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Effect of Feeding Enriched Formulated Diet and Live Feed on Growth, Survival and Fatty Acid Profile of Deccan Mahseer, *Tor Khudree* (Sykes) First Feeding Fry

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Abstract

Deccan mahseer (*Tor khudree*) has recently been included in the IUCN list of threatened species due to its declining population. Intervention of nutritional strategies for better growth and survival of early stages for grow out and ranching is essential. The present investigation intended to study the effect of various dietary combinations containing live and inert feed enriched with cod liver oil on growth and survival of *Tor khudree* first feeding fry, while effects on whole body fatty acid composition of fry were also studied. Cod liver oil as a source of essential fatty acids was used for enriching both live and inert formulated feed. The experimental design consist of four treatments viz. T0 (inert diet without enrichment), T1 (inert diet enriched with cod liver oil), T2 (inert diet enriched with cod liver oil+unenriched *Artemia*) and T3 (unenriched inert diet+enriched *Artemia*) fed to four distinct experimental groups for a period of 60 days. At the end of the experiment growth, survival and fatty acid profile of the fry were examined. Fry fed combination of unenriched inert diet and enriched *Artemia* (T3) had the highest growth and feed efficiency in terms of percentage weight gain (PWG), specific growth rate (SGR), average daily growth (ADG) and feed conversion ratio (FCR). Survival percentage was also significant in T3 compared to other groups. Enrichment of dietary components had a direct effect on the whole body fatty acid profile of mahseer fry with significant ($P<0.05$) changes in the eicosapentaenoic acid (EPA 20:5 n-3) and docosahexaenoic acid (DHA 22:6 n-3) composition. This study provides avenues for improving the growth and survival of mahseer fry through enrichment and concurrent co-feeding strategies with dietary formulated diet which can further reduce feed cost during fry rearing.

Keywords: *Tor khudree*; Enrichment; Co-feeding; EPA; DHA; First feeding fry; Cod liver oil; Enrichment

Abbrevations

IUCN (International Union for Conservation of Nature); PWG (Percentage weight gain); SGR (Specific Growth Rate); ADG (Average Daily Growth); FCR (Feed Conversion Ratio); EPA (Eicosapentaenoic Acid); DHA (Docosahexaenoic Acid)

Introduction

Mahseers are considered as potential candidate species for aquaculture, especially in the wake of the recent push toward the culture of indigenous species. Deccan Mahseer (*Tor khudree*) is one of the important coldwater fish species having market demand as a food fish and for recreational fisheries in India [1]. Recently the fish is paid special attention due to its rapid disappearance from natural environment and also ecological alterations and physical changes in natural environment [2]. The latest version of the IUCN Red List of Threatened Species has listed *Tor khudree* for the first time in the year 2007 as endangered because of a 60-70% decline in the native wild population during the past three generations (20 years), as a result of exploitation and habitat degradation. Commercial expansion of this species for aquaculture and stock enhancement in the natural waters requires special attention. The main bottleneck in the process is the limitation of optimum sized fingerlings for grow out or ranching into the natural waters. For promotion of mahseer, one alternative could be the development of suitable breeding and rearing technology which requires knowledge of their nutritional requirement from hatchlings to adult stage. Weaning success from live food to formulated feed remains a very critical period in which there is a gradual change from live prey to formulated [3]. Use of live food (*Artemia metanauplii*) during early developmental stages of *T. khudree* is expensive; therefore weaning in co-feeding with *Artemia nauplii* provides alternative weaning strategy for ensuring lower feed cost [4]. An overlapping co-feeding period during which live food is gradually replaced by increasing quantities of formulated feed has been shown to improve growth and survival of fish larvae compared to the use of live food only [5]. Moreover, formulated diets can balance the nutritional composition of live food especially with respect to amino acids which are not easy to modify in live food [6]. Live food may influence ingestion, digestion and assimilation of formulated diets [7] and also influences digestion by stimulating endocrine responses [8].

