Changes in fatty acid profiles during maturation and fatty acid composition of eggs and embryos of female guppy *Poecilia reticulata* (Peters) fed on different diets

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Abstract

Changes in the fatty acid profiles of muscle during maturation and the fatty acid composition of eggs and embryos in the female guppy, *Poecilia reticulata,* fed different diets were investigated. Three feed types currently being used in guppy farming, namely Diet 1, 2 and 3, containing 18.26%, 29.27% and 43.60% crude protein and 4.17%, 4.55% and 9.47% crude lipid, respectively, were used in this experiment. The percentage of lipid in fry was 25.03 and varied between 26.56- 29.83 in mature fish and between 6.30 and 7.28 in their eggs and embryos. The fish fed Diet 3 had significantly ($p<0.05$) higher lipid in muscle, eggs and embryos than the fish fed other two diets. Significantly higher levels of EPA, DHA, HUFA, (n-3) PUFA, (n-3) HUFA and (n-3)/n-6 levels were recorded with Diet 3 and in the muscle and the eggs of the fish fed this diet than the other two. There were no significantly different levels of (n-6) PUFA and (n-6) HUFA in the muscle of the fish fed Diets 2 and 3, though the levels were higher than those in the fish fed Diet 1. There were some significantly different levels of fatty acids recorded in the eggs and embryos of the fish fed the same diet, but there were no significantly different levels of fatty acids recorded between the eggs and embryos. The results showed, therefore, that the fatty acid composition of fish muscle, eggs and embryos reflected those in the diet and that the fatty acid profiles of guppies can therefore be modified by altering the source of fats and oils used in formulating fish feed.

Keywords: *Poecilia reticulata,* fatty acids, (n-3) PUFA, (n-3) HUFA, (n-6) HUFA

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Introduction

The guppy *Poecilia reticulata* is an omnivorous and viviparous fish of the family Poeciliidae which is a very popular ornamental fish species due to it's variety of body colours, fin patterns and also because they can tolerate a wide range of environmental conditions. Studies have shown that reproductive performances of these live breeders are influenced by nutrition (Dzikowski *et al.*, 2001; Kruger *et al.*, 2001).

The proportion of lipids and fatty acids in the diet is of fundamental significance since, being eminently carnivorous, they are the main, if not the only available source of energy (Bell *et al.*, 1986). The fatty acid composition of fish tissue lipids usually reflects those of the dietary lipids (Hendersion and Tocher, 1987; Sargent *et al.*, 2002; Bell, 1998; Higgs and Dong, 2000; Jobling, 2001) even though there is potential for modification and metabolism of fatty acids sequestered from the diet (Hendersion, 1996; Sargent *et al.*, 2002; Bell, 1989). Although tissue fatty acid profiles can be modified by altering the sources of fat, highly unsaturated fatty acids (HUFA) such as arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are important structural and physiological components of cell membranes and are thought to play important roles in permeability, enzyme activity and other functions in polar lipids of membranes (Bell *et al.*, 1986; Lee, 2001). In addition, some species have the ability to elongate and desaturate C18 fatty acids to higher molecular weight n-3 HUFA, while other species do not have such an ability (Ibeas *et al.*, 1994, 1996). It has been reported that HUFA content of brood fish feed significantly affects fecundity, fertility, hatching and viability of fish eggs, egg quality and larval growth (Mourente and Odriozola, 1990, Fernandez-Palacios *et al.*, 1995; Izquierdo *et al.*, 2001).

Rainbow trout (*Oncorhynchus mykiss*) fed on n-3 deficient diet during the last 3 months of vitellogenesis produced a moderate effect on the incorporation of DHA into egg lipid whereas EPA concentration decreased by 50%. Selective retention of DHA has also been found during embryogenesis (Izquierdo, 1996) and during starvation (Tandler *et al.*, 1989), denoting the importance of this fatty acid for the developing embryo and larvae. Poly unsaturated fatty acids can also regulate eicosanoid production, particularly prostaglandins, which are involved in several reproductive processes (Moore, 1995), including the production of steroid hormones and gonadal development such as ovulation.

