whereas glutamic acid predominated in enriched copepods.

The total saturated fatty acids content was found significantly higher in enriched copepods than in *M. littorale* (Independent *t*-test, $t_4 = 27.90$, $p < 0.001$) while total monounsaturated fatty acids were observed significantly higher in *M. littorale* compared to enriched copepods (Independent *t*-test, $t_4 = -60.24$, $p < 0.001$) (Fig. 8). The total polyunsaturated fatty acids did not vary among enriched copepods and *M. littorale* (Independent *t*-test, $t_4 = 1.38$, $p = 0.241$). Among PUFAs, alpha-linoleic acid (C18:2n-6) and eicosapentaenoic acid (C20:5n-3) were found to be the most abundant in enriched copepods where arachidonic acid (C20:4n-6) was most abundant in *M. littorale* (Table 3).

3.3. Effects of different diets on the growth performance of the larvae of Nile tilapia

The median values of GL differed significantly among tilapia larvae fed six different diets (Kruskal-Wallis Test, $\chi_5^2 = 15.02$, $p = 0.01$)
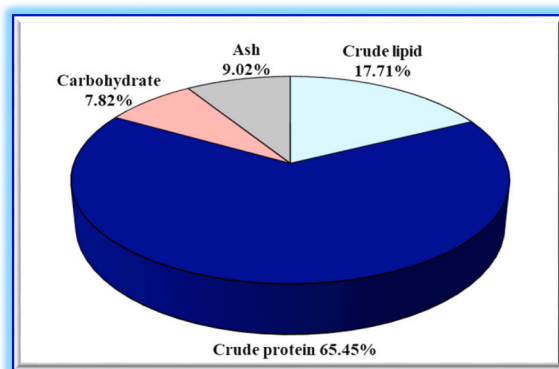

Fig. 5. Proximate composition of enriched copepods (*Diaptomus* sp. and *Cyclops* sp.) in terms of crude protein, crude lipid, carbohydrate and ash content (% dry weight basis).
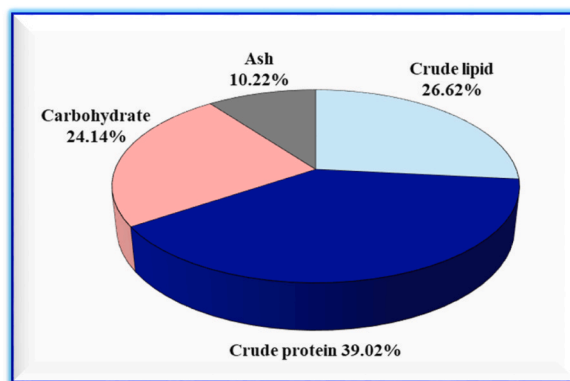
Fig. 6. Proximate composition of green microalga, *Monoraphidium littorale* in terms of crude protein, crude lipid, carbohydrate and ash content (% dry weight basis).
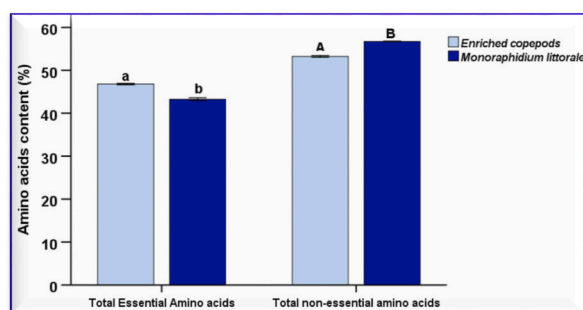

Fig. 7. Mean (±SD) total essential and non-essential amino acids content (%) of enriched copepods and *Monoraphidium littorale* (n = 3). Different letters represent statistical differences at α = 0.05.

Table 2
Amino acid composition of enriched copepods and *Monoraphidium littorale* on the dry matter basis.

