



Biochemical composition and growth performances of Malaysian Mahseer *Tor tambroides* larvae fed with live and formulated feeds in indoor nursery rearing system

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ARTICLE INFO

Article history:

Received 17 July 2016

Received in revised form

22 September 2016

Accepted 28 September 2016

Available online 24 October 2016

Keywords:

Malaysian mahseer

Live feed

Formulated feed

Fatty acids composition

DNA/RNA ratio

Nursery rearing

ABSTRACT

The consequences of live and formulated feeds on carcass biochemical composition and growth performances of Malaysian mahseer, *Tor tambroides* larvae were assessed in indoor nursery rearing systems. Quadruplicate groups of *T. tambroides* larvae (0.07 ± 0.01 g, mean \pm SE) were stocked in sixteen aquaria ($60 \times 30 \times 30$ cm), randomly arranged in four dietary treatments: larvae fed artemia (LA), moina (LM), daphnia (LD) and formulated feed (FF) with stocking density of 34 larvae per aquarium. An additional experiment was conducted with the same dietary treatments in four circular tanks (1000 L capacity) with stocking density of 100 larvae per tank. In both experiments, the larvae were fed to visually near satiation in two equal feedings per day, seven days per week for 75 days. Treatments results were compared using ANOVA ($\alpha = 0.05$). At the end of the feeding trial, the significantly highest whole body crude protein and lipid contents were found in larvae fed FF compared to those fed live feeds. Whole body polyunsaturated fatty acids were highest in the larvae fed FF compared to those fed live feeds. The significantly highest RNA/DNA ratio was observed in larvae fed FF, followed by those fed LA, and the lowest values were observed in larvae fed LM or LD. The growth-related parameters (mean final weight, mean weight gain and specific growth rate) were significantly highest in treatment FF, followed by treatment LA and lowest in treatment LD or LM. The results of the present experiment demonstrated that the nursery rearing of *T. tambroides* larvae with formulated feed gave better results than feeding live feeds.

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1. Introduction

The success of larval rearing of a particular fish species depends mainly on the availability of suitable diets that are readily consumed, efficiently digested, and provide essential nutrients needed for their growth and health (Giri et al., 2002). Despite recent progress in the production of artificial feeds for fish larvae (Lazo et al., 2000; Cahu and Infante 2001; Koven et al., 2001), feeding of most species of interest for aquaculture still relies on live feeds

during the early life stages due to a number of advantages over artificial feeds. Independently of their nutritional value, live feeds are easily detected and captured due to their swimming movements in the water column and efficiently digested, given their lower nutrient concentrations (water content >80%) (Conceicao et al., 2010). Although live feeds are the preferred choice at early stages of fish rearing, they are costly and not as convenient as formulated feeds. Live feeds are difficult to sustain and require considerable space and expenses. Artificial diets are easier to manage and have lower production costs, but have not proven successful for raising the majority of fish larvae. The dependence on live feeds and lack of nutritional information for fish larvae have hindered progress in developing early stages of fish rearing diets. Therefore, development of artificial feed for fish larvae capable of supporting good survival and growth would be a tremendous benefit for early stages of fish rearing in aquaculture.

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Malaysian Mahseer, *Tor tambroides*, locally known as “kelah”, is a new potential aquaculture species because: (1) it is one of the most important and expensive freshwater food fish in Malaysia, (2) it has a great demand as a game and ornamental fish (Ng, 2004), and (3) successful induced spawning of captive broodstock was developed (Ingram et al., 2005). As the natural abundance and distribution of this species declined, there is great interest in its artificial propagation, both for aquaculture production and for conservation purposes. With the development of successful induced spawning, a ready supply of *T. tambroides* larvae is now available for commercial aquaculture production (Ingram et al., 2007a,b).

The dietary requirements of *T. tambroides* larvae are distinctly different from those of juvenile and adult fish. In general, the early stages of *T. tambroides* require higher protein, lipids and highly unsaturated fatty acids for growth, survival and neural development. In feeding of freshwater larvae, live *Artemia*, *Moina* and *Daphnia* are the most important live feed organisms due to their favorable protein sources for larval development, and their broad spectrum of digestive enzymes and fatty acids (Lavens and Sorgeloos, 1996; Macedo and Pinto-Coelho, 2001). Especially cladocerans such as *Moina* and *Daphnia* have been widely used as larval feeds in ponds (Qin and Culver, 1996). *Artemia* nauplii have also been widely used as live feeds in both freshwater and marine water aquaculture. There are no data on the protein and lipid requirements of early stage *T. tambroides* larvae (average individual weight 0.07 g). Ng et al. (2008a,b) reported that dietary protein and lipid requirements of *T. tambroides* fingerlings between 20 and 55 g are estimated to be 48% and 10%, respectively. Therefore, a formulated feed consisting of 50% protein and 15% lipid was used in this experiment.

Length and weight measurements are poor indicators of the physiological and nutritional status of an organism (Weber et al., 2003). Various combinations of biochemical indices can represent the organism's physiological status and enable an accurate assessment of condition and growth potential. Carcass proximate and fatty acids (FAs) composition have proven to be important in relation to new muscle development and growth, and to the physiological condition of fish especially during larval and early juvenile stages (Cahu et al., 2003; Villeneuve et al., 2005; Lall and Lewis-McCrea, 2007). The RNA/DNA ratio reflects metabolic activity and protein synthesis potential in fish (Tong et al., 2010), especially at early stages of fish rearing. Many studies have shown that the RNA/DNA ratio can be considered a good indicator of physiological and nutritional status for larvae and early juveniles of fish and crustaceans (Westerman and Holt, 1988; Wagner et al., 2001; Desai and Anil 2002).

Although feeding of most species of interest still relies on live feeds during the early life stages, our research objective was to evaluate the performance of *T. tambroides* larvae fed artificial formulated feed compared to those fed live feeds in a nursery rearing system. To the best of our knowledge, there are no published reports yet about any aspect of nursery rearing of *T. tambroides* larvae, particularly on how the biochemical composition and growth performances are affected by different live and formulated feeds at early rearing stages. Therefore, the present experiment was conducted to evaluate the effect of live and formulated feeds on growth performance and carcass biochemical composition of *T. tambroides* larvae in indoor nursery rearing systems.