Certain dietary nutrients also exert a marked effect on fertilization. Dietary EPA and ArA levels show correlation with fertilization rates in gilthead bream broodstock

(Fernandez-Palacios, *et al.*, 1995). Studies on broodstock nutrition revealed that several nutrients are essential for the normal development of the embryo, and their optimum level in broodstock diets improves egg morphology and hatching rates. The percentage of morphologically normal eggs (as a parameter to determine egg viability) has been found to increase with an increase in the n-3 HUFA levels in broodstock diets and an incorporation of these fatty acids in to the eggs (Fernandez-Palacios *et al.*, 1995). Improved egg quality has been associated with higher total n-3 fatty acids content in European seabass fed on a pelleted diet enriched with high quality fish oil (Navas *et al.*, 1996). Increased n-3 HUFA (particularly DHA) levels in broodstock diets were reported to significant enhance the weight of fish larvae and their resistance to osmotic shock (Aby-ayad *et al.*, 1997). In a similar way, increasing n-3 HUFA levels in broodstock diets for gilthead seabream significantly improved the percentage of live larvae after yolk sack re-absorption.

Most studies related to fatty acids have been carried out with the food fish and there has been little work reported on the fatty acid profile of tropical ornamental fish.

Therefore, the present study was designed to examine the changes of fatty acid composition of muscle, eggs and embryos, and to find out whether there is an effect of dietary fatty acids on the muscle, eggs and embryo fatty acid profiles of guppy fed on three different types of feeds currently being used in the guppy farming which contain varying dietary nutrient levels.

Materials and methods

Experimental setup, animals and diets

The experiment was conducted at the Central Institute of Fisheries Education, Mumbai, India. There were three treatment groups, each with three replicates, and nine 160 L rectangular plastic tubs (763 X 521 X 410 mm).

Two experiments were carried out in sequential order namely growth and breeding trials. In the first experiment the sexes were separated to obtain virgin females for the growth experiment. Each tub was stocked with sixty *P. reticulata* female fry of almost uniform size (0.077-0.087 g). The tubs were covered with transparent acrylic sheets to prevent escape of the fishes and the water volume was maintained at 140 L. Aeration

was provided using a 2 HP air blower. Tubs were cleaned by siphoning fecal matter and uneaten food and 50% of the water was replaced with fresh chlorine free bore well water every second day. The fish were cultured separately till they became mature. Males were also cultured with the same food to be used for breeding.

Three diets were used. Feed 1 was made of mainly wheat flour, wheat bran and maize bran. Feed 2 was a closed formula commercial feed and feed 3 was made of rice bran, wheat flour, fish meal and ground chicken feed.

Preparation of tanks for obtaining fishes with embryos

After carrying out the growth experiment 20 female fishes were separated from each treatment and were used for breeding purpose. Three replicates for each diet group were used and each tank was stocked with 20 female fishes and 10 males of the same size. All tanks were provided with mild aeration during the breeding period. After a gestation period of 21-25 days (Depeche and Schoffeniels, 1975; Stolk, 1951) the fishes were taken out for extraction of embryo lipid and fatty acid content. The embryos were identified by the presence of developed eyes.

Feeding of fish

Feeding was carried out until satiation twice a day at 0800 and 1700 hr throughout the experimental period. Each time a small amount of feed was dropped in to the tank and this process was repeated until satiation was observed.

Biochemical analysis of diets

The proximate compositions of all diets were determined following standard methods (AOAC, 1995).

Lipid extraction

Total lipid was extracted by the Folch (1957) method. The lipids were extracted from the diets, the fry and the muscle of fish after maturation (Stage 1) and eggs and embryos of female guppy (stage 2). Dissections were carried out under a binocular stereoscopic microscope. After excision of the gonads, the oviduct and mesovarium were separated and removed and directly used for estimation of lipid and for the preparation of fatty acid methyl esters (FAME).

