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Evaluation of potential use of feeder guppies as a source of highly unsaturated fatty acids for high value fishes

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Abstract

The present study was carried out to find out whether the fatty acid profiles of mature guppies (*Poecilia reticulata*) and the resulting fry could be altered through feeding different lipid sources with a view to use them as a highly unsaturated fatty acids (HUFA) enriched feeder fish. Four iso-calorific, iso-lipidic and iso-proteic diets were formulated using coconut oil, sunflower oil, linseed oil, and cod liver oil as lipid sources for preparing Diet-CO, Diet-SO, Diet-LO and Diet-FO respectively. The experimental diets were designed in such a way that each diet had different classes of fatty acids such as saturated fatty acids (SAFA), n-6 poly unsaturated fatty acids (PUFA), n-3 PUFA and HUFA. Three replicates were used for each dietary treatment and the fish were fed twice a day until satiation. The fish fed with Diet-CO were characterized by SAFA, 18:1 n-9, 18:2 n-6 and 20:4 n-6 fatty acids. The fish fed with Diet-SO contained significantly higher amounts of 18:2 n-6, 18:3 n-6, 20:3 n-6, 20:4 n-6, 22:4 n-6, 22:5 n-6, n-6 PUFA and n-6 HUFA than the fish fed with the other three diets. The fish fed with Diet-FO contained a significantly higher amount of 16:1 n-7, 20:1 n-9, 22:1 n-9, monounsaturated fatty acids (MUFA), 20:3 n-3, eicosa pentaenoic acid (EPA), docosa hexaenoic acid (DHA), HUFA, n-3 HUFA and n-3/n-6 than the fish fed with the other three diets. The present study demonstrated that the muscle fatty acid profiles of guppy can be altered through feeding with different lipid sources. The guppy fed with Diet-CO and Diet-SO can be used as SAFA, MUFA and n-6 PUFA source while the guppy fed with Diet-LO and Diet-FO could be used as SAFA, MUFA and n-3 PUFA sources for feeding high value fish. The resulting fry from all diets can be used as n-3 PUFA and n-6 PUFA sources for feeding both freshwater as well as marine fish. The resulted fry fed with Diet-CO and Diet-FO can be used as HUFA source to feed marine fish since they do not have the ability to synthesize

HUFAs. The present study demonstrated that plant based oil sources particularly coconut oil, sunflower oil and linseed oil can be used as a cheap lipid sources for preparation of feed for guppy to alter the fatty acid profile and their resulting fry could be used as good sources of n-3 and n-3 PUFA for feeding high value fishes.

Introduction

Broodstock nutrition is vital to produce high-quality eggs and larvae with fatty acid contents optimized to give the developing embryos and larvae the best chance of success (Tandler et al. 1989). Gonadal development and fecundity in fish are greatly affected by broodstock nutrition (Bromage 1995). Regarding larval nutrition, feeding of marine fish larvae is often limited to the administration of a few live prey organisms such as rotifers and Artemia. This reduced range of food organisms available for the larvae may lead to nutritional imbalances or nutritional deficiencies (Watanabe 1993). One of the principal factors affecting the nutritional value of live prey organisms is their essential fatty acids (EFA) content which in turn could fulfill the EFA requirement of fish larvae (Watanabe et al. 1984).

Fresh water fishes have the ability to desaturate and elongate short chain n-3 and n-6 fatty acids to their higher chain fatty acids. Marine fish, however require a dietary supply of EPA and DHA due to their relative deficiency in eitherΔ5 desaturate or one of the two enzymes of the elongase multi-enzyme complex (Tocher 2001). Generally fresh water fish require 18:2 n-6 as a precursor to produce arachidonic acid (ARA) and 18:3 n-3 is required as a precursor to produce EPA and DHA while marine fishes should be provided with those essential fatty acids with their diets as marine fishes do not have the ability to produce them using precursors. ARA, EPA and DHA play a major role in growth and reproduction of fish (Sargent et al. 2002). Studies on the essentiality of polyunsaturated fatty acids in the diets (Bell et al. 1986; Sargent et al. 1989) have demonstrated that marine species of finfish require n-3 highly unsaturated fatty acids (n-3 HUFA), especially EPA and DHA in their diet for normal growth.