Type of amino acids	Amino acids	Amount (%) in enriched copepods	Amount (%) in *M. littorale*
Essential amino acids	Lysine (LYS)	7.30 ± 0.20	8.42 ± 0.01
	Arginine (ARG)	7.20 ± 0.10	1.80 ± 0.02
	Leucine (LEU)	7.20 ± 0.10	9.87 ± 0.03
	Histidine (HIS)	6.69 ± 0.07	2.13 ± 0.03
	Threonine (THR)	4.62 ± 0.17	5.10 ± 0.04
	Valine (VAL)	4.50 ± 0.10	5.60 ± 0.05
	Phenylalanine (PHE)	3.60 ± 0.08	3.49 ± 0.03
	Isoleucine (ILE)	3.20 ± 0.12	3.45 ± 0.06
	Methionine (MET)	2.50 ± 0.08	3.40 ± 0.09
Non-essential amino acids	Glutamic acid (GLU)	14.60 ± 0.03	12.50 ± 0.12
	Aspartic acid (ASP)	9.40 ± 0.03	8.71 ± 0.03
	Alanine (ALA)	7.70 ± 0.10	13.23 ± 0.02
	Cystine (CYS)	6.50 ± 0.02	5.70 ± 0.03
	Glycine (GLY)	5.00 ± 0.01	7.30 ± 0.03
	Tyrosine (TYR)	5.40 ± 0.01	3.80 ± 0.05
	Serine (SER)	4.60 ± 0.10	5.50 ± 0.001
Total essential amino acids		**46.81 ± 0.09[a]**	**43.26 ± 0.17[b]**
Total non-essential amino acids		**53.2 ± 0.10[A]**	**56.74 ± 0.04[B]**

The data are presented as mean ± SD (n = 3).

(Fig. 9a). Tilapia larvae showed significantly higher GL (mm) when fed T_2 diet in comparison to the T_1 diet (p = 0.009). However, no statistically significant difference in median GL was found between larvae fed T_2 diet and those on other diets (T_2 vs T_3: p = 0.265, vs T_4: p = 0.698, vs T_5: p = 1.000, vs T_6: p = 0.993). The GL was 1.62 times higher in tilapia larvae fed T_2 diet compared to those fed T_1 diet.
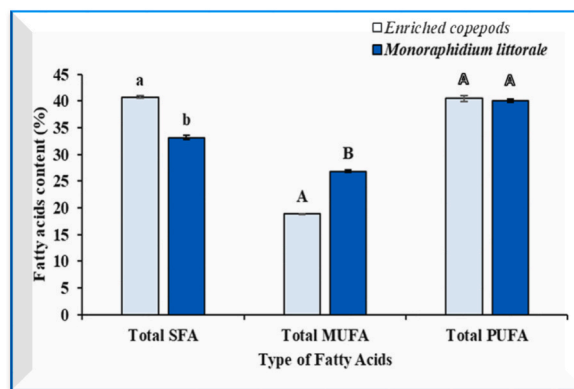
Fig. 8. Mean (±SD) fatty acids content (%) (total SFA = total saturated fatty acids; total MUFA = total monounsaturated fatty acids, and total PUFA = total polyunsaturated fatty acids) of enriched copepods and *Monoraphidium littorale* (n = 3). Different letters represent statistical differences at α = 0.05.

Table 3
Fatty acid profile (% of total fatty acid) of enriched copepods and *M. littorale* on a dry matter basis.