2. Materials and methods

2.1. Experimental fish, system preparation and research design

T. tambroides larvae were collected by tribal fishermen along the Pahang River in Malaysia. Since the larvae were collected from the

Table 1
Dietary ingredients and feed formulation.

Ingredients (% fed basis)	Formulated feed
Fishmeal ^a	65.00
Squid meal ^b	2.00
Shrimp meal ^b	2.00
Wheat flour ^c	15.00
Cod liver oil ^d	5.00
Soybean lecithin ^a	3.00
Vit Mix ^d	3.00
Min Mix ^e	3.00
Carboxymethyl cellulose ^a	2.00

^a Sri Purta Trading, Alor Star, Kedah.

^b Collected raw material from local market, dried and made squid and shrimp meal in laboratory.

^c Collected from the local market in Terengganu.

^d Rovithai, DSM Nutritional Products Ltd. Scotland; composition (IU/g/mg per kg): vitamin A 50 IU, vitamin D₃ 10 IU; vitamin E130 g, vitamin B₁ 10 g, vitamin B₂ 25 g, vitamin B₆ 16 g, vitamin B₁₂ 100 mg, biotin 500 mg, pantothenic acid 56 g, folic acid 8 g, niacin 200 g, anticake 20 g, antioxidant 0.2 g and vitamin K₃ 10 g.

^e Rovithai, DSM Nutritional Products Ltd. Scotland; composition (g per kg): copper 7.50 g, iron 125.0 g, manganese 25.0 g, zinc 125.0 g, cobalt 0.50 g, iodine 0.175 g, selenium 0.300 g and anticake 10.0 g.

wild, they were identified as *T. tambroides* based on the tribal fishermen expertise and Azuadi et al. (2013). The larvae (0.06 ± 0.02 g) were post-yolk sac stage and dependent on exogenous feeding. Upon arrival in the Mahseer Hatchery, School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, Malaysia (UMT), about 2000 larvae were kept in rectangular fiberglass tanks (240 × 45 × 30 cm) and acclimated to laboratory conditions for 1 week. During this period, *T. tambroides* larvae were fed frozen moina (Tianjin Intra Technologies Co. Ltd., China).

Three different live feeds were used to study their effect on the performance of *T. tambroides*: live *Artemia* (LA); live *Moina* (LM); live *Daphnia* (LD); and an artificial formulated feed (FF). Two feeding trials were conducted with these experimental feeds (LA, LM, LD and FF) for 75 days. In Experiment I, quadruplicate groups of 34 *T. tambroides* larvae (0.07 ± 0.01 g) were stocked in sixteen aquaria (60 × 30 × 30 cm) and assigned randomly to the four dietary treatments. Experiment II was conducted with the same diets in four circular tanks (1000 L capacity) with a stocking density of 100 larvae per tank. This setup allowed to compare the growth performance of *T. tambroides* in two different nursery rearing systems and to get enough sample for whole body proximate analysis. Sand-filtered de-chlorinated freshwater was supplied in each aquarium and tank, which were also equipped with individual air-stones for continuous aeration. To maintain good water quality, uneaten feed and feces were removed every morning by siphoning. About 10% of the water was replaced every day and 100% of the water was replaced biweekly during sampling.

2.2. Feed preparation and feeding of *T. tambroides* larvae

The ingredients and feed formulation of FF is shown in Table 1. All of the dry ingredients were weighed, sieved (mesh size 150 μm) and thoroughly mixed. Subsequently, soybean lecithin, cod liver oil and water were added and mixed again. The final thick dough-like product was dried in an oven at 40 °C. The dried thick dough-like product was rubbed on the surface of a metallic sieve net (mesh size 212 μm) to make larval feed and kept at -20 °C until use. LA nauplii were hatched by incubating 2 g of cysts (red jungle brand, Ocean International Inc., USA) per liter of 32 ppt sea water with sufficient aeration. The hatched nauplii were siphoned out into a 100 μm mesh size plankton net and then washed thoroughly with dechlorinated water. The LD and LM were collected everyday from a green-water based mass culture unit continuously in operation at our hatchery. The larvae were hand-fed with the formulated feed

to visually near satiation level in two equal feedings daily at 09:00 and 17:00 h.

2.3. Water quality measurement

Water quality parameters such as temperature, dissolved oxygen and pH were monitored *in situ* (YSI meter model no 550A) daily at 10:00 h. The concentrations of different cations (ammonium, sodium, potassium, magnesium and calcium) and anions (chloride, nitrate, nitrite, phosphate, sulphate) of culture water were measured fortnightly by using a Metrohm Professional Compact Ion Chromatography (IC) system 881 (Metrohm, Switzerland) with conductivity detector and packed bed suppressor unit and Metrohm 858 Professional Sample Processor. The instrument was used for ion chromatography of anions with sequential suppression and without the suppression for the determination of cations. All water samples were filtered (2 µm) before analysis with IC. Filtered, unacidified samples were analyzed using a Metrospe A Supp 5–250 column for anion analysis with an eluent of sodium carbonate and bi-carbonate. Filtered acidified samples were analyzed using a Metrosep C4 150 column for cation analysis using an eluent of 1% nitric acid and 4.6 mM H₃PO₄. Prior to injection, the samples were passed through the ultrafiltration cell mounted directly on the 858 Professional IC Sample Processor. Sample preparation and analysis are fully automatic. The ultra-pure water type 1 was used as blank. Data acquisition and instrument settings were performed by Magic Net software (version 2.1; Metrohm, Switzerland).