The fishes which are used as feed for other fishes are known as feeder fishes. In recent years, the feeder fish industry has also been growing, catering to aquarists who keep predatory fishes like Oscar Cichlid and Dragon fish, etc. The feeder fishes used in this industry are mainly mollies and guppies. They are obtained mostly from catchers who scour the drains and canals for these fishes, which can be very abundant in some places. Rejects (deformed, drab or potentially unsaleable animals) from guppy, goldfish and tropical fish breeding farms also are used as feeder fish for high value, carnivorous cultured fish to a large extent. Since guppies can tolerate brackish and saline water conditions, they can be used successfully as feed for freshwater fish as well as marine fishes.

Presently in the fish farming industry various types of live feeds such as tubifex worm, Moina, Artemia, mosquito larvae and earth worms are used as high quality feed for brooders and fish fry. Tamaru et al. (1997) reported that those live feed contain only a small amount of essential fatty acids and DHA has not been reported in any live feed organism while little EPA level has been reported in all live feed varieties. Therefore, studies have been conducted to find out the other suitable live feeds to improve lipid composition of Artemia, Moina etc. by prefeeding them with n-3 HUFA-rich diets (bioencapsulation, nutritional enrichment or boosting). It is important to investigate whether there is a possibility to enhance nutritional quality of low value fishes such as guppies by enriching them with essential fatty acids Therefore, the present study was carried out to find out whether the fatty acid profile of guppy and the resulting fry can be altered through feeding different lipid sources with a view to using them as essential fatty acids enriched feeder fish.

Materials and Methods

Four iso-calorific, iso-proteic and iso-lipidic diets were formulated with a constant lipid content of 10%. Coconut oil (CO), sunflower oil (SO), linseed oil (LO), and cod liver oil (FO) was used as the different lipid sources (Diet-CO, Diet-SO, Diet-LO and Diet-FO). The feed ingredients such as casein, starch, and cellulose were obtained from a reputed feed ingredients producing company. Cod liver oil, sunflower oil, linseed oil and coconut oil were procured from the local market. Vitamin and mineral mixture (Agrimin), vitamin C, vitamin B complex (Becosules) and carboxy methylcellulose (CMC) and soybean meal (Hi Media labs, India.) were also used for feed formulation. The composition of experimental diets is presented in Table 1. Each feed was prepared as a dry powder and then sufficient amount of water was added to make the feed into a paste. Feeding of fish was carried out until satiation twice a day at 0800 and 1700 hr throughout the experimental period of six months.

Fish

The experiment was carried out at the Central Institute of Fisheries Education (CIFE), Mumbai, India. Guppies were obtained from ornamental fish breeding and culture section of CIFE. Plastic tanks were used for rearing fish. All tanks were filled with water and conditioned for 48 hrs with aeration. The female guppies (0.095-0.097 g in body weight) were randomly distributed to the plastic tanks (60 virgin female fry per tank). The tanks were covered with transparent acrylic sheets to allow light penetration and also to prevent the fishes from jumping out. A uniform volume of 140 L of water was maintained in each tank throughout the experimental period. Water in each tank was aerated for 24 hours with a uniform air pressure using a 2HP

air blower and regulators. Three replicate tanks were used for each dietary treatment.

Table 1. Composition of experimental diets

Ingredients	%
Soybean Meal	30.00
Casein	25.00
Cellulose powder	15.9
Starch	14.00
Lipid source[1]	10.00
CMC	2.00
Vitamin-Mineral mix[2]	2.90
Vitamin B Complex[3]	0.10
Vitamin C	0.10

[1] Coconut oil (in Diet-CO) or sunflower oil (in Diet-SO) or linseed oil (in Diet-LO) or cod liver oil (in Diet-FO)

[2] Composition of vitamin-mineral mix (Agrimin)(quantity kg^{-1}):Vitamin A-6,25,000 IU; Vitamin D_3-62,500 IU; Vitamin E- 250 mg; Nicotinamide-1 g; Cu-312 mg; Co- 45 mg; Mg- 6 g; Fe- 1.5 g; Zn- 2.13 g; I- 156 mg; Se- 10 mg; Mn- 1.2 g; Ca- 247.34 g; P- 114.68 g; S-12.2 g; Na-5.8 mg; K-48.05 mg.

[3]Composition of vitamin B complex (quantity g^{-1}):Thiamine mononitrate-20 mg; Riboflavin-20 mg; Pyridoxine hydrochloride-6 mg; Vitamin B_{12}-30 µg; Niaciamide-200 mg; Ca pantothenate-100 mg; Folic acid-3 mg; Biotin-200 µg.