Essential fatty acids are unsaturated fatty acids that must be provided preformed in the diet [9]. The active forms in n-3 family are eicosapentaenoic acid (EPA 20:5 n-3) and docosahexaenoic acid (DHA 22:6 n-3). In general, warm water fishes require polyunsaturated n-6 fatty acids or a mixture of n-3 and n-6 fatty acids, while cold water species require n-3 forms [10]. Coldwater fishes have a higher requirement for n-3 PUFAs, whereas warm water fishes tend to have a greater requirement for n-6 fatty acids [11]. This is because; n-3 structure permits a greater degree of unsaturation, which is necessary

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in the membrane phospholipids to maintain flexibility and permeability characteristics at low temperatures [12]. During fry rearing, live feed are the main food during live prey feeding. However, it is very difficult to reach optimal enrichment levels as ingested EPA/DHA is catabolised and used as an energy source by *Artemia* [9,13,14]. Therefore, the supplementation of formulated diets could be a solution to satisfy the nutritional requirements. During such transitional period, enriched live food is partially replaced by artificial diets until the fry can be fed solely on formulated diets. Early co-feeding is beneficial, since it reduces the use of live food, which are cumbersome to produce and difficult to manipulate nutritionally [15]. In addition, co-feeding is expected to improve the nutritional condition of the larvae and might facilitate earlier transition into dry feeds; only [16] of early co-feeding has been widely documented in various species such as tongue sole, *Cynoglosssus semilaevis* [17], barramundi, *L. calcarifer* [18], turbot, *Scophthalmus maximus* [19], and winter flounder, *Pseudopleuronectes americanus* [20].

While knowledge of the exact nutrient requirements of *T. khudree* is limited, lipids and essential fatty acids are generally recognised to play critical roles in larval development. Recent advances have led to the possible replacement of live feed organisms with micro diets from first feeding in Haddock, *Melanogrammus aeglefinus* [21]. *Artemia metanauplii* as starter diet may be too small and might not contain enough nutrients for the larval development. It has been established that high levels of (n-3) HUFA in the live feed can improve growth and survival of fish larvae in a number of species. Starting an early co-feeding with appropriate enriched diets, formulated diets or *Artemia* biomass can be a solution to improve and balance the larval nutrition and to shorten the live prey feeding period. Meanwhile, the appropriate nutritional composition, especially fatty acid contents of the formulated diets for co-feeding will be crucial for development of mahseer fry. The present work was intended to compare the effect of different dietary formulation combining both live feed and inert diet after enrichment using EPA/DHA sources on growth, survival and fatty acid profile of *T. khudree* first feeding fry.

Materials and Methods

Maintenance of experimental fishes

First feeding fry of *T. khudree* (Average weight: 0.10 ± 0.12 g) were procured from Tata Power Company Ltd. (TPCL), Lonavla, Maharashtra, India during October month and maintained at the rearing unit of Aquaculture Division, Central Institute of Fisheries Education, Mumbai, India. Fry were maintained with optimum conditions for another 20 days and fed on live feed. Round the clock aeration was provided to maintain an optimum dissolved oxygen. The entire tank bottom was siphoned clean every third day. The water quality parameters were checked once in three days and were found within the optimum range (dissolved oxygen: 7.0-7.2 mg L^{-1}; pH: 7.5-7.8; temperature: 20.4-24.8°C; ammonia nitrogen: 0.14-0.23 mg L^{-1}; nitrite nitrogen: 0.001-0.005 mg L^{-1}; nitrate nitrogen: 0.02-0.07 mg L^{-1}) throughout the experimental period as observed earlier by Sangma and Basavaraj [22].

Experimental design and trial

The experimental design consist of four treatments viz. T0 (inert diet without enrichment), T1 (inert diet enriched with cod liver oil), T2 (inert diet enriched with cod liver oil+unenriched *Artemia*) and T3 (unenriched inert diet+ enriched *Artemia*) with three replicates each following a completely randomized design (CRD). The experimental trial was conducted for a period of 60 days at the Aquaculture facility of the Institute. Plastic tubs of 70 L capacity covered with perforated lids were used for the trial. The tubs were filled with 50 L of chlorine free water and round the clock aeration was provided in all tanks. A total of 240 uniformly sized fry having an average weight of 0.40 g (20 fry×4 treatments×3 replicates) were uniformly distributed into the experimental groups and acclimatised for two days prior to the start of the experiment. Each diet (inert diet and live feed) was fed twice daily (09:00 and 17:00 hour) to triplicate group of *T. khudree* fry at 5% of the body weight. Siphoning of the uneaten feed was carried out daily.

Hatching and separation of *Artemia* nauplii

Artemia cyst used in the study was supplied from Supreme Plus ™, Ogden, USA. Hatching of *Artemia* cysts were carried out as suggested by Sorgeloos et al. [23]. Prior to hatching, cysts were washed in active bleach liquor for six minutes followed by rinsing in chlorine free water. The cysts were incubated in a glass jar at density of 0.3 g 500 mL^{-1} for 24 h using saline water (25 ppt). Temperature of water during hatching of *Artemia nauplii* was maintained at 26°C and vigorous aeration was provided throughout the hatching period for efficient hatching of *Artemia* cyst into nauplii. After hatching, the nauplii were separated from the cyst using a rubber tube and a piece of blotting cloth and were washed for five minutes in clean chlorine free running water. The nauplii were then transferred to fresh saline water (12 ppt).