Types of fatty acids	Fatty acids	Content (%) in enriched copepods	Content (%) in *M. littorale*
Saturated fatty acids	Hexanoic acid (C6:0)	0.31 ± 0.02	ND
	Octanoic acid (C8:0)	0.54 ± 0.01	ND
	Decanoic acid (C10:0)	0.04 ± 0.01	0.17 ± 0.10
	Undecanoic acid (C11:0)	0.00 ± 0.00	0.28 ± 0.04
	Lauric acid (C12:0)	0.54 ± 0.02	0.37 ± 0.09
	Tridecanoic acid (C13:0)	0.19 ± 0.01	ND
	Myristic acid (C14:0)	4.11 ± 0.02	0.76 ± 0.10
	Pentadecyclic acid (C15:0)	1.13 ± 0.04	ND
	Palmitic acid (C16:0)	23.12 ± 0.05	17.08 ± 0.30
	Margaric acid (C17:0)	2.16 ± 0.04	ND
	Stearic acid (C18:0)	4.09 ± 0.04	13.79 ± 0.20
	Arachidic acid (C20:0)	0.28 ± 0.05	0.71 ± 0.07
	Heneicosanoic acid (C21:0)	0.24 ± 0.01	ND
	Behenic acid (C22:0)	0.63 ± 0.01	ND
	Tricosanoic acid (C23:0)	2.42 ± 0.03	ND
	Lignoceric acid (C24:0)	0.91 ± 0.03	ND
Monounsaturated fatty acids	Tetradecenoic acid (C14:1)	0.56 ± 0.02	ND
	Pentadecenoic acid (C15:1)	0.16 ± 0.05	ND
	Palmitoleic acid (C16:1)	8.60 ± 0.10	6.25 ± 0.10
	Heptadecenoic acid (C17:1)	0.91 ± 0.12	0.68 ± 0.09
	Oleic acid (C18:1, Cis)	6.69 ± 0.04	6.34 ± 0.20
	Eicosenoic acid (C20:n-9)	0.79 ± 0.05	ND
	Eicosenoic acid (C20:n-7)	ND	13.57 ± 0.30
	Erucic acid (C22:1)	0.40 ± 0.02	ND
	Nervonic acid (C24:1)	0.72 ± 0.03	ND
Polyunsaturated fatty acids	Linoleic acid (C18:2n-6)	5.25 ± 0.04	6.83 ± 0.21
	Gamma-Linolenic acid (C18:3n-6)	0.41 ± 0.01	0.41 ± 0.07
	Alpha-Linolenic acid (C18:3n-3)	13.33 ± 0.21	4.23 ± 0.05
	Eicosadienoic acid (C20:2)	0.52 ± 0.13	ND
	Eicosatrienoic acid (C20:3n-3)	0.88 ± 0.02	ND
	Dihomo-gamma-linolenic acid (C20:3n-6)	0.13 ± 0.03	ND
	Arachidonic acid (C20:4n-6)	2.05 ± 0.04	13.16 ± 0.10
	Eicosapentaenoic acid (C20:5n-3)	11.95 ± 0.02	3.52 ± 0.13
	Docosapentaenoic acid C21:6n-3	ND	2.71 ± 0.20
	Docosadienoic acid (C22:2n-9)	0.25 ± 0.06	4.86 ± 0.30
	Docosahexaenoic acid (C22:6n-3)	5.70 ± 0.09	4.28 ± 0.10
Total saturated fatty acids		40.70 ± 0.25[a]	33.16 ± 0.40[b]
Total monounsaturated fatty acids		18.83 ± 0.05[A]	26.84 ± 0.22[B]
Total polyunsaturated fatty acids		40.47 ± 0.51[C]	40.00 ± 0.30[C]

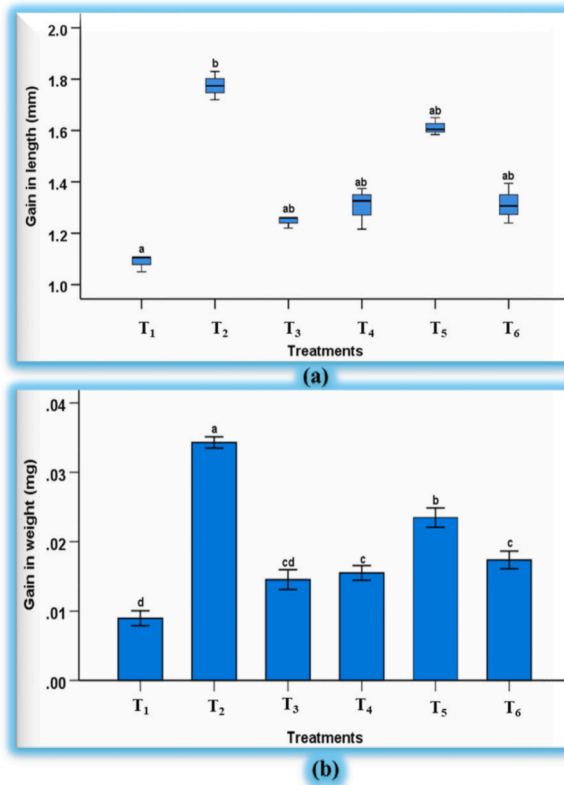The data are presented as mean ± SD (n = 3). ND denotes not detected.