After three months rearing period, 20 female fish were selected from each tank for breeding and the rest were used for lipid extraction. The selected 20 female fishes and 10 males were stocked in each tank for breeding. Since the male mortality is known to be higher, a sex ratio of 2 females: 1 male was chosen (Breder and Coates 1932; Shoemaker 1947). Three replicate tanks were used for testing each feed. Breeding tanks were provided with polythene strips arranged in bundles to offer shelter for new swimming fry from parental cannibalism. Each bunch of polythene strips was tied to a weight, which helped to keep it in a fixed position in the tank. All tanks were provided with mild aeration during the breeding period. After a gestation period of 21-25 days the newly born fry in each tank were collected at three days intervals and they were also used for lipid extraction.

Lipid extraction

Crude fat content was estimated by Soxtec method (1045 Soxtec Extraction unit, Tecator, Sweden) using diethyl ether (boiling point, 40-60°C) as a solvent. Total lipid was extracted by following the method described by Folch (1957). Lipid was extracted from initial fry, feeds, fish muscle after maturation and the resulting fry collected from the breeding tanks. The tissue was homogenized in (1:10 w/v) methanol fortified with butylated hydroxyl

toluene (BHT) (0.01%) followed by chloroform (1:20 w/v) in a Teflon coated tissue homogenizer (Superfit, India). (BHT was added to inhibit the oxidative degradation of lipids during analysis). After dispersion, the whole mixture was agitated for 15-20 minutes in an orbital shaker at room temperature.

The homogenate was filtered to recover the liquid phase and the filter residue was re-homogenized with a second volume of chloroform-methanol. The filtered solvent was washed with 0.2 volume (4 ml for 20 ml) of 0.9% NaCl solution and phases were vigorously mixed. The mixture was poured into a separating funnel and allowed to separate into two phases. The lower chloroform phase containing lipids was collected and evaporated under vacuum in a rotary evaporator to bring down to a concentration of 2-3 ml. Further evaporation of chloroform was done under a nitrogen stream and residues were weighed to quantify the amount of lipid extracted. The lipid residue was redissolved in chloroform/methanol (2:1, v/v) and then stored in a 25ml conical flask with glass stopper under nitrogen at –20°C until needed.

Preparation of Fatty Acid Methyl Esters

The AOAC (1995) method was followed to esterify the lipid extract. Fatty acid methyl esters were prepared from the isolated lipids by heating with the 0.5N methanolic NaOH attached to a condenser reflux. The solution was heated for 5 minutes from when steam evaporation and condensation was observed. Then 2.5 ml of boron tri fluoride (BF_3) methanol was added from the top of the condenser and boiled for two minutes and 5 ml of n-heptane was added to recover the methyl esters in organic phase. The mixture was washed with saturated NaCl solution and the two phases were separated. The upper n-heptane phase was pipetted out and put into 10 ml glass vials. Then a small amount of preheated sodium sulphate was added and stored until further analysis.

Gas Chromatography-Mass Spectrometry

The extracted lipids from fish tissues and feed were esterified with BF_3 methanol and recovered in heptane (AOAC 1995). The Fatty acid methyl esters were analyzed by GC-MS (QP 2010, quadruple mass-spectrometer with ionization energy of 70eV) equipped with DB-WAX capillary column (30 m x 0.25 mm i.d., 0.5 µm film thickness, J & W Scientific, USA) with helium gas as carrier gas. The sample was injected at split mode injection port with 1:15 split ratio at 250°C and oven temperature was programmed from 50-230°C at 10°C/min and held for 35 min. The mass spectrometer was tuned to get relative abundance of m/Z ranging from 40.00 to 550.00. The values of fatty acids are presented in area percentage of total identified fatty acids.

Data analysis

All data are presented as means ±SEM. The effects of diets on fatty acid composition of mature guppies and fry were analyzed by one-way analysis of variance (ANOVA) using SPSS statistical package (SPSS 2005).

Duncan's test was used to determine significant differences ($p<0.05$) between individual treatments where appropriate.

Results and Discussion

The crude fat levels in the initial guppy fry, muscle of guppy after maturation and resulting fry are given in Table 2. Significantly higher levels of crude fat contents were observed in the matured fish fed with each experimental diet compared to the initial fry and resulted fry. This may be due to the deposition of excess fat during the grow-out period.