Enrichment of *Artemia* nauplii

The hatched nauplii were collected together in a tank containing saline water (12 ppt) and the enrichment emulsion was added at the rate of 0.5 mL L^{-1} of the *Artemia* nauplii containing water with a density of 100-150 nauplii ml^{-1}. The enrichment was conducted for 12 h. The *Artemia* nauplii containing water was provided with vigorous aeration to ensure thorough mixing. The composition of the emulsion used for enriching the *Artemia* is given in Table 1.

Formulation of inert diet

A purified inert diet containing 45% protein was formulated using ingredients obtained from Himedia Laboratories, Mumbai, India. Composition of diet and their proximate analysis are presented in Table 2. The required amount of the ingredients were weighed and mixed properly. Cod liver oil as a source of EPA/DHA was added gradually to assure the homogeneity of the ingredients. The mixed ingredients were passed through a single screw pelletizer with 1 mm dia.

Growth and survival

Fry in each tub were bulk weighed every week to monitor the growth rates. The growth performance of the fingerlings was evaluated in terms of percentage weight gain, specific growth rate (SGR), feed efficiency ratio (FER), feed conversion ratio (FCR), feed conversion efficiency (FCE), average daily growth (ADG) based on the following standard formulae:

Weight gain % = (final weight–initial weight)/(initial weight)x100

Ingredients	Composition
Water	100 ml
Cod liver oil	20 ml
Egg yolk	11 ml
Gelatine	3.7 g
Vitamin E	40 mg

Table 1: Composition of emulsion used for enrichment.

	Enriched Diet	Unenriched Diet
Ingredients		
*Casein	37.78	37.78
*Gelatin	10.00	10.00
¶Cod liver oil	5.00	-
¶Sunflower oil	-	5.00
Vitamin[1]	2.00	2.00
Mineral[2]	4.00	4.00
*Carboxy Methyl Cellulose	1.00	1.00
*Dextrin	25.22	25.22
*α-cellulose	10.00	10.00
Proximate composition		
Dry matter	93.94 ± 0.87	92.97 ± 0.13
Crude protein	45.92 ± 0.27	45.06 ± 0.35
Lipid	9.96 ± 0.05	9.47 ± 0.08
Ash	4.60 ± 0.01	4.68 ± 0.02
Crude fibre	8.21 ± 0.08	8.12 ± 0.03

[1]Mineral premix (%): $KAl(SO_4)_2$, 0.159; $CaCO_3$, 18.101; $MgSO_4$, 5.216; $CoCl_2$, 0.07; KCl, 16.553; ferric citrate ($5H_2O$), 1.338; sodium selenite, 0.004; $MnSO_4.H_2O$, 0.07; KI, 0.014; $ZnSO_4$, 0.192; NaH_2PO_4, 13.605; $CuSO_4.5H_2O$, 0.075

[2]Vitamin premix: thiamine hydrochloride, 10 mg kg^{-1}; riboflavin, 20 mg kg^{-1}; calcium pantothenate, 40 mg kg^{-1}; nicotinic acid, 50 mg kg^{-1}; pyridoxine hydrochloride, 10 mg kg^{-1}; folic acid, 5 mg kg^{-1}; inositol, 400 mg kg^{-1}; choline chloride, 2000 mg kg^{-1}; menadione, 10 mg kg^{-1}; cholecalciferol, 1500 IU; biotin, 1 mg kg^{-1}; vitamin B12, 0.02 mg kg^{-1}; vitamin A, 3000 IU; vitamin E, 50 IU; vitamin C, 200 mg kg^{-1}

¶ Procured from local market

*Himedia Laboratories, Mumbai, India

Data are expressed as mean ± SE

Table 2: Formulation of experimental inert diet (g/100 g) for *T. khudree* fry and proximate composition (% dry weight).

SGR = 100 (ln average final weight–ln average initial weight)/ number of culture days

FCR = total dry feed intake (g)/live weight gain (g)

ADG = (mean final weight– mean initial weight)/days of culture

Survival (%) = (total number of animals harvested/total number of animals stocked)x100

Biochemical analysis

The biochemical compositions of formulated diet, emulsion, *Artemia* nauplii and *T. khudree* fry were measured using standard methods [23] for moisture, ash, crude protein, lipid and crude fibre.