The tissue was homogenized in 10 volumes (of tissue w/v) methanol fortified with BHT (0.01%) (which was added to inhibit the oxidative degradation of lipids during analysis), followed by 20 volume (of tissue w/v) chloroform in a Teflon coated tissue homogenizer (Superfit, India). After dispersion, the whole mixture was agitated for 15-20 minutes in an orbital shaker at room temperature.

The homogenate was filtered using a Buchner funnel with a folded defatted filter paper to recover the liquid phase and the filter residue re-homogenized with a second volume of chloroform-methanol. The filtered solvent was washed with 0.2 volumes (4 ml for 20 ml) of 0.9% NaCl solution and the two phases were vigorously mixed. The mixture was poured into a separating funnel and allowed to decant. The lower chloroform phase containing lipids was collected and evaporated under vacuum in a rotary evaporator 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 re-dissolved in chloroform/methanol (2:1 v/v) and then stored under nitrogen at –20 °C for further analysis.

Preparation of Fatty Acid Methyl Esters (FAME)

The AOAC (1995) method was followed to esterify the lipid extract. The FAMEs were prepared from the isolated lipids by heating with the methanolic NaOH first and then with BF_3 Methanol for esterification. 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 using a separating funnel. The upper N-heptane phase was pipetted out and stored in 10 ml glass vials with Teflon lids until further analysis.

Gas Chromatography-Mass Spectrometry

The Fatty acid methyl esters (FAME) were analyzed by GC-MS (QP 2010, quadruple mass-spectrometer with ionization energy of 70eV) equipped with DB-WAX column (30 m x 0.25 mm internal diameter, 0.5 ìm film thickness, J & W Scientific, USA) with helium gas as the carrier. The sample was injected at a split mode injection port with 1:15 split ratio at 250°C; the 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 were presented as area percentages of the total identified fatty acids.

Data and statistical analysis

All data were presented as means ±S.E. The effects of diets on muscle fatty acid composition, eggs and embryo fatty acid composition were analyzed by one-way analysis of variance (ANOVA) using SPSS statistical package (SPSS, 2005) followed where appropriate by Duncan's test to determine significant differences ($p<0.05$) between individual treatments.

Results and Discussion

Proximate composition of diets

The proximate compositions of the diets are given in Table 1. Three diets contained three different levels of nutrients. Protein and crude lipid percentages in the Diet-3 were found to be significantly higher ($p<0.05$) than Diet-1 and 2. Diet-2 also contained significantly higher levels ($p<0.05$) of protein and lipid than Diet-1. Diet-1 had the lowest levels of crude protein and lipid compared to other diets. Therefore Diet-3 fulfilled the required amount of protein and lipid while Diet-1 did not fulfill the required amount for growth and reproduction of guppy as reported by Shim and Leng (1986). Diet-2 had only the required protein amount and it did not fulfill the required amount of lipid.

Table 1. Proximate composition of experimental diets.

Nutrient	**Percentage composition**		
	Diet-1	**Diet-2**	**Diet-3**
Moisture	6.40	7.20	6.80
Crude protein	18.26	29.27	43.60
Crude lipid	4.17	4.55	9.47
Ash	8.43	12.69	8.45
Total carbohydrates	69.14	53.49	38.48

Lipid levels in carcass, eggs and embryos

Lipid percentages in initial fish fry, after maturation and eggs and embryos are given in Table 2. The lipid content in the fishes changes according to species, diet, geographical location and season. In a same species, sex maturity and age contribute to significant differences (Jean *et al.*, 2005). Lipid class composition of freshwater fish eggs varies between species, but in general freshwater fish eggs have lipid contents in the range 2.5-10% of the wet weight (Henderson and Tocher, 1987). Kairanta and Ackman, (1981) reported that the fat content in the fish eggs varied from 1.5% to 10% wet weight Lipid and fatty acid composition of broodstock diets have been identified as major dietary factors that determine successful reproduction and survival of offspring (Sampath and Pandean 1984, James *et al.*, 1993, Jobling, 1998). The general composition of egg lipid classes is a conservative trait of the species, even if broodstock nutrition affects both percentage lipid content and fatty acid composition (Sargent *et al.*, 1989).