Fig. 9. (a) A box plot illustrating the median gain in length (mm) of tilapia larvae fed 6 different diets (T_1 = live *M. littorale*, T_2 = live copepods enriched with *M. littorale*, T_3 = powdered *M. littorale*, T_4 = powdered enriched copepods, T_5 = live *M. littorale* + live enriched copepods, and T_6 = powdered *M. littorale* + powdered enriched copepods) on the 16th day of the rearing period (n = 3/treatment). The horizontal center line in the boxes represents the median. The box reflects the median contained in the first and third quartiles (Q1 and Q3), with whiskers extending to minimum and maximum values; **(b)** Mean (±SD) values of gain in total weight (mg) of *O. niloticus* larvae on the 16th day of the rearing period at the 6 different diets (n = 3/treatment). In both graphs, different letters represent statistical differences at α = 0.05.

In addition, mean values of GW (mm) differed among tilapia larvae fed on six different diets (One-way ANOVA, $F_{5, 12}$ = 55.00, p < 0.001) (Fig. 9b). Larvae provided T_2 diet demonstrated markedly higher GW compared to all other dietary groups (p < 0.001 for all comparisons). Specifically, the mean GW of larvae fed T_2 diet exceeded that of larvae fed T_1 diet by 3.83, T_3 diet by 2.36, T_4 diet by 2.21, T_5 diet by 1.46, and T_6 diet by 1.97 times.

The tilapia larvae fed 6 different diets significantly differed in growth in terms of LG% (One-way ANOVA, $F_{5, 12}$ = 50.47, p < 0.001)
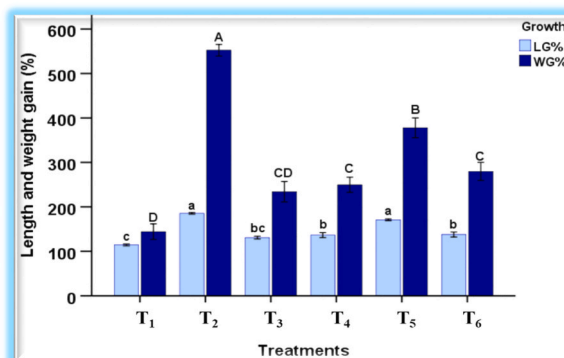

Fig. 10. Mean (±SD) values of length gain LG (%) and weight gain WG (%) of *O. niloticus* larvae on the 16th day of the rearing period at the 6 different diets (n = 3/treatment) (T_1 = live *M. littorale*, T_2 = live copepods enriched with *M. littorale*, T_3 = powdered *M. littorale*, T_4 = powdered enriched copepods, T_5 = live *M. littorale* + live enriched copepods, and T_6 = powdered *M. littorale* + powdered enriched copepods). Different letters represent statistical differences at α = 0.05.

and WG% (One-way ANOVA, $F_{5, 12} = 55.00$, $p < 0.001$) (Fig. 10). The LG% was significantly higher in the T_2 diet in comparison to T_1, T_3, T_4, and T_6 diets ($p < 0.001$ for all comparisons) except T_5 diet ($p = 0.139$). However, larvae-fed T_2 diet gained 1.2 times higher length than the T_5 diet. In addition, WG (%) was also significantly higher in the T_2 than in the other 5 diets ($p < 0.001$ for all comparisons). Specifically, the WG% in the T_2-fed diet was 3.83 times higher than T_1 followed by 2.36 times than T_3, 2.21 times than T_4, 1.45 times higher than T_6-fed diets.

The SGR (% day^{-1}) of tilapia larvae exhibited significant variation across six distinct dietary treatments (One-way ANOVA, $F_{5, 12} = 13.04$, $p < 0.001$) (Fig. 11). Larvae provided with the T_2 diet exhibited the highest SGR compared to all other dietary groups ($p < 0.001$) while, there was no significant difference observed in SGR between T_2 and T_5-fed diet ($p = 0.143$).

3.4. Unraveling the effects of different experimental diets on the survival rate of tilapia larvae

The survival rate (%) of *O. niloticus* larvae varied significantly across the six different diets (One-way ANOVA, $F_{5,12} = 11.01$, $p < 0.001$) (Fig. 12). Larvae fed the T_2 diet showed notably higher survival rates (95 %) in comparison to those fed T_1 diet while, there was no significant difference in survival rates observed among larvae fed T_3 (82 %), T_4 (93 %), T_5 (73 %), and T_6 diet (93 %) ($p > 0.05$ for all comparisons). The lowest survival rate (43.33 %) was recorded in T_1 diet, with a peak in larval mortality observed from the 10th to the 16th day of the rearing period.