Fatty acid analysis

Total lipid was extracted using Folch method [24] with some modification from the enriched and unenriched *Artemia nauplii,* formulated feed and the larval tissue. The AOAC [25] method was followed to esterify the lipid extract. Fatty acid methyl esters (FAME) was prepared from the extracted lipids by heating with methanolic NaOH and then with BF_3 Methanol for esterification. N-heptane (5 mL) was added to recover the methyl esters in organic phase. Saturated NaCl solution was added to the mixture and the aqueous and organic layers were separated using a separating funnel. The upper N- heptane phase was pipetted out and stored in 10 mL glass vials in refrigerator until further analysis. Gas chromatography-Mass spectrometry (GC-MS) measurements were performed using a Shimadzu QP2010 quadrupole Gas Chromatography Mass Spectrometer equipped with a carbowax (30 m×0.25 mm ID; 0.25 µm film thickness) capillary column (Cromlab S.A). Helium was used as the carrier gas. Injector and detector temperatures were set at 250°C. Injection was performed in split mode (1:15). The column temperature was programmed initially at 50°C for 2 min and then to increase at a rate of 10°C per min to a final temperature of 230°C. FAM esters were separated at constant pressure (23.1 kPa) and peaks were identified by comparing the mass spectra with the mass spectral data base.

Principal component analysis (PCA)

Principal component analysis (PCA) of samples fed with different experimental diets was performed by using Unscrambler (Version 9.5, CAMO, Norway).

Statistical analysis

All the data were analysed using SPSS version 16.0 for windows. One-way analysis of variance (One-way ANOVA) was used to compare all the treatments. Significant difference between two means was measured by Duncan's multiple range test. All the differences were considered significant at $P<0.05$ and the results are presented as mean ± standard error (SE).

Results

Growth and survival

Effect of dietary treatment on important growth parameters viz. PWG, SGR, ADG and FCR are given in Table 4. Groups fed with enriched *Artemia* and unenriched inert diet (T3) recorded highest percentage weight gain (297.00 ± 0.42) which was significant ($P<0.05$) whereas groups fed with unenriched inert diet (T0) exhibited lowest value (137.2 ± 0.20). SGR and ADG was highest in T3 (2.28 ± 0.00; 22.21 ± 0.00) and lowest in T0 (1.45 ± 0.00; 9.55 ± 0.00) which was significant ($P<0.05$). FCR followed a similar pattern which was

Fatty acids	Before enrichment		After enrichment	
	[1]Inert diet	Artemia	[2]Inert diet	Artemia
12:0	0.70	0.28	0.33	0.20
13:0	ND	0.24	ND	0.07
14:0	3.30	1.78	5.77	5.40
15:0	0.33	0.90	0.54	0.82
16:0	18.4	14.48	19.27	19.13
16:1 n-9	0.03	6.34	4.06	7.48
16:1 n-7	0.63	ND	0.55	0.86
17:0	0.21	1.50	0.34	0.72
18:0	5.26	0.62	3.98	0.27
18:1 n-9	24.70	24.29	31.92	25.00
18:1 n-7	0.13	3.31	3.16	3.75
18:1 n-5	9.73	ND	11.02	0.61
18:2 n-6	0.10	8.90	3.08	8.50
19:0	0.56	ND	0.25	0.16
18:3 n-3	0.66	4.73	3.73	11.74
20:0	ND	0.54	0.11	0.55
20:1 n-9	ND	0.65	0.77	6.04
20:2 n-6	ND	1.01	0.13	1.03
20:3 n-3	ND	0.89	1.36	1.29
20:4 n-6	ND	1.30	0.32	1.40
20:4 n-3	ND	1.40	0.72	1.49
20:5 n-3	0.12	2.45	3.45	4.53
22:1 n-9	0.12	0.53	1.75	1.83
22:6 n-3	0.10	1.30	3.39	4.14

[1] Inert diet with sunflower oil as lipid source represents unenriched diet

[2] Inert diet with cod liver oil as lipid source represents enriched diet

*ND: Not detected

Table 3: Fatty acid profile (area %) of enriched and unenriched *Artemia nauplii* and inert diet.