Table 2. Crude fat content in initial guppy fry and muscle of mature guppy and resulting fry fed with experimental diets*

Diet type	% Crude fat in dry weight basis		
	Initial fry	After maturation	Resulting fry
	23.46 ^{a}±0.12		
Diet-CO		27.54 ^{b}±0.19	22.89 ^{a}±0.21
Diet-SO		26.10 ^{b}±0.10	22.57 ^{a}±0.38
Diet-LO		26.38 ^{b}±0.14	22.06 ^{a}±0.27
Diet-FO		27.37 ^{b}±0.15	22.38 ^{a}±0.32

*Composition of the experimental diets is given in Table 1.Data are presented as Mean±SEM (n=20). Data with different superscript letters are significantly different from each other ($p<0.05$).

The fatty acid profile of the experimental diets are presented in Table 3. The dominant fatty acids in all the diets were 16:0, 18:0, 18:1 n-9 and 18:2 n-6. Diet-CO had higher amount of 12:0, 14:0, 16:0 resulting in high amounts of SAFA compared to other diets. The Diet-SO and Diet-LO contained higher amount of 18:2 n-6 and 18:3 n-3 respectively. Diet-FO had significantly higher amount of EPA and DHA than the other three diets. Diet-CO, Diet-SO, Diet-LO did not contain any EPA and DHA levels.

The fatty acid profiles of the guppy after maturation are presented in Table 4. Thirty one fatty acids were identified in the muscle of the fish fed with Diet-SO, Diet-LO and Diet-FO while 32 fatty acids were identified in the muscle of guppy fed with Diet-CO. The fatty acid composition of fish tissue lipids usually reflect those of the dietary lipids (Henderson and Tocher 1987; Bell 1998; Jobling 1998; Higgs and Dong 2000; Sargent et al. 2002) even though there is potential for modification and metabolism of fatty acids sequestered from the diet (Henderson 1996; Bell 1998; Sargent et al2002).

Table 3. Fatty acid profiles (% of individual fatty acids among total identified fatty acids) of the experimental diets*

Fatty acid	Diet-CO	Diet-SO	Diet-LO	Diet-FO
6:0	1.46	0.14	0.06	0.12
8:0	8.66	0.44	0.08	0.10
10:0	7.54	0.43	0.10	0.17
12:0	24.21	2.63	0.19	0.31
13:0	0.13	ND	ND	0.04
14:0	15.03	1.91	0.76	5.44
15:0	0.22	0.18	0.13	1.15
16:0	11.27	10.45	9.04	9.73
17:0	ND	0.19	0.18	0.43
18:0	7.86	11.18	12.20	4.17
20:0	0.28	0.95	0.57	0.12
22:0	ND	2.13	0.62	ND
SAFA	**76.66**	**30.63**	**23.90**	**21.78**
16:1n-9	0.11	0.53	0.44	1.12
16:1n-7	0.47	0.63	0.61	7.82
18:1n-9	13.48	24.93	20.71	19.83
18:1n-7	ND	ND	ND	0.44
20:1n-9	0.28	0.70	0.62	11.85
22:1n-9	ND	ND	1.61	8.07
MUFA	**14.43**	**26.79**	**23.99**	**49.13**
18:2n-6	7.62	41.87	16.14	5.07
18:3n-3	0.54	0.53	35.84	2.09
18:4n-3	ND	ND	ND	2.37
20:2n-7	ND	ND	0.06	0.31
20:2n-9	0.34	ND	ND	ND
20:3n-9	0.41	ND	ND	ND
20:3n-3	ND	0.18	0.08	0.95
20:4n-6	ND	ND	ND	0.61
EPA	ND	ND	ND	7.92
DHA	ND	ND	ND	9.77
PUFA	**8.91**	**42.58**	**52.11**	**29.09**
HUFA	**ND**	**ND**	**ND**	**18.30**
n-3PUFA	0.54	0.71	35.91	23.10
n-3HUFA	ND	ND	ND	17.69
n-6PUFA	7.62	41.87	16.14	5.68
n-6HUFA	ND	ND	ND	0.61
n-3/n-6	0.07	0.02	2.22	4.07

* Composition of the experimental diets is given in Table 1.
ND=Not Detected, SAFA=Saturated Fatty Acids, MUFA=Mono Unsaturated Fatty Acids, PUFA=Poly Unsaturated Fatty Acids, HUFA=Highly Unsaturated Fatty Acids