2.4. Estimation of growth parameters

Growth performances of *T. tambroides* larvae were monitored in terms of mean final weight (g), weight gain (%), specific growth rate (% day⁻¹) and survival rate (%). At the end of the experiment, individual weight and standard length of all fish from each aquarium and tank were measured. The formula for the calculation of these parameters are as follows:

$$\text{Weight gain(\%)} = \left\{ \frac{\text{(final weight - initial weight)}}{\text{initial weight}} \right\} \times 100$$

$$\text{Specific growth rate(\%day}^{-1}\text{)} = \left\{ \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{75 \text{ days}} \right\} \times 100$$

$$\text{Survival(\%)} = 100 \times \left\{ \frac{\text{final no. of } T. \text{tambroides}}{\text{initial no. of } T. \text{tambroides}} \right\}$$

2.5. Proximate composition analysis

Proximate compositions of formulated feed, live feeds and *T. tambroides* whole body samples were analyzed in triplicate using standard AOAC methods (AOAC, 1990). The moisture was determined by drying the sample at 105 °C to constant weight. The ash was analyzed by combustion at 550 °C for 12 h. The crude protein content was determined by measuring the nitrogen content (N × 6.25) using the Kjeldahl method (KBL 40 S Digestion System, Gerhardt GmbH & Co. Königswinter, Germany and Kjeltec 2100 Distilling unit, FOSS Tecator AB, Högendäs, Sweden). Crude lipids were analyzed according to the soxhlet method (FOSS Labtec ST310, Högendäs, Sweden).

2.6. Fatty acids analysis

The freeze dried samples of LA, LM, LD and FF, and whole body of *T. tambroides* were analyzed for fatty acid (FA) composition. Quadruplicate samples (200–300 mg) were taken and the one-step method of FA analysis was carried out by combining the extraction and esterification processes using a single tube following the method described by [Abdulkadir and Tsuchiya \(2008\)](#). The fatty acid methyl esters (FAMES) were separated and quantified by gas chromatography equipped with mass spectrometry (GCMS-QP2010 Ultra). Qualitatively (as a percentage), composition in terms of individual FAs was calculated by comparing the peak area of each FA with the total peak area of all FAs in the sample.

2.7. RNA/DNA ratio analysis

Total RNA and DNA were extracted on Day-1, Day-30, Day-50 and Day-75 of the feeding trial from the four average representative size of fish (very close to mean individual weight) from each treatment using NucleoSpin[®] RNA and NucleoSpin[®] Tissue DNA isolation kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) according to the manufacturer's protocol. During RNA isolation, the muscle samples from larvae/early juveniles were transferred into RNAlater[™] solution (Ambion Inc., Texas, USA) to prevent RNA degradation. About 2 mg and 20 mg of sample were taken from each fish for RNA and DNA analysis, respectively. Then the samples were homogenized using Medigene WT130 Hand –held Homogenizer until complete mixing. The quantities of RNA and DNA were determined by using Eppendorf BioPhotometer plus (Eppendorf AG, Hamburg, Germany) and finally calculated as µg of RNA or DNA mg⁻¹ of each sample. The ratio of RNA and DNA of each sampling date was determined by dividing the concentrations of RNA (µg mg⁻¹) and DNA (µg mg⁻¹).

2.8. Statistical analysis

All experimental data were subjected to one-way analysis of variance (ANOVA) to determine if significant differences occurred among different treatments. The assumptions of normal distributions and homogeneity of variances were checked before analysis. All ANOVA were tested at 5% level followed by Tukey's test in IBM SPSS Statistics software version 22.

3. Results

3.1. Proximate and fatty acid composition of live and formulated feeds

The live feeds (LA, LM and LD) contained only 7.30 to 10.55% dry matter compared to 88.25% in FF ([Table 2](#)). However, the crude protein and crude lipid contents (on dry matter basis) of all live feeds were comparable with FF, except protein content in LM. The ash content was about four times higher in LD compared to the others. The FA composition of the experimental feeds ([Table 3](#)) showed that saturated FAs (SFAs) were highest in LA (30.7%) followed by LD (25.6%) and LM (26.4%), and the lowest was observed in FF (22.1%). On the other hand, FF contained the highest levels of eicosapentaenoic acid (EPA, C20:5n-3; 5.32%), docosahexanoic acid (DHA, C22:6n-3; 4.32%) and total amount of n-3 poly unsaturated fatty acids (PUFA) (17.16%) compared to the live feeds. LD and LA contained lower levels of total n-3 PUFA (10.26% and 8.54%, respectively) with very low levels of EPA (0.17% and 0.89%, respectively) and DHA (0.07% and 0.12%, respectively). Similar with n-3 PUFA, the highest level of n-6 PUFA (17.44%) was found in FF compared to the live feeds (11.24% to 13.77%) which all contained a major percentage (70.3% to 88.7%) of linoleic acid (C18:2n-6). The n-3:

Table 2
Proximate composition of experimental feeds for nursery rearing of *T. tambroides* larvae^a.

Proximate composition (% dry matter basis)	Experimental feeds ^b			
	LA	LM	LD	FF
Dry matter	10.55 ± 0.67	7.30 ± 0.51	7.82 ± 0.56	88.25 ± 0.38
Crude protein	54.95 ± 1.16	66.55 ± 0.84	48.52 ± 0.85	50.39 ± 0.37
Crude lipid	16.93 ± 0.43	18.29 ± 0.67	14.42 ± 0.37	15.56 ± 0.48
Ash	7.53 ± 0.36	5.61 ± 0.43	26.28 ± 0.99	6.77 ± 0.34

^a Values are mean ± SE of triplicate measurements (n = 3).

^b LA, treatment with artemia as live food; LM, treatment with moina as live food; LD, treatment with daphnia as live food; FF, treatment with formulated feed.

Table 3
Fatty acid composition of experimental feeds for nursery rearing of *T. tambroides* larvae¹.