Treatments	PWG	SGR	ADG	FCR
T0	137.20 ± 0.20[a]	1.45 ± 0.005[a]	9.55 ± 0.003[a]	2.71 ± 0.003[a]
T1	177.41 ± 0.25[b]	1.73 ± 0.003[b]	12.05 ± 0.030[b]	1.95 ± 0.003[b]
T2	196.45 ± 0.26[c]	1.80 ± 0.003[c]	16.13 ± 0.003[c]	1.95 ± 0.003[c]
T3	297.02 ± 0.42[d]	2.28 ± 0.003[d]	22.21 ± 0.003[d]	1.61 ± 0.003[d]

PWG: Percentage weight gain; SGR: Specific Growth Rate; ADG: Average daily growth (mg/day); FCR: Feed Conversion Ratio; FER: Feed Efficiency ratio; FCE: Feed conversion Efficiency; Mean values in the same column with different superscript (a, b, c, d) differ significantly (P<0.05). Data expressed as Mean ± SE, n=3

Table 4: Growth parameters of *T. khudree* fry fed different experimental diets for 60 days.

Treatments	Moisture	Crude Protein	Lipid	Ash	TC*
T0	72.20 ± 0.01	15.80 ± 0.02	2.78[a] ± 0.02	4.60 ± 0.03	4.62 ± 0.01
T1	72.45 ± 0.03	15.50 ± 0.02	4.27[b] ± 0.03	3.50 ± 0.03	5.28 ± 0.02
T2	71.76 ± 0.02	15.02 ± 0.01	5.05[b] ± 0.01	3.40 ± 0.02	4.77 ± 0.01
T3	70.63 ± 0.03	16.70 ± 0.03	6.27[c] ± 0.01	3.02 ± 0.01	3.38 ± 0.03

*TC- Total Carbohydrate
Mean values in the same column with different superscript (a, b, c, d) differ significantly (P<0.05). Data expressed as Mean ± SE, n=3

Table 5: Proximate composition of *Tor khudree* (% wet weight) fry after 60 days experimental feeding.

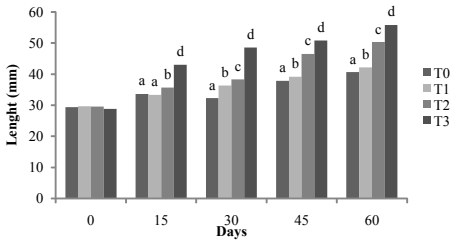

Figure 1: Growth of *T. khudree* fry measured as total length (mm) measured at different sampling times during the experimental trial at 5% significance level.

significant ($P<0.05$) among the groups. Rearing of *T. khudree* fry fed with different diets showed significant variation in survival (Figure 3). Percent survival did not show any variation up to 15 days but was significant ($P<0.05$) after 15 days of experimental feeding. At the end of the trial, significant variation ($P<0.05$) in survival was observed with better survival in T3 followed by T2, T1 and T0.

Fry whole body biochemical composition

Proximate composition of *T. khudree* fry after 60 days experimental feeding is presented in Table 5. No significant variation in whole body composition was observed due to different experimental diets ($P>0.05$) except lipid content which was significantly different among experimental groups ($P<0.05$). Higher lipid content was observed in T2 and T3 (5.05 ± 0.01 and 6.27 ± 0.01 respectively) which was significant ($P<0.05$) from other groups. Whole body moisture content varied from 70.63 ± 0.03 to 72.45 ± 0.03 with highest in T1 and lowest in T3. Lowest crude protein was observed in T2 (15.02 ± 0.01) whereas highest in T3 (16.70 ± 0.03). Total carbohydrate was highest in T1 (5.28 ± 0.02) and lowest in T3 (3.38 ± 0.03). T0 exhibited highest ash content and T2 had lowest value.

Fatty acid profile of *Artemia*/inert diet after enrichment

The fatty acid profile of the *Artemia nauplii* and inert diet before and after enrichment is summarized in Table 3. In the inert diet no

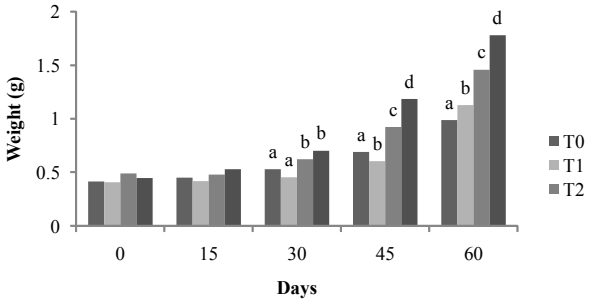

Figure 2: Growth of *T. khudree* fry measured as weight (g) measured at different sampling times during the experimental trial at 5% significance level.