The mature fish fed with Diet-CO contained significantly higher amounts of 12:0, 14:0, 16:0 and SAFA levels in the muscle compared to the fish fed with other three diets. The fish fed with Diet-SO contained significantly higher amount of 18:2 n-6, 18:3 n-6, 20:3 n-6, 20:4 n-6, 22:4 n-6, 22:5 n-6, n-6 PUFA and n-6 HUFA than the fish fed with other three diets. The fish fed with Diet-LO had significantly higher amount of 18:1n-7 and 18:3 n-3 levels than the fish fed with other three diets. The muscle of guppy fed with Diet-CO contained significantly higher level of 20:2 n-9 than the fish fed with other three diets and 20:3 n-9 fatty acid was reported only in the fish fed with Diet-CO while those fatty acid was not reported in the fish fed with Diet-SO, Diet-LO and Diet-FO. The fish fed with Diet-FO contained significantly higher amount of 16:1 n-7, 20:1 n-9, 22:1 n-9, MUFA, 20:3 n-3, EPA, DHA, HUFA, n-3 HUFA and n-3/n-6 than the fish fed with other three diets.

The present study demonstrated that the muscle fatty acid profiles of guppy can be altered through feeding with different lipid sources. The guppy fed with Diet-CO and Diet-SO can be used as SAFA, MUFA and n-6 PUFA sources while the guppy fed with Diet-LO and Diet-FO could be used as SAFA, MUFA and n-3 PUFA source for feeding high value fish. The mature fish of guppy fed Diet-FO can be used as a n-3 HUFA source for feeding marine fish brooders since n-3 HUFA cannot be biosynthesized de novo by marine fish (Halver and Hardy 2002).

It was also observed that the dietary fatty acids levels of mature female guppy influence the fatty acid profile of resulting fry (Table 5). The fry of the fish fed with Diet-CO contained significantly higher amount of 12:0, 14:0, SAFA, than the fish fed with other three diets while the fish fed with Diet-SO had significantly higher amount of fry fatty acid levels of 18:2 n-6, 20:3 n-6, 22:4 n-6, 22:5 n-6, n-6 PUFA and n-6 HUFA. The fry of the fish fed with Diet-LO contained significantly higher levels of 18:3 n-3, 18:4 n-3 and n-3 PUFA than the fish fed with other three diets while significantly higher levels of 16:1 n-7, 20:1 n-9, 22:1 n-9, MUFA, EPA, DHA, HUFA, n-3HUFA and n-3/n-6 PUFA were observed in the fry of the fish fed with Diet-FO than other three diets. In addition, fry of the fish fed with Diet-CO contained higher amount of AA and DHA than the fish fed Diet-SO and Diet-LO. Therefore the resulting fry from all diets can be used as n-3 PUFA and n-6 PUFA sources for feeding both freshwater as well as marine fish. The resulting fry fed with Diet-CO and Diet-FO can be used as HUFA source to feed marine fish since they do not have the ability to synthesize HUFAs.

Table 4. Fatty acid profile (% of individual fatty acids among total identified fatty acid) of guppy after maturation*