Fatty acids (% of total fatty acids)	Experimental feeds ²			
	LA	LM	LD	FF
C14:0	0.85 ± 0.01 ^d	2.18 ± 0.03 ^c	2.56 ± 0.04 ^b	4.34 ± 0.05 ^a
C15:0	0.35 ± 0.01 ^b	–	–	0.51 ± 0.02 ^a
C16:0	19.71 ± 0.37 ^a	17.34 ± 0.05 ^b	16.39 ± 0.12 ^c	13.41 ± 0.11 ^d
C17:0	0.75 ± 0.04 ^a	0.22 ± 0.01 ^c	0.55 ± 0.01 ^b	–
C18:0	8.75 ± 0.05 ^a	5.67 ± 0.21 ^b	4.65 ± 0.11 ^c	3.82 ± 0.02 ^d
C20:0	0.25 ± 0.01 ^b	0.27 ± 0.01 ^b	0.49 ± 0.01 ^a	–
C22:0	–	0.68 ± 0.02 ^b	0.99 ± 0.04 ^a	–
Total saturates	30.66 ± 0.43 ^a	26.38 ± 0.21 ^b	25.63 ± 0.11 ^b	22.09 ± 0.17 ^c
C14:1n5	0.55 ± 0.02 ^a	–	0.44 ± 0.02 ^b	–
C16:1n7	9.06 ± 0.07 ^b	16.57 ± 0.17 ^a	0.76 ± 0.02 ^d	8.54 ± 0.07 ^c
C16:1n9	0.88 ± 0.02 ^a	–	–	0.52 ± 0.02 ^b
C18:1n7	7.34 ± 0.28 ^a	–	–	2.20 ± 0.04 ^b
C18:1n9	18.34 ± 0.07 ^c	18.34 ± 0.05 ^c	36.32 ± 0.45 ^a	22.61 ± 0.28 ^b
C20:1n9	0.32 ± 0.02 ^{bc}	0.42 ± 0.03 ^b	0.27 ± 0.02 ^c	1.32 ± 0.03 ^a
C22:1n9	–	–	–	0.89 ± 0.01
Total monoenes	36.50 ± 0.24 ^{ab}	35.33 ± 0.24 ^b	37.79 ± 0.44 ^a	36.09 ± 0.27 ^b
C18:2n6	8.64 ± 0.10 ^b	12.21 ± 0.04 ^a	8.71 ± 0.08 ^b	12.68 ± 0.20 ^a
C18:3n6	0.33 ± 0.01 ^c	0.27 ± 0.01 ^d	0.46 ± 0.01 ^b	0.55 ± 0.02 ^a
C20:3n6	–	1.07 ± 0.06 ^b	1.84 ± 0.05 ^a	1.26 ± 0.03 ^b
C20:4n6	2.27 ± 0.08 ^a	–	0.09 ± 0.01 ^b	2.26 ± 0.01 ^a
C22:5n6	1.05 ± 0.05	0.23 ± 0.01	0.15 ± 0.01	0.69 ± 0.01
Total n-6 PUFA	12.29 ± 0.16 ^c	13.77 ± 0.01 ^b	11.24 ± 0.11 ^c	17.44 ± 0.22 ^a
C18:3n3	6.76 ± 0.11 ^b	6.65 ± 0.09 ^b	8.14 ± 0.08 ^a	4.42 ± 0.13 ^c
C18:4n3	0.61 ± 0.02 ^a	0.27 ± 0.01 ^b	0.27 ± 0.02 ^b	0.28 ± 0.02 ^b
C20:3n3	–	1.75 ± 0.07 ^a	1.51 ± 0.04 ^b	0.88 ± 0.02 ^c
C20:5n3	0.89 ± 0.03 ^c	2.82 ± 0.03 ^b	0.17 ± 0.01 ^d	5.32 ± 0.04 ^a
C22:5n3	–	–	0.10 ± 0.01 ^b	1.93 ± 0.03 ^a
C22:6n3	0.12 ± 0.01 ^c	0.34 ± 0.02 ^b	0.07 ± 0.01 ^c	4.32 ± 0.04 ^a
Total n-3 PUFA	8.54 ± 0.05 ^d	11.92 ± 0.15 ^b	10.26 ± 0.03 ^c	17.16 ± 0.21 ^a
Total PUFA	20.83 ± 0.21 ^c	25.71 ± 0.15 ^b	21.5 ± 0.14 ^c	34.61 ± 0.24 ^a
Total n-3 HUFA	1.05 ± 0.03 ^c	3.24 ± 0.06 ^b	0.34 ± 0.02 ^d	11.57 ± 0.11 ^a
n-3:n-6	0.69 ± 0.01 ^c	0.86 ± 0.02 ^b	0.91 ± 0.01 ^b	0.98 ± 0.02 ^a
Total fatty acid	88.00 ± 0.55 ^b	87.4 ± 0.18 ^b	84.92 ± 0.28 ^c	92.79 ± 0.51 ^a

¹ Values are the mean ± SE of triplicate measurements (n = 3). The mean values followed by the different superscript letter in each parameter indicate significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test.

² LA, treatment with artemia as live food; LM, treatment with moina as live food; LD, treatment with daphnia as live food; FF, treatment with formulated feed.

n-6 PUFA ratios were highest in FF (0.98) followed by the LD and LM (0.91 and 0.86, respectively) and the lowest was observed in LA (0.69). All differences described here were significant ($P < 0.05$; see Table 3).

3.2. Water quality parameters

Ion chromatography data showed that ammonium, nitrate, nitrite, phosphate and sodium ion concentration in the aquaria nursery rearing system significantly varied among different treatments; whereas other water quality parameters were not significantly affected by the dietary treatments (Table 4). All nitrogenous and phosphate ion concentrations were significantly higher in treatment FF than in the other treatments. However, all the water quality parameters in different dietary treatments were within the acceptable range of fish culture.

3.3. Effects on growth parameters

In aquaria rearing system, mean final weight, weight gain and SGR values were highest in treatment FF, followed by the treatment LA and LM; while the lowest values were observed in treatment LD (Table 5). The mean final standard length of fish was also highest ($P < 0.05$) in treatment FF. The survival was lowest in LD and highest in LA ($P < 0.05$), while slight variations ($P > 0.05$) were found among the other treatments. Similar to the aquaria nursery rearing system, the growth parameters of *T. tambroides* in the tank nursery system were significantly higher in the FF group than in all the live feed groups. In addition, the values for all the observed growth performance parameters were higher in all the tank treatments than in the aquaria. However, the survival in the tank system was slightly lower than in the aquaria system.

Table 4
Water quality parameters (mean \pm SE) observed in indoor aquarium nursery rearing system of *T. tambroides* larvae fed with different experimental feeds for 75 days¹.