3.5. The water quality parameters during the O. niloticus larvae rearing period

The water quality parameters (temperature, pH, and dissolved oxygen) measured during the study period are shown in Table 4. Values of DO (mg L^{-1}) varied significantly (One-way ANOVA, $F_{5,18} = 4.109$, $p = 0.012$) among the treatments although DO levels were in the acceptable range throughout the study period in all treatment groups. The mean range of DO concentration was 6.15 ± 0.13 to 6.51 ± 0.17 mg L^{-1}. Moreover, there was no significant difference in the pH (One-way ANOVA, $F_{5,18} = 0.535$, $p = 0.747$) and temperature (One-way ANOVA, $F_{5,18} = 0.042$, $p = 0.999$) among the treatment groups throughout the experimental period.

4. Discussion

The first feeding of fish larvae is the critical transition time when their internal nutrient sources are depleted, making it essential for them to start consuming external food [54,55]. The success of the first feeding of fish larvae mostly relies on food availability, quality and quantity [54]. Previous studies showed that the utilization of freshwater live copepods as supplementary feed can increase the growth and survival rate of many fish larvae [56–58]. In the present study the highest values of GL, GW, LG %, WG %, SGR, and survival rate of *O. niloticus* larvae were found in enriched copepods (T_2) fed diet. Although the LG % and SGR of larvae were statistically identical in the T_2 and T_5 diets, the T_2 diet caused 1.08 times higher LG % and 1.20 times higher SGR in comparison to the T_5 diet. The best performance of tilapia larvae in T_2 diet might have resulted from the swimming motion, balanced nutritional profile and higher content of digested enzymes and phospholipids of the copepods [54,59]. The present study also highlighted that the higher content of crude protein in enriched copepods underscores its suitability as a primary feed source for larval fish, facilitating optimal growth and survival rates of *O. niloticus* larvae. Moreover, enriched copepods also exhibited higher amino acids and fatty acids content. Notably, enriched copepods had higher levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential omega-3 fatty acids known for their beneficial effects on growth and development in aquatic organisms. Enriched copepods also contained higher levels of oleic acid (18:1n-9), which might have enhanced energy availability and promoted the growth of *O. niloticus* larvae, similar to the findings of Yanes-Roca et al. [8]. Such enrichment procedure of live food should be immensely prioritized for the successful larval rearing of *O. niloticus* larvae in fish hatcheries. Still, it is very challenging to develop a mass culture of copepods although numerous improved culture techniques of copepods have been already explored [60,61]. Rahmati et al. [35] reported that incorporating copepods in the fish larval rearing techniques resulted in significantly better larval survival than different traditional live feeds. Adding copepods as a live feed supplement increased Asian seabass larvae's specific growth rate (6.35 % ± 0.45 % per day) and survival rate (64.20 % ± 3.56 %) [34]. Another copepod, *Acartia clausi* demonstrated higher survival rates (58.13 %) in *L. calcarifer* larvae in comparison to those fed rotifers (39.93 %) and *Artemia* nauplii (41.62 %) [62]. Additionally, the growth of catfish larvae, *Clarias gariepinus* was improved when fed a mixed diet of freshwater cyclopoid copepods and *Artemia* nauplii [63]. Similarly, freshwater fish larvae of *Pterophyllum scalare* exhibited higher specific growth rates when fed a mixed diet containing the cyclopoid copepod *Eucyclops serrulatus* and the cladoceran *Ceriodaphnia quadrangular* [64]. Therefore, a combination diet of *Cyclops* sp. and *Diaptomus* sp. appears to be the most effective for sustainably rearing tilapia larvae. From an aquaculture perspective, copepod species with a balanced nutritional profile and higher fecundity rate are highly desirable for the growth of fish larvae [65,66].