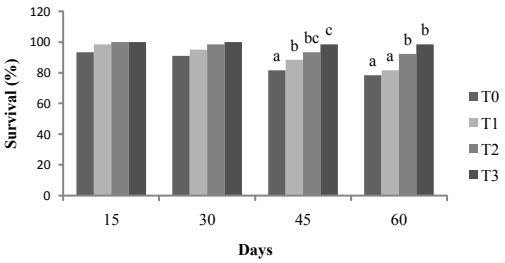

Figure 3: Survival rate (%) of *T. khudree* fry in the different experimental groups at 15 days interval during the 60 days experimental trial at 5% significance level.

considerable changes in composition of short chain fatty acids (12:0, 14:0, 15:0, 16:0, 17:0 and 18:0) occurred due to dietary enrichment with cod liver oil. Monounsaturated fatty acids (MUFA) content in inert diet increased slightly. The Polyunsaturated fatty acids (PUFA) content of unenriched inert diet was 10.05% whereas that of enriched micro diet was 22.3%. PUFA viz. EPA and DHA showed higher levels in enriched diet. Enrichment process increased the PUFA level in *Artemia* with EPA and DHA in *Artemia* nauplii ranging from 8.48% (unenriched *Artemia*) to 20.41% (enriched *Artemia*).

Fry whole body fatty acid profile

After 60 days experimental trial, the whole body fatty acid composition of *T. khudree* fry reflected dietary effect and is given in Table 6. Significant ($P<0.05$) increase in n-3 PUFA such as EPA (20:3 n-3) and DHA (22:6 n-3) was observed in T1, T2 and T3 compared to control due to higher EPA and DHA content in diet after enrichment. Highest EPA content was observed in T3 followed by T1 and T2. DHA content was highest in T3 followed by T2 and T1.

Principal component analysis (PCA)

Principal component analyses (PCA) of samples with the different fatty acids are plotted in score plot and bi-plot and are presented in Figure 4. Score plot describes change in position of T3 from the initial position whereas T1 and T2 fall on the same area indicating similar effect due to dietary treatments. Bi-plot analysis also reveals shift in position and the shift was found to be due to dietary PUFA viz. 18:3 n-3; 22:6 n-3; 20:5 n-3. T3 (unenriched inert diet+enriched Artemia) showed the best performance with highest level of PUFA.

Discussion

Although live foods are often good sources of nutrition, for many fish species, these traditional food sources have been found to

Fatty acids	Diet 1 (T0)	Diet 2 (T1)	Diet 3 (T2)	Diet 4 (T3)
12:0	0.04 ± 0.00^{a}	0.13 ± 0.01^{a}	0.21 ± 0.03^{ab}	0.36 ± 0.16^{b}
14:0	3.31 ± 0.00^{a}	5.50 ± 0.07^{c}	3.99 ± 0.09^{b}	3.28 ± 0.09^{a}
15:0	0.73 ± 0.01^{a}	0.84 ± 0.08^{a}	0.97 ± 0.21^{a}	0.79 ± 0.13^{a}
16:0	0.73 ± 0.01^{a}	0.84 ± 0.08^{a}	0.97 ± 0.21^{a}	0.79 ± 0.13^{a}
16:1 n-9	6.25 ± 0.14^{c}	3.47 ± 0.08^{b}	1.96 ± 0.05^{a}	2.13 ± 0.14^{a}
16:1 n-7	0.46 ± 0.02^{a}	1.08 ± 0.15^{b}	0.60 ± 0.06^{a}	1.14 ± 0.07^{b}
17:0	1.25 ± 0.14^{b}	0.67 ± 0.63^{a}	0.94 ± 0.16^{a}	0.78 ± 0.05^{a}
18:0	12.25 ± 0.87^{b}	2.86 ± 0.17^{a}	2.63 ± 0.18^{a}	3.20 ± 0.21^{a}
18:1 n-9	16.15 ± 0.03^{a}	31.77 ± 0.15^{d}	29.60 ± 0.13^{c}	28.63 ± 0.36^{b}
18:1 n-7	2.35 ± 0.14^{a}	2.32 ± 0.20^{a}	2.94 ± 0.12^{b}	4.91 ± 0.32^{c}
18:2 n-6	18.02 ± 0.29^{c}	10.39 ± 0.22^{a}	12.69 ± 0.30^{b}	10.53 ± 0.13^{a}
18:3 n-3	4.35 ± 0.14^{b}	3.55 ± 0.26^{a}	4.37 ± 0.08^{b}	6.97 ± 0.14^{c}
19:0	0.14 ± 0.14	ND	ND	0.18 ± 0.02
20:0	0.23 ± 0.02^{a}	0.61 ± 0.15^{b}	0.30 ± 0.01^{a}	0.34 ± 0.04^{a}
20:1 n-9	1.70 ± 0.12^{a}	3.72 ± 0.03^{c}	3.96 ± 0.08^{c}	2.34 ± 0.07^{b}
20:2 n-9	0.43 ± 0.01	ND	ND	ND
20:2 n-6	0.11 ± 0.01^{a}	0.18 ± 0.02^{b}	0.25 ± 0.25^{b}	0.26 ± 0.03^{c}
20:2 n-7	0.12 ± 0.01^{a}	0.68 ± 0.02^{c}	0.47 ± 0.01^{b}	0.39 ± 0.08^{b}
20:3 n-9	0.26 ± 0.01	ND	ND	ND
20:3 n-7	0.75 ± 0.09^{b}	0.29 ± 0.03^{a}	0.47 ± 0.07^{a}	0.94 ± 0.05^{b}
20:4 n-6 (AA)	1.24 ± 0.01^{a}	1.31 ± 0.00^{ab}	1.35 ± 0.07^{ab}	1.41 ± 0.00^{b}
20:3 n-3	1.60 ± 0.06^{d}	0.73 ± 0.03^{b}	0.55 ± 0.02^{a}	0.93 ± 0.04^{c}
20:4 n-3	1.31 ± 0.01^{a}	0.56 ± 0.06^{b}	0.47 ± 0.35^{b}	1.20 ± 0.01^{a}
20:5 n-3 (EPA)	4.01 ± 0.00^{a}	4.45 ± 0.20^{b}	4.40 ± 0.01^{b}	6.50 ± 0.05^{c}
22:1 n-9	1.30 ± 0.02^{b}	1.42 ± 0.09^{bc}	1.55 ± 0.07^{c}	0.28 ± 0.04^{a}
22:6 n-3 (DHA)	3.21 ± 0.00^{a}	3.55 ± 0.02^{b}	4.15 ± 0.01^{c}	5.40 ± 0.06^{d}