Water quality parameters	Experimental feeds ²				Desired range ³
	LA	LM	LD	FF	
Temperature ($^{\circ}$ C)	26.92 \pm 0.05	27.15 \pm 0.67	26.81 \pm 0.11	26.98 \pm 0.90	25–35
Dissolved O ₂ (mg l ⁻¹)	5.48 \pm 0.17	4.60 \pm 0.46	5.59 \pm 0.43	4.68 \pm 0.04	5–15
pH	7.42	7.51	7.52	7.38	6.5–9
Ammonium ion (mg l ⁻¹)	0.47 \pm 0.03 ^b	0.47 \pm 0.04 ^b	0.48 \pm 0.03 ^b	0.65 \pm 0.05 ^a	0.2–2
Sodium ion (mg l ⁻¹)	3.84 \pm 0.27 ^{ab}	4.75 \pm 0.45 ^a	3.35 \pm 0.23 ^b	3.28 \pm 0.22 ^b	2–100
Potassium ion (mg l ⁻¹)	2.43 \pm 0.25	2.49 \pm 0.29	2.77 \pm 0.26	2.56 \pm 0.23	1–10
Magnesium ion (mg l ⁻¹)	7.18 \pm 0.38	7.04 \pm 0.38	6.85 \pm 0.39	6.87 \pm 0.35	5–100
Calcium ion (mg l ⁻¹)	7.62 \pm 0.46	8.27 \pm 0.56	7.60 \pm 0.54	7.57 \pm 0.47	5–100
Chloride ion (mg l ⁻¹)	6.22 \pm 0.36	5.40 \pm 0.30	6.14 \pm 0.45	6.04 \pm 0.35	1–100
Nitrate ion (mg l ⁻¹)	5.41 \pm 0.48 ^{ab}	5.24 \pm 0.41 ^{ab}	4.89 \pm 0.39 ^b	6.72 \pm 0.30 ^a	0.2–10
Nitrite ion (mg l ⁻¹)	0.12 \pm 0.005 ^b	0.13 \pm 0.008 ^{ab}	0.13 \pm 0.007 ^{ab}	0.15 \pm 0.011 ^a	<0.3
Phosphate ion (mg l ⁻¹)	0.45 \pm 0.02 ^b	0.44 \pm 0.02 ^b	0.47 \pm 0.03 ^{ab}	0.54 \pm 0.02 ^a	0.005–0.8
Sulphate ion (mg l ⁻¹)	4.18 \pm 0.24	4.37 \pm 0.31	4.64 \pm 0.34	4.70 \pm 0.31	2–100

¹ Values are the mean \pm SE of four replicates and five sampling dates (n = 20). The mean values followed by the different superscript letter in each parameter indicates significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test.

² LA, treatment with artemia as live food; LM, treatment with moina as live food; LD, treatment with daphnia as live food; FF, treatment with formulated feed.

³ The range of desired concentrations of water quality parameters was considered based on the reports of Boyd (1990, 1998).

Table 5
Growth performance of *T. tambroides* larvae fed with different experimental feeds in indoor aquarium¹ and tank² nursery rearing systems for 75 days.

Parameters	Experimental feeds ³			
	LA	LM	LD	FF
In aquarium nursery rearing system				
Mean initial weight (g)	0.07 \pm 0.01	0.07 \pm 0.01	0.07 \pm 0.01	0.07 \pm 0.01
Mean final weight (g)	0.60 \pm 0.01 ^b	0.37 \pm 0.01 ^c	0.33 \pm 0.02 ^c	0.82 \pm 0.02 ^a
Mean final standard length (cm)	2.90 \pm 0.11 ^a	2.16 \pm 0.11 ^b	2.02 \pm 0.03 ^b	3.13 \pm 0.04 ^a
Weight gain (%)	569.2 \pm 13.9 ^b	307.7 \pm 12.2 ^c	267.2 \pm 13.4 ^c	807.2 \pm 27.6 ^a
Specific growth rate (% day ⁻¹)	2.53 \pm 0.03 ^b	1.87 \pm 0.04 ^c	1.73 \pm 0.05 ^c	2.93 \pm 0.04 ^a
Survival (%)	96.3 \pm 1.4 ^a	88.2 \pm 2.68 ^{ab}	87.5 \pm 2.5 ^b	91.9 \pm 1.4 ^{ab}
In tank nursery rearing system				
Mean initial weight (g)	0.07 \pm 0.01	0.07 \pm 0.01	0.07 \pm 0.01	0.07 \pm 0.01
Mean final weight (g)	0.67 \pm 0.08 ^b	0.43 \pm 0.07 ^c	0.38 \pm 0.05 ^c	0.88 \pm 0.11 ^a
Weight gain (%)	857.1 \pm 15.6 ^b	428.5 \pm 12.6 ^c	371.4 \pm 14.8 ^c	1157 \pm 33.4 ^a
Specific growth rate (% day ⁻¹)	2.67 \pm 0.06 ^b	2.07 \pm 0.05 ^c	1.93 \pm 0.05 ^c	3.25 \pm 0.07 ^a
Survival (%)	94.5	88.5	85.4	90.6

¹ Aquarium nursery rearing system: the values are the mean \pm SE of four replicates (n = 4). The mean values followed by the different superscript letter in each parameter indicates significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test.

² Tank nursery rearing system: the values represented here are the mean \pm SE of three identical subsamples of fish (n = 3) from a population of 100 fish in a single replicate and statistics was performed as described in footnote 1.

³ LA, treatment with artemia as live food; LM, treatment with moina as live food; LD, treatment with daphnia as live food; FF, treatment with formulated feed.

3.4. Proximate and fatty acid composition of whole body samples of *T. tambroides*

After 75 days of feeding trial, whole body moisture, crude protein, crude lipid and ash of fish from the tank system were significantly different among the dietary treatments (Table 6). The moisture content was lowest, and the crude protein and crude lipid contents were highest in larvae fed with FF compared to larvae fed the live feeds. Significantly lower levels of protein and lipid were found in treatments LM and LD. The ash content was significantly higher in fish fed LD compared to the other treatments.