Furthermore, a mixed diet containing live *M. littorale* + enriched live copepods (T_5) resulted in a significantly higher growth rate in larvae in comparison to a diet supplemented with only live *M. littorale* (T_1) which may be due to the synergistic effect of the combined diet's nutritional value. Al-Abdul-Elah et al. [67] also observed significantly higher survival rates in *Pampus argenteus* larvae when fed *Chlorella* sp., *Isochrysis* sp., and *Nannochloropsis* sp. along with rotifers, rather than microalgae alone, which supports the findings of the present study. Microalgae are primarily used for zooplankton enrichment and subsequently supplied for larval-rearing purposes [33, 68].

In the T_1-fed diet, larvae were observed floating at the water surface of the experimental bowls, leading to high mortality, consistent with the findings of Al-Abdul-Elah et al. [67]. The lower growth and survival rates in tilapia larvae fed live *M. littorale* may be attributed to the inadequate supplementation of microalgal cells during the experimental period, despite *M. littorale* being rich in
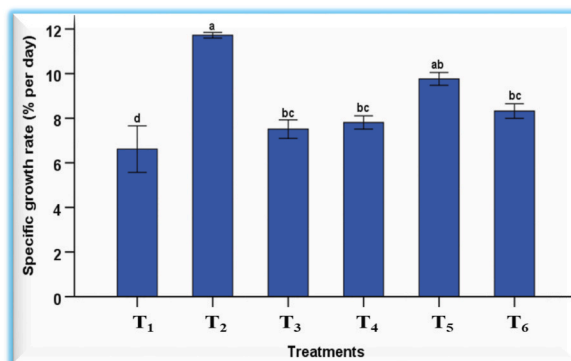
Fig. 11. Mean (±SD) values of specific growth rate, SGR (% day^{-1}) of *O. niloticus* larvae on the 16th day of the rearing period at the 6 different diets (n = 3/treatment) (T_1 = live *M. littorale*, T_2 = live copepods enriched with *M. littorale*, T_3 = powdered *M. littorale*, T_4 = powdered enriched copepods, T_5 = live *M. littorale* + live enriched copepods, and T_6 = powdered *M. littorale* + powdered enriched copepods). Different letters represent statistical differences at α = 0.05.
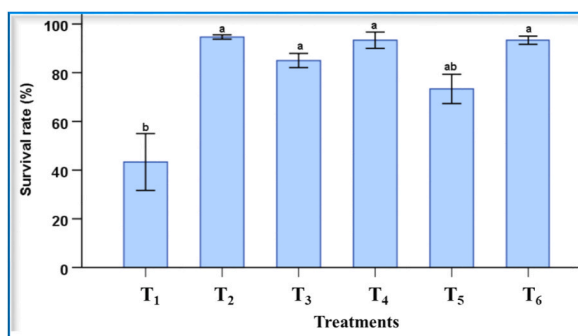

Fig. 12. Mean (±SD) values of survival rate (%) of *O. niloticus* larvae on the 16th day of the rearing period at the 6 different diets (n = 3/treatment) (T_1 = live *M. littorale*, T_2 = live copepods enriched with *M. littorale*, T_3 = powdered *M. littorale*, T_4 = powdered enriched copepods, T_5 = live *M. littorale* + live enriched copepods, and T_6 = powdered *M. littorale* + powdered enriched copepods). Different letters represent statistical differences at α = 0.05.

Table 4
Values (mean ± SD) of water quality parameters after exposure to different food conditions during the experimental period.

Diets	Water quality parameters		
	pH	Temperature (°C)	DO (mg L^{-1})
T_1	9.26 ± 0.12	24.07 ± 0.33	6.48 ± 0.08[a]
T_2	9.20 ± 0.0	24.07 ± 0.11	6.15 ± 0.13[b]
T_3	9.30 ± 0.04	23.92 ± 0.10	6.47 ± 0.14[a]
T_4	9.26 ± 0.01	23.73 ± 0.21	6.51 ± 0.17[a]
T_5	9.18 ± 0.03	23.73 ± 0.12	6.43 ± 0.12[ab]
T_6	9.28 ± 0.03	23.9 ± 0.16	6.34 ± 0.06[ab]

(Means in a column with different letters are significantly different from one another (p < 0.05). T_1: live *M. littorale*; T_2: live copepods enriched with *M. littorale*, T_3: powdered *M. littorale*; T_4: powdered enriched copepods; T_5: live *M. littorale* + live enriched copepods; and T_6: powdered *M. littorale* + powdered enriched copepods).

essential amino acids, monounsaturated and polyunsaturated fatty acids, similar to enriched copepods. Although tilapia larvae showed high survivability in the first 10 days of the rearing period, this changed drastically between days 11 and 16. More research is needed to evaluate the potential of utilizing the green microalga *M. littorale* and to determine the appropriate cell density requirements for the first feeding of various fish and shellfish species.