Fatty acid	Diet-CO	Diet-SO	Diet-LO	Diet-FO
10:0	0.78^{b}±0.10	0.07^{a}±0.02	0.04^{a}±0.01	0.06^{a}±0.01
12:0	7.00^{b}±0.30	0.61^{a}±0.04	0.46^{a}±0.34	0.26^{a}±0.02
14:0	9.75^{d}±0.58	5.59^{c}±0.62	3.07^{b}±0.15	3.62^{b}±0.24
15:0	2.89^{c}±0.33	0.68^{a}±0.05	1.94^{b}±0.16	2.75^{c}±0.13
16:0	14.91^{c}±0.77	$10.87^{a,b}$±0.6	11.73^{b}±0.68	8.81^{a}±0.24
17:0	3.0^{c}±0.22	1.12^{a}±0.20	$2.45^{b,c}$±0.21	1.37^{a}±0.19
18:0	11.02^{b}±0.40	10.93^{b}±0.50	9.40^{b}±0.28	7.20^{a}±0.22
20:0	0.56^{a}±0.09	0.68^{a}±0.04	0.43^{a}±0.04	0.51^{a}±0.04
22:0	0.06^{a}±0.01	0.63^{c}±0.02	0.06^{a}±0.01	0.17^{b}±0.05
SAFA	**49.97^{d}±1.92**	**31.18^{b}±2.19**	**$29.58^{a,b}$±0.63**	**24.71^{a}±0.44**
16:1 n-9	2.97^{c}±0.11	1.10^{b}±0.10	1.15^{b}±0.05	0.25^{a}±0.05
16:1 n-7	4.79^{b}±0.58	1.82^{a}±0.03	4.07^{b}±0.38	7.42^{c}±0.26
18:1 n-9	$18.40^{b,c}$±0.75	18.71^{b}±0.97	$17.53^{a,b}$±0.17	16.03^{a}±0.85
18:1 n-7	0.68^{a}±0.04	0.28^{a}±0.04	3.59^{b}±0.28	0.69^{a}±0.02
20:1 n-9	2.62^{b}±0.28	1.71^{b}±0.26	2.56^{b}±0.12	10.10^{c}±0.46
22:1 n-9	0.66^{a}±0.06	0.16^{a}±0.03	0.78^{a}±0.06	7.64^{b}±0.48
MUFA	**30.12^{b}±1.13**	**23.78^{a}±0.78**	**29.68^{b}±0.14**	**42.13^{c}±1.07**
18:2 n-9	1.25^{b}±0.21	0.48^{a}±0.07	0.26^{a}±0.05	0.25^{a}±0.04
18:2 n-6	4.12^{a}±0.45	20.28^{d}±0.85	11.11^{b}±0.75	5.78^{a}±0.20
18:3 n-6	$0.79^{a,b}$±0.07	4.94^{c}±0.58	1.60^{b}±0.22	$0.60^{a,b}$±0.03
18:3 n-3	0.21^{a}±0.03	0.43^{a}±0.02	10.85^{c}±0.21	1.58^{b}±0.40
18:4 n-3	0.55^{b}±0.07	$0.41^{a,b}$±0.03	0.50^{b}±0.08	0.24^{a}±0.03
20:2 n-9	1.46^{b}±0.21	0.27^{a}±0.02	0.18^{a}±0.02	0.26^{a}±0.02
20:3 n-6	0.36^{a}±0.06	2.47^{c}±0.39	$0.67^{a,b}$±0.05	1.12^{b}±0.12
20:3 n-3	0.48^{b}±0.03	0.30^{a}±0.07	0.28^{a}±0.04	0.92^{c}±0.03
20:2 n-7	0.97^{b}±0.05	2.22^{c}±0.11	0.58^{a}±0.01	0.56^{a}±0.04
20:3 n-9	1.11±0.02	ND	ND	ND
20:4 n-6	3.13^{b}±0.34	4.94^{c}±0.38	$2.45^{a,b}$±0.34	1.48^{a}±0.16
20:4 n-3	0.16^{a}±0.03	0.11^{a}±0.02	0.81^{b}±0.17	0.36^{a}±0.03
EPA	0.16^{a}±0.04	0.03^{a}±0.01	0.80^{b}±0.12	4.67^{c}±0.21
22:4 n-6	0.25^{a}±0.04	1.76^{b}±0.20	0.37^{a}±0.05	0.17^{a}±0.02
22:5 n-6	0.06^{a}±0.01	5.00^{b}±0.38	0.25^{a}±0.03	0.04^{a}±0.01
22:5 n-3	1.47^{b}±0.26	0.15^{a}±0.03	0.97^{b}±0.08	0.35^{a}±0.04
DHA	2.99^{b}±0.63	1.25^{a}±0.22	7.55^{c}±0.42	14.81^{e}±0.17
PUFA	**19.91^{a}±0.49**	**45.04^{d}±1.42**	**40.74^{c}±0.49**	**33.16^{b}±0.63**
HUFA	**8.22^{a}±0.55**	**13.24^{b}±0.19**	**13.20^{b}±0.21**	**21.85^{d}±0.67**
n-3 PUFA	6.02^{b}±0.78	2.68^{a}±0.34	21.76^{d}±0.18	22.90^{d}±0.08
n-3 HUFA	4.78^{b}±0.91	1.54^{a}±0.38	10.13^{c}±0.47	20.16^{e}±0.48
n-6 PUFA	8.71^{a}±0.79	38.88^{d}±2.10	16.45^{b}±0.78	9.19^{a}±0.45
n-6 HUFA	3.44^{b}±0.36	11.70^{d}±0.56	3.07^{b}±0.26	1.69^{a}±0.19
n-3/n-6 PUFA	0.69^{b}±0.15	0.07^{a}±0.01	1.32^{c}±0.07	2.40^{d}±0.13

*Composition of the experimental diets is given in Table 1. The data are presented as mean±SEM, (n=20), ND=Not Detected Values within a row with different superscript letters are significantly different ($p<0.05$), SAFA=Saturated Fatty Acids, MUFA=Mono Unsaturated Fatty Acids, PUFA=Poly Unsaturated Fatty Acids, HUFA=Highly Unsaturated Fatty Acids