Among saturated fatty acids (SFAs), the concentrations of palmitic acid (C16:0) were the highest (27.31–29.91%) followed by the oleic acid (C18:0) (7.29–8.56%) in the carcass lipid (Table 7). Total monoenes concentrations of the whole body samples were consistent with those of the live feeds and found to differ significantly among treatments (Tables 3 and 7). The total n-6 PUFA was significantly highest in larvae fed the FF diet while lowest in the LD group. Similarly lower values (3.31–5.05%) of total n-3 PUFA were found in whole body samples of fish fed the live feeds compared to those in FF (10.17%). The EPA and DHA followed similar trends. The n-3: n-6 ratio in carcass lipid ranged from 0.44 for fish fed LA to 1.03 for FF.

3.5. Muscle RNA and DNA concentrations and their ratios

RNA concentrations consistently increased over the total experimental periods in all treatments ranging from 29.6% (LD) to 106% (FF) (Table 8). On Day-75, the highest RNA was found in treatment FF (9.85 μ g mg⁻¹), followed by treatment LA (7.93 μ g mg⁻¹) and the lowest was observed in treatments LD (5.91 μ g mg⁻¹) and LM (5.95 μ g mg⁻¹). On the other hand, DNA concentrations were almost similar over the experimental period and were not affected by the dietary treatments. As the DNA concentrations were not affected by the dietary treatments and remained similar ($P > 0.05$) over the different sampling periods, RNA/DNA ratios varied significantly among treatments and followed the same trend as RNA concentrations of increasing gradually over the experimental period. At the end of the experiment, RNA/DNA ratio was highest in fish muscle fed FF (2.73), followed by the fish fed LA (2.28) and the lowest were found in fish fed LD (1.73) and LM (1.74).

4. Discussion

Addressing the challenges of larval nutrition is complex but necessary to make progress in developing diets for optimum growth and development of the early life stages of fish. The present study

Table 6Proximate composition of whole body samples of *T. tambroides* fed with different experimental feeds in indoor tank nursery rearing system for 75 days¹.

Proximate composition (% wet basis)	Experimental feeds ²			
	LA	LM	LD	FF
Moisture	73.64 ± 0.27 ^{ab}	75.51 ± 0.21 ^a	74.01 ± 0.32 ^{ab}	72.68 ± 0.34 ^b
Crude protein	14.08 ± 0.14 ^b	13.62 ± 0.12 ^{bc}	13.18 ± 0.16 ^c	14.79 ± 0.13 ^a
Crude lipid	6.08 ± 0.08 ^{ab}	5.71 ± 0.03 ^b	5.83 ± 0.07 ^b	6.32 ± 0.16 ^a
Ash	3.04 ± 0.07 ^b	3.15 ± 0.07 ^b	3.89 ± 0.12 ^a	3.09 ± 0.07 ^b

¹ Values are the mean ± SE of triplicate measurements (n = 3). The mean values followed by the different superscript letter in each parameter indicates significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test.

² LA, treatment with artemia as live food; LM, treatment with moina as live food; LD, treatment with daphnia as live food; FF, treatment with formulated feed.

Table 7Fatty acid composition of whole body samples of *T. tambroides* early juveniles fed with different experimental feeds in indoor aquarium nursery rearing systems for 75 days¹.

Fatty acid (% of total fatty acids)	Experimental feeds ²			
	LA	LM	LD	FF
C14:0	3.81 ± 0.07	3.26 ± 0.53	3.47 ± 0.20	2.74 ± 0.34
C16:0	28.74 ± 1.56	29.91 ± 1.47	28.15 ± 0.96	27.31 ± 1.66
C17:0	0.44 ± 0.06 ^a	0.24 ± 0.06 ^{bc}	0.36 ± 0.01 ^{ab}	0.07 ± 0.01 ^c
C18:0	8.45 ± 0.24	7.29 ± 0.44	7.42 ± 0.07	8.56 ± 0.34
C20:0	0.15 ± 0.03 ^{bc}	0.25 ± 0.02 ^a	0.23 ± 0.01 ^{ab}	0.07 ± 0.01 ^c
Total saturates	41.59 ± 1.77	40.94 ± 1.11	39.63 ± 1.07	38.75 ± 1.86
C14:1n5	0.37 ± 0.03	0.30 ± 0.02	0.31 ± 0.03	0.29 ± 0.03
C16:1n7	6.77 ± 0.06 ^{ab}	7.74 ± 0.56 ^a	3.84 ± 0.59 ^c	4.68 ± 0.64 ^{bc}
C18:1n7	2.45 ± 0.19 ^a	0.81 ± 0.02 ^b	0.62 ± 0.02 ^b	1.02 ± 0.90 ^b
C18:1n9	22.64 ± 0.25 ^b	25.01 ± 0.61 ^b	31.78 ± 0.31 ^a	25.11 ± 0.94 ^b
C20:1n9	2.71 ± 0.01 ^b	2.52 ± 0.02 ^b	2.72 ± 0.01 ^b	6.76 ± 0.01 ^a
Total monoenes	34.94 ± 0.21 ^b	36.37 ± 0.88 ^{ab}	39.27 ± 0.51 ^a	35.43 ± 1.50 ^{ab}
C18:2n6	6.52 ± 0.03 ^b	7.76 ± 0.01 ^a	6.51 ± 0.09 ^b	7.82 ± 0.03 ^a
C18:3n6	0.26 ± 0.01 ^b	–	0.24 ± 0.05 ^b	0.39 ± 0.01 ^a
C20:3n6	0.21 ± 0.01 ^c	0.31 ± 0.01 ^b	0.37 ± 0.03 ^b	0.51 ± 0.01 ^a
C20:4n6	0.48 ± 0.02 ^b	0.12 ± 0.01 ^d	0.32 ± 0.01 ^c	0.97 ± 0.01 ^a
Total n-6 PUFA	7.46 ± 0.05 ^c	8.21 ± 0.02 ^b	7.44 ± 0.11 ^c	9.78 ± 0.05 ^a
C18:3n3	1.12 ± 0.01	1.11 ± 0.02	1.05 ± 0.05	1.09 ± 0.01
C20:3n3	0.15 ± 0.02 ^d	0.75 ± 0.01 ^a	0.63 ± 0.01 ^b	0.41 ± 0.01 ^c
C20:5n3	0.35 ± 0.01 ^d	0.88 ± 0.03 ^b	0.47 ± 0.01 ^c	2.29 ± 0.04 ^a
C22:5n3	0.33 ± 0.01 ^{bc}	0.41 ± 0.02 ^b	0.29 ± 0.02 ^c	1.16 ± 0.04 ^a
C22:6n3	1.36 ± 0.03 ^c	1.91 ± 0.04 ^b	0.95 ± 0.06 ^d	5.21 ± 0.01 ^a
Total n-3 PUFA	3.31 ± 0.05 ^c	5.05 ± 0.06 ^b	3.39 ± 0.13 ^c	10.17 ± 0.08 ^a
Total PUFA	10.78 ± 0.08 ^c	13.25 ± 0.04 ^b	10.84 ± 0.17 ^c	19.95 ± 0.09 ^a
Total n-3 HUFA	2.04 ± 0.05 ^c	3.18 ± 0.08 ^b	1.70 ± 0.07 ^d	8.67 ± 0.08 ^a
n-3:n-6	0.44 ± 0.01 ^c	0.62 ± 0.01 ^b	0.46 ± 0.02 ^c	1.03 ± 0.01 ^a