The present study also demonstrated higher survival rates (>90 %) of *O. niloticus* larvae in T_2, T_4 and T_6. This suggests that dried powdered enriched copepods (T_4) and *M. littorale* + dried powdered enriched copepods (T_6) could be served as viable alternatives to on-site microalgal culture. Laing and Millican [69] found that dried *Tetraselmis suecica* can effectively replace live algal diets to some extent. Similarly, freeze-dried algae completely replaced fresh *Nannochloropsis gaditana* and *Isochrysis galbana* without compromising

growth and survival rates in mass rearing of larval sea bream (*Sparus aurata*) [70]. Therefore, substituting live microalgae and zooplankton with preserved dried powder shows promise as an effective technique for expanding and specializing aquaculture practices in the future. To achieve this, optimizing processing techniques is crucial to preserve the nutritional integrity of powdered food. In our study, we used an oven-drying technique for preparing dried powder of enriched copepods and *M. littorale*. While drying processes are necessary to preserve algae biomass, they are often associated with a loss of quality compared to the fresh product. The drying phase can be carried out through various methods such as freeze-drying, convective and oven drying, infrared, and spray-drying. Desmorieux and Hernandez [71] studied various methods of drying *Spirulina*, finding that freeze-drying resulted in the lowest protein loss (<10 % of the initial proteins), whereas oven drying at 40 °C showed a 10 % protein loss. For oven drying, maintaining a temperature of 40 °C is optimal, as higher temperatures lead to increased protein loss. Although freeze-drying is the best technique for preparing dried microalgae powder, offering minimal protein loss, we did not employ this method due to the un-availability of the necessary equipment. The research findings carry significant implications for the aquaculture sector, particularly in Nile tilapia larval rearing practices. By demonstrating the superior performance of live copepods enriched with *M. littorale* as a starter food, this study highlights a promising approach to improve hatchery production efficiency. The observed high survival rates and enhanced growth suggest that incorporation of enriched copepods into larval diets can be crucial for successful hatchery operations. Additionally, the nutritional analysis underscores the importance of providing larvae with balanced nutrition during early stages for optimal development. These findings emphasize the potential of utilizing enriched live food to address challenges in providing nutritionally balanced and sustainable feed for fish larvae. Hence, for the enduring development of the aquaculture industry, the inclusion of live copepods (*Diaptomus* sp. and *Cyclops* sp.) enriched with *M. littorale* for rearing *O. niloticus* larvae is indispensable in a way of attaining the highest success in larval first-feeding. Thus, integrating live food production techniques into aquaculture can generate a resource-efficient, environment-friendly approach to the progression of the sustainable aquaculture industry.

5. Conclusion

The establishment of a reliable food regime for the small, developing, and extremely vulnerable larvae has turned out to be an emergent impediment in uplifting the production of many commercially important fish. The present study demonstrated that live copepods enriched with *M. littorale* resulted in higher growth, and survival rate of *O. niloticus* larvae and emerged as a promising live food owing to its enriched nutritional profile. The results underscore the practical significance of incorporating enriched copepods into the feeding regime for tilapia larvae, presenting a significant step forward in larval nutrition. The implications of this study for aquaculture are profound. The demonstrated effectiveness of live enriched copepods with *M. littorale* offers a promising direction for enhancing larval fish production, which is crucial for the aquaculture industry. Future research should focus on optimizing the nutritional value of zooplankton through the utilization of various microalgae; investigating the synergistic effects of different microalgae on copepod enrichment and subsequent larval nourishment which will help in refining the feeding protocol. By doing so, the aquaculture industry can achieve greater efficiency and sustainability, ultimately contributing to global food security.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Jinnath Rehana Ritu: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Saleha Khan:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Md Helal Uddin:** Writing – review & editing, Investigation. **Jasmin Akter Poly:** Writing – review & editing, Investigation. **Md Sakhawat Hossain:** Writing – review & editing. **Md Mahfuzul Haque:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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