Values expressed as mean ± standard deviation, n=3.
Superscripts (a, b, c, d) denotes significant differences among the diets (P<0.05)
ND=not detected.
AA: Arachidonic acid
EPA: Eicosapentaenoic acid
DHA: Docosahexaenoic acid

Table 6: Fatty acid profile of *T. khudree* fry fed with different experimental diets for a 60 days period.

be inadequate to support nutritional requirement. It has been well established that one of the limiting factors in nutrition of early stages of fishes is the level of n-3 HUFA. Hence certain feeds containing HUFAs (especially DHA, 22:6 n-3 and EPA 20:5 n-3) can be valuable as food sources for enrichment. Also, feed ingestion and digestion do not appear to be regulated by total lipid content, but by the lipid source and fatty acid composition [26].

In the present study, EPA/DHA enriched diet (T1, T2 and T3) exerted significant effect (P<0.05) on percentage weight gain of *T. khudree* fry compared to unenriched inert diet (T0). Fry co-fed with enriched *Artemia* and unenriched inert diet promoted highest percentage weight gain, specific growth rate and average daily growth (Figures 1-3). These may be explained by higher percentage of EPA and DHA accumulated in enriched *Artemia* and growth promoting effect of these fatty acids. Significantly lower growth in fry fed only inert diet (T0 and T1) signifies that mahseer fry at this stage are unable to metabolise the diet and may be due to partially functional digestive system. Moreover, physical aspects such as particle size, distribution and attractiveness of the formulated diets can affect larval ingestion [26]. According to Cahu and Zambonino [27] the enzyme activity pattern is age-dependent, but can be modulated by the inert diet. Live food given together with a formulated diet is reported to enhance the efficiency of the formulated diet by promoting the assimilation and deposition of dietary nutrients in the larval body [7].

In the present study, low feed intake (data not shown) was observed in groups fed only inert diet which may be due to low palatability of the inert diet as explained by higher FCR. In treatments T2 and T3, better feed consumption when 50% of the live prey are substituted by inert diet provides future avenues for formulating low feed cost for this species. EPA and DHA (HUFA) play a major role growth process during early stages of this fishes. In the present experiment, EPA/DHA levels were comparatively higher in enriched *Artemia* than present in the inert diets. This result may explain a major beneficial role of live food as an effective carrier vehicle for EPA/DHA during early stages. Fry percent survival was significant after 45th day of experimental trial (Figure 3). Fry fed completely on inert diet exhibited low survival rate compared to fry co-fed with both inert and live feed. This may be due to higher supplementation of dietary lipid in formulated diet which would impact the water quality as seen in our study. Oil films were observed in tanks which would choke the gills of developing fry and increases stressful conditions. Mahseer fry co-fed on inert diet and enriched live feed had the highest survival rate and this could be explained by better assimilation of dietary EPA/DHA through live food enrichment. It has been established that high levels of (n-3) HUFA in the live feed can improve survival of fish larvae in a number of species [28]. The higher survival percentage may be due to the enhancement of ability of fry to withstand stressful environment by feeding on HUFA enriched *Artemia* nauplii.