¹ Values are the mean ± SE of four replicates (n = 4). The mean values followed by the different superscript letter in each parameter indicates significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test.

² LA, treatment with artemia as live food; LM, treatment with moina as live food; LD, treatment with daphnia as live food; FF, treatment with formulated feed.

revealed that *T. tambroides* larvae performed better with FF compared to any of the live feeds tested in terms of growth performance, body composition and RNA/DNA ratio.

All of the water quality parameters in different dietary treatments were within the acceptable range of fish culture (Boyd, 1998). The dietary protein requirement of *T. tambroides* fingerlings (20–55 g body weight) is estimated to be 48% (Ng et al., 2008a,b). The protein content of the live and formulated feeds used in this experiment ranged from 48.5% (LD) to 66.5% (LM) on a dry matter basis, indicating that protein availability was comparable. Since larval stages of fish require comparatively higher protein content than the juvenile or later stages, the different live and formulated feeds in the present study can be assumed to satisfy the dietary protein requirement of *T. tambroides*.

In general, all the live and formulated feeds were well accepted by the larvae of *T. tambroides* and the growth performances of the larvae were satisfactory for all the dietary treatments. The final weight as well as weight gain and SGR were higher in the tank nursery system than in the aquarium system, which might be attributed to the larger size of the tank nursery system providing more space. Most importantly, the trends of growth performance (final weight, weight gain and SGR) were similar between the two nursery systems and in both systems the highest growth was found

in larvae fed with FF compared to any of the live feeds. Similarly, larvae of several freshwater fish species, such as goldfish (*Carassius auratus*) (Szlaminska et al., 1993) and pike perch (*Sander lucioperca*) (Ostaszewska et al., 2005), have been successfully reared entirely on artificial formulated diets. In contrast, co-feeding with live (artemia or mixture of artemia and rotifer, respectively) and formulated feeds was suggested for the larvae of southern flounder (*Paralichthys lethostigma*) (Alam et al., 2015) and spider crab (*Maja brachydactyla*) (Andrés et al., 2011).

Significantly higher whole body protein content was observed in fish fed the FF. Fish growth primarily consists of deposition of muscle protein. Therefore, the higher percentages of carcass protein in FF might be related to the high growth rate of *T. tambroides*. The lipid content of live and formulated feeds used in this experiment ranged from 14.4% (LD) to 18.29% (LM) on dry matter basis. Previous studies have shown that the dietary lipid requirement of juvenile *T. tambroides* is only 5% and increasing dietary lipid level beyond 6% up to 19% did not result any significant changes in growth performances (Ng and Andin, 2011). The lipid content of live and formulated feeds used in this experiment did not consistently reflect the whole body lipid contents of *T. tambroides*. However, consistent with the protein content, whole body lipid content might be related with the growth rate of *T. tambroides*, and was highest

Table 8
RNA and DNA concentration ($\mu\text{g mg}^{-1}$) and RNA/DNA ratio of muscle tissues of *T. tambroides* fed with different experimental feeds in indoor aquarium nursery rearing systems at different experimental periods¹.

Variables	Experimental feeds ²			
	LA	LM	LD	FF
RNA concentration ($\mu\text{g mg}^{-1}$)				
Day-1	4.56 ± 0.21	4.56 ± 0.21	4.56 ± 0.21	4.56 ± 0.21
Day-30	6.66 ± 0.08 ^a	4.89 ± 0.11 ^b	4.95 ± 0.19 ^b	7.06 ± 0.13 ^a
Day-50	7.02 ± 0.08 ^b	5.46 ± 0.28 ^c	5.69 ± 0.09 ^c	8.83 ± 0.25 ^a
Day-75	7.93 ± 0.13 ^b	5.95 ± 0.09 ^c	5.91 ± 0.19 ^c	9.85 ± 0.23 ^a
DNA concentration ($\mu\text{g mg}^{-1}$)				
Day-1	3.27 ± 0.04	3.27 ± 0.04	3.27 ± 0.04	3.27 ± 0.04
Day-30	3.41 ± 0.41	3.33 ± 0.08	3.29 ± 0.06	3.37 ± 0.03
Day-50	3.44 ± 0.10	3.39 ± 0.06	3.50 ± 0.04	3.45 ± 0.06
Day-75	3.48 ± 0.06	3.42 ± 0.06	3.42 ± 0.08	3.60 ± 0.06
RNA/DNA ratio				
Day-1	1.39 ± 0.08	1.39 ± 0.08	1.39 ± 0.08	1.39 ± 0.08
Day-30	1.95 ± 0.04 ^a	1.47 ± 0.05 ^b	1.50 ± 0.06 ^b	2.09 ± 0.04 ^a
Day-50	2.04 ± 0.05 ^b	1.61 ± 0.10 ^c	1.62 ± 0.03 ^c	2.55 ± 0.11 ^a
Day-75	2.28 ± 0.07 ^b	1.74 ± 0.01 ^c	1.73 ± 0.04 ^c	2.73 ± 0.02 ^a

¹ Values are the mean ± SE of four replicates (n = 4). The mean values followed by the different superscript letter in each parameter indicates significant difference at 0.05. If the effects were significant, ANOVA was followed by Tukey test.