Table 5. Fatty acid profiles (% of individual fatty acids among total identified fatty acids) of guppy fry fed with different experimental diets*

Fatty acid	Diet-CO	Diet-SO	Diet-LO	Diet-FO
10:0	$0.50^{c}\pm0.02$	$0.27^{b}\pm0.06$	$0.26^{b}\pm0.05$	$0.02^{a}\pm0.01$
12:0	$6.09^{b}\pm0.51$	$0.59^{a}\pm0.06$	$0.17^{a}\pm0.02$	$0.15^{a}\pm0.02$
14:0	$8.68^{c}\pm0.36$	$1.83^{a}\pm0.13$	$1.55^{a}\pm0.10$	$3.96^{b}\pm0.28$
15:0	$2.36^{a,b}\pm0.26$	$1.81^{a}\pm0.09$	$1.84^{a}\pm0.13$	$2.58^{b}\pm0.10$
16:0	$10.66^{b}\pm0.60$	$9.01^{a,b}\pm0.20$	$9.82^{b}\pm0.42$	$7.77^{a}\pm0.45$
17:0	$2.33^{b}\pm0.21$	$2.06^{b}\pm0.15$	$4.05^{c}\pm0.06$	$1.41^{a}\pm0.16$
18:0	$12.65^{c}\pm0.44$	$10.32^{b}\pm0.17$	$10.84^{b,c}\pm0.48$	$8.13^{a}\pm0.46$
20:0	$0.44^{b}\pm0.03$	$0.51^{b}\pm0.02$	$0.45^{b}\pm0.05$	$0.32^{a}\pm0.01$
22:0	$0.05^{a}\pm0.01$	$0.40^{c}\pm0.03$	$0.24^{b}\pm0.03$	$0.31^{b}\pm0.01$
SAFA	**$43.76^{c}\pm1.17$**	**$26.80^{a}\pm0.55$**	**$29.22^{b}\pm0.26$**	**$24.65^{a}\pm0.67$**
16:1 n-9	$0.45^{a}\pm0.04$	$1.70^{b}\pm0.49$	$0.22^{a}\pm0.01$	$0.83^{a,b}\pm0.05$
16:1 n-7	$5.58^{b}\pm0.38$	$2.43^{a}\pm0.31$	$2.62^{a}\pm0.03$	$7.39^{c}\pm0.41$
18:1 n-9	$15.50^{a}\pm0.49$	$21.18^{b}\pm0.60$	$17.61^{a}\pm1.29$	$21.96^{b}\pm0.65$
18:1 n-7	$3.27^{b}\pm0.15$	$0.14^{a}\pm0.02$	$2.71^{b}\pm0.25$	$0.42^{a}\pm0.01$
20:1 n-9	$1.61^{a}\pm0.35$	$0.75^{a}\pm0.05$	$1.54^{a}\pm0.11$	$7.95^{b}\pm0.36$
22:1 n-9	$0.47^{a}\pm0.02$	$0.07^{a}\pm0.02$	$0.31^{a}\pm0.02$	$3.23^{b}\pm0.25$
MUFA	**$26.88^{a}\pm0.35$**	**$26.27^{a}\pm1.39$**	**$25.01^{a}\pm0.88$**	**$41.78^{b}\pm1.71$**
18:2 n-9	$0.72^{b}\pm0.06$	$0.25^{a}\pm0.05$	$0.63^{b}\pm0.01$	$0.18^{a}\pm0.06$
18:2 n-6	$4.75^{a}\pm0.46$	$23.07^{c}\pm0.51$	$11.21^{b}\pm1.21$	$5.01^{a}\pm0.03$
18:3 n-6	$0.57^{a}\pm0.01$	$3.75^{b}\pm0.75$	$0.89^{a}\pm0.03$	$0.46^{a}\pm0.02$
18:3 n-3	$0.29^{a}\pm0.02$	$0.42^{a}\pm0.04$	$15.92^{c}\pm0.40$	$1.54^{b}\pm0.05$
18:4 n-3	$0.30^{a}\pm0.02$	$0.49^{a}\pm0.03$	$2.48^{c}\pm0.26$	$1.07^{b}\pm0.08$
20:2 n-9	$0.47^{b}\pm0.05$	$0.14^{a}\pm0.03$	$0.13^{a}\pm0.02$	$0.18^{a}\pm0.06$
20:2 n-7	$0.59^{a}\pm0.05$	$1.55^{c}\pm0.02$	$0.89^{b}\pm0.06$	$0.54^{a}\pm0.05$
20:3 n-9	1.21 ± 0.02	ND	ND	ND
20:3 n-6	$1.44^{b}\pm0.21$	$1.93^{c}\pm0.05$	$0.54^{a}\pm0.03$	$0.40^{a}\pm0.03$
20:3 n-3	$1.30^{a,b}\pm0.09$	$0.27^{a}\pm0.01$	$0.58^{a}\pm0.04$	$2.00^{b}\pm0.71$
20:4 n-6	$5.47^{b}\pm0.50$	$4.98^{b}\pm0.04$	$2.44^{a}\pm0.06$	$1.31^{a}\pm0.26$
20:4 n-3	$0.11^{a}\pm0.02$	$0.04^{a}\pm0.01$	$0.45^{c}\pm0.02$	$0.19^{b}\pm0.02$
EPA	$0.27^{a}\pm0.02$	$0.13^{a}\pm0.02$	$0.58^{a}\pm0.05$	$3.88^{b}\pm0.71$
22:4 n-6	$0.26^{b}\pm0.03$	$1.54^{c}\pm0.02$	$0.13^{a}\pm0.01$	$0.13^{a}\pm0.02$
22:5 n-6	$3.03^{c}\pm0.18$	$5.15^{d}\pm0.17$	$0.80^{b}\pm0.06$	$0.28^{a}\pm0.01$
22:5 n-3	$2.52^{b}\pm0.08$	$1.60^{a}\pm0.05$	$1.97^{a}\pm0.01$	$2.48^{b}\pm0.04$
DHA	$6.46^{b}\pm0.36$	$1.62^{a}\pm0.08$	$6.13^{b}\pm0.05$	$13.92^{c}\pm0.29$
PUFA	**$29.36^{a}\pm0.83$**	**$46.93^{b}\pm0.34$**	**$45.77^{b}\pm0.38$**	**$33.57^{a}\pm2.37$**
HUFA	**$18.12^{c}\pm0.12$**	**$14.93^{b}\pm0.51$**	**$12.50^{a}\pm0.05$**	**$22.19^{c}\pm1.35$**
n-3 PUFA	$11.25^{b}\pm0.77$	$4.57^{a}\pm0.10$	$28.11^{d}\pm0.79$	$25.08^{c}\pm1.9$
n-3 HUFA	$9.36^{c}\pm0.65$	$3.39^{a}\pm0.17$	$9.13^{b}\pm0.09$	$20.47^{d}\pm1.06$
n-6 PUFA	$15.52^{b}\pm0.11$	$40.42^{c}\pm0.35$	$16.01^{b}\pm1.25$	$7.59^{a}\pm0.31$
n-6 HUFA	$8.76^{b}\pm0.77$	$13.60^{c}\pm0.05$	$3.37^{a}\pm0.04$	$1.72^{a}\pm0.29$
n-3/n-6 PUFA	$0.72^{b}\pm0.06$	$0.11^{a}\pm0.01$	$1.76^{c}\pm0.17$	$3.30^{d}\pm0.12$