The present experiment was also designed to evaluate HUFA retention capacity of cold water fish species, *T. khudree* fry after experimental feeding with both live feed and inert diet. As the early life stages of fishes have limited capacity to elongate and desaturate 18-carbon PUFA, freshwater species require supplemented PUFA in their diet. In the present study, dietary lipid levels in both inert and

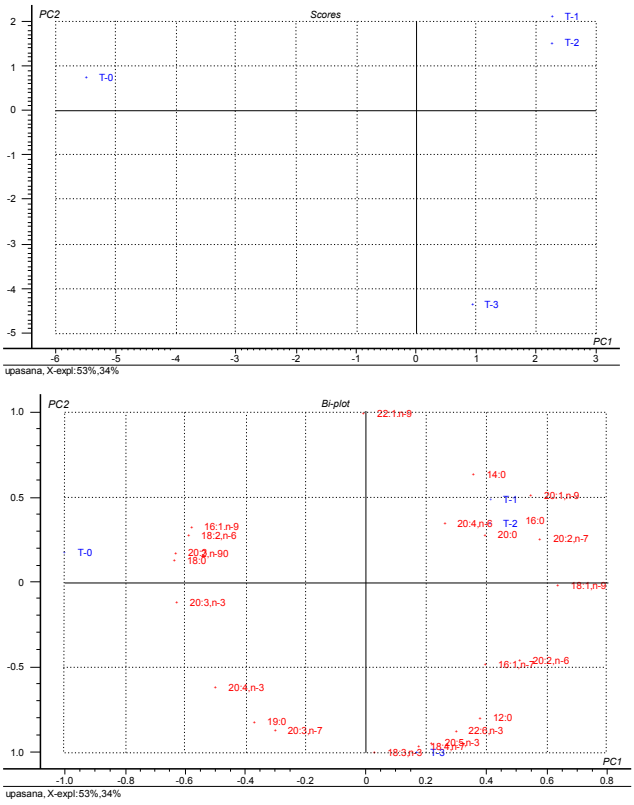

Figure 4: Score plot and bi plot showing the difference in the fatty acid profiles of *T. khudree* fry fed with different experimental diets.

live feed exerted significant effect on some class of fatty acid in the fish. Highest EPA level was observed in fry fed combination of enriched *Artemia* and unenriched diet (T3). This observation suggests that beneficial EPA is transformed more efficiently into tissue when live feed are enriched with EPA rich sources. Fry fed combination of unenriched *Artemia* and enriched *Artemia* had less EPA in tissue compared to T3. Dietary DHA levels had similar effect on the tissue fatty acid level with lowest value observed in control (T0) and highest value in T3. From the above results it can be concluded that enrichment process using live feed as vehicle for transport of essential fatty acids serves the purpose of maintaining the fatty acid requirement of the early young stages of *T. khudree* fry and subsequently higher accumulation in fish would provide avenues for fish products as source of these fatty acids.

Principal components analysis reveals that irrespective of the diet, lipid fractions shows distinct fatty acid patterns. The fish fed with PUFA enriched diet and enriched *Artemia* for 60 days were different from initial position based on fatty acid composition. PUFA, n-3 PUFA, MUFA, 18:2 n-6, 18:1 n-9 and 22:6 n-3 were found to be responsible for causing the differences among the samples and T3 showed the best performance with high level of PUFA in this experiment. This was supported by Karanth et al. [29] where the PCA shows the differences in the muscle fatty acid profiles of fish fed with different dietary groups enriched with PUFA which were found to be responsible for causing the differences among the samples.

Conclusion

Result from the present study reveals that nutritional quality of *Artemia* can be improved by enriching with EPA and DHA rich sources such as cod liver oil which is easily available in market. EPA and DHA being essential for early life stages can improve growth and survival as observed in our study. Further, co-feeding early stages of cold water species with enriched live feed in combination with inert diet can further reduce the live feed cost. Thus this study provides an opportunity for growth enhancement and better survival in cold water species, *Tor khudree* through nutritional manipulation so that optimum sized fingerlings can be produced to make its culture a viable commercial activity and protect the species from extinction in the wild.

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