² LA, treatment with artemia as live food; LM, treatment with moina as live food; LD, treatment with daphnia as live food; FF, treatment with formulated feed.

in FF where the growth rate of *T. tambroides* was also the highest. Other study reported similar observations in *T. tambroides* (Ng and Andin, 2011). The significantly higher whole carcass ash content in treatment LD might be related to the very high ash content (26.3%) in the LD diet.

The main roles of fatty acids in larval fish are consistent with those in juveniles and adults. In general, it is known that the dietary fatty acid composition reflects the fatty acid composition of the tissue lipids in fish, and this statement applies more to for PUFA than to SFA (Turchini et al., 2009; Ng and Andin, 2011). In general, SFAs are integrated in muscle lipids at a narrower range because these fatty acids have the ability to be oxidized depending on dietary levels (Ng and Andin, 2011). In the present study, the range of SFA (42.5% to 45.6% of the total fatty acids) content observed in the whole body of *T. tambroides* was much narrower compared to the formulated and live feeds (22.1 to 30.7% of the total fatty acids). In another experiment with juvenile *T. tambroides* (Ng and Andin, 2011) the SFA content varied from 5.9% to 45.8% of the total fatty acids in the diet, but only from 28.5% to 32.4% in muscle lipid. Other studies reported similar observations in red hybrid tilapia *Oreochromis sp.* (Bahurmiz and Ng, 2007), grass carp (Du et al., 2008) and Chinook salmon *Oncorhynchus tshawytscha* (Mugrditchian et al., 1981). In contrasts to SFAs, whole body PUFA content of *T. tambroides* consistently reflected the PUFA content of live and formulated feeds used in this experiment. Other studies reported similar observations that the PUFA content of the tissue lipid in fish is closely related to dietary PUFA composition (Bahurmiz and Ng, 2007; Du et al., 2008; Turchini et al., 2009; Ng and Andin, 2011).

The low carbon PUFAs (LCPUFAs) – EPA, DHA and ARA – in the feeds largely determine the efficacy of many physiological processes during rapid growth and development of fish larvae and larvae. Fatty acid analysis showed that the live feeds used in this experiment contained very low level of LCPUFAs compared to the formulated feed. Unlike marine fish species, *T. tambroides* and other carps do not require very high levels of dietary n-3 PUFA or n-3 LCPUFAs for optimal growth (Turchini et al., 2009). Freshwater fish such as *T. tambroides* (Ng and Andin, 2011), grass carp (Du et al., 2008) and tilapia (Teoh et al., 2011) seem to possess sufficient $\Delta 6$ - and $\Delta 5$ -desaturates and elongase capability to produce LCP-

UFAs from their shorter-chain precursor linoleic acid (C18:2n-6) and linolenic acid (C18:3n-3) if present in the diet. As *T. tambroides* is capable of bioconversion and its dietary requirement of LCPUFAs acids is lower, the observed growth differences in *T. tambroides* might be related to the sensory and behavioral role of these fatty acids which influences the feeding behavior and consumption of prey and food particles. A number of authors have reported that increased LCPUFAs level in diets improved larval visual acuity, which improves hunting success, feed consumption and feeding behavior, and ultimately increases the biomass of fish larvae (Bell et al., 1995).

The ratio of RNA to DNA (RNA/DNA) is a widely used biochemical parameter in larval studies to estimate the relative activation of new protein synthesis that in turn is correlated with growth rate. This is based on the assumption that RNA content varies according to the rate of protein synthesis, whereas the amount of DNA in the larvae is approximately fixed and depends mainly on the body size (Zhou et al., 2001; Buckley et al., 2008). The DNA concentrations over the experimental periods were stable and RNA concentrations increased linearly in all treatments, which suggests that somatic growth of Malaysian mahseer mostly occurs by hypertrophy resulting from active protein synthesis. The high values of growth parameters and RNA/DNA ratio in FF suggests that the differences in nutritive values of the supplied feeds and feeding conditions were mainly responsible for such variations among different treatments (Smith and Buckley, 2003). The RNA/DNA ratios in fish were stated to be positively correlated with the growth rate in three-spined sticklebacks *Gasterosteus aculeatus* (Ali and Wootton, 2003; Pottinger et al., 2011). In the larvae of Atlantic cod *Gadus morhua* and winter flounder *Pseudopleuronectes americanus* reared under laboratory condition, the RNA/DNA ratio was positively correlated with the growth rates achieved and food densities offered (Buckley, 1984). Smith and Buckley (2003) extracted RNA and DNA from tissues, and found that the ratio of RNA/DNA reflects the feeding conditions and growth of juvenile Atlantic cod.

In conclusion, the data of the present study confirmed that *T. tambroides* larvae are able to assimilate the formulated diets efficiently during early stages of their development, thus supporting the highest growth performances compared to the live feeds tested in this experiment. In this study, various combinations of biochemical indices consistently and reliably depict the improved physiological condition and growth performance of *T. tambroides* larvae fed formulated feed compared to the larvae fed live feeds. Formulated feed enhanced the growth performances of *T. tambroides* larvae by supplying the essential dietary nutrients and enhancing metabolic activity and new protein synthesis as confirmed by the RNA/DNA ratio analysis. In summary, nursery rearing of *T. tambroides* larvae with formulated feed gives better results than feeding live feeds.

Acknowledgements

This research was financially supported by the Fundamental Research Grants Scheme (FRGS), Ministry of Higher Education (MOHE), Malaysia to Dr. Md Asaduzzaman (Grant No. 59343). We would like to give thanks to Balqis Romlan and Mohd Taufik (Research Assistant) and Lim Chee Wei and Terence Tito (Undergraduate students), School of Fisheries and Aquaculture Sciences, University Malaysia Terengganu for managing the experiment, assisting in sampling and laboratory analysis. The authors are also grateful to the science officers of the Institute of Marine Biotechnology (IMB), University Malaysia Terengganu for assisting with the fatty acid analysis.

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