*Composition of the diets are given in Table 1. Data are presented as mean±SEM, n=20, ND=Not Detected, Values within a row with different superscript letters are significantly different ($p<0.05$), SAFA=Saturated Fatty Acids, MUFA=Mono Unsaturated Fatty Acids, PUFA=Poly Unsaturated Fatty Acids, HUFA=Highly Unsaturated Fatty Acids

Tamaru et al. (1997) have reported that the live feeds presently used in the aquarium industry do not contain any DHA levels and contain only little EPA levels. Therefore those live feeds cannot be used especially for marine fish which cannot synthesize EPA and DHA in vivo. For those fishes feeder guppies are an ideal source of essential fatty acids. The results of the present study clearly demonstrated that the mature guppy fed with Diet-FO and their fry contained significantly higher levels of n-3 HUFA compared to the initial fry. Those fry can be used as n-3 HUFA source to feed marine fish brooders and larval stages since n-3 HUFA cannot be biosynthesized de novo by marine fish. Moreover, that the plant based oil sources particularly coconut oil, sunflower oil and linseed oil can also be used as cheap lipid sources for preparation of feed for guppy to alter the fatty acid profile and resulting fry which could be used as good source of n-6 and n-3 PUFA for feeding high value fishes. Furthermore, in comparison with the other live food organisms fish oil enriched feeder guppies contained significantly higher amount of n-3 HUFA levels and the early larval stages could be used to feed fingerlings and juvenile stages, and mature feeder guppies can be used to feed brooders of high value carnivorous fish.

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