Jobling (2004) found that the initial fatty acid content became diluted as the fish grew and deposited increasing amount of lipid. In the present study, it was found that lipid levels of fish after maturation were significantly ($p<0.05$) higher than initial fry. The lipid levels in muscle and pre and post fertilized eggs were significantly ($p<0.05$) higher in the fish fed Diet-3 than other 2 diets probably due to the presence of significantly ($p<0.05$) high amount of lipid levels in that diet. The fish fed Diet-1 had the lowest lipid levels.

Table 2. Percentage lipids content in initial fish fry, in the muscle after maturation, and eggs and embryos.

	Initial fry	**After maturation**	**Eggs**	**Embryos**
Diet 1	$25.03^{a}\pm0.07$	$26.56^{a}\pm0.15$	$6.30^{a}\pm0.07$	$6.08^{a}\pm0.04$
Diet 2		$27.20^{b}\pm0.08$	$6.90^{b}\pm0.05$	$6.38^{b}\pm0.05$
Diet 3		$29.83^{c}\pm0.19$	$7.28^{c}\pm0.04$	$6.95^{c}\pm0.03$

Values within a column with different superscript letters are significantly different

Fatty acid profile of experimental diets

The fatty acid profiles of the experimental diets are presented in Table 3. The most available fatty acids in the all diets were 16:0, 18:0 18:1 n-9, 18:2 n-6, 18:3 n-3. In addition, Diet-2 and Diet-3 were also richer in EPA and DHA and hence their n-3 total PUFA, HUFA and n-3 HUFA were also significantly higher ($p<0.05$) than Diet-1. Diet-1 had the significantly ($p<0.05$) highest PUFA and n-6 PUFA levels since it contained significantly ($p<0.05$) highest level of 18:2 n-6 compared to other diets. Diet-3 recorded the significantly highest ($p<0.05$) levels of EPA, DHA, and n-3 PUFA. Diet-1 contained the significantly highest ($p<0.05$) levels of 16:0 (palmitic acid) 18:1 n-9 (oleic acid) 18:2 n-6 (linoleic acid), 18:3n-3 (á-linolenic acid). Diet-2 contained significantly ($p<0.05$) higher level of 20:4 n-6 than Diets 1 and 3. The n-3/n-6 ratios varied in the three experimental feeds and the Diet-3 had significantly ($p<0.05$) higher ratio than other two diets.

Fatty profile of initial fry and muscle of guppy after maturation

The guppy fry used in this experiment contained 35.65% SAFA, 34.35% MUFA, 30.00% PUFA and 15.08% HUFA (Table 4). The levels of PUFA were significantly ($p<0.05$) higher in fish fry than in matured fish. After feeding experimental diets throughout the study period, the guppy fed with Diet-1 contained almost 47.58% SAFA, 38.59% MUFA, 13.83% PUFA and 2.8% HUFA while the guppy fed Diet-2 contained 41.09% SAFA, 41.10% MUFA, 17.81% PUFA and 4.79 HUFA. The fish fed Diet-3 had 33.68% SAFA, 37.04% MUFA, 29.28% PUFA and 14.7 HUFA. The most available fatty acids in the muscle were 16:0, 18:0, 16:1 n-7, 18:1 n-9, 18:2 n-6. The levels of 18:3 n-3, 20:4 n-6, 20:5 n-3 and 22:6 n-3 in the guppy fed Diet-3 were significantly ($p<0.05$) higher than the fish fed other two diets. The total n-3 PUFA and n-3 HUFA were significantly higher in the guppy fed Diet-3 than other 2 diets (Figure 2). n-3/ n-6 ratios in muscle varied considerably (0.12-1.12) among the guppy fed different diets and the fish fed Diet-3 had significantly ($p<0.05$) higher ratio.

Table 3. Fatty acid profile (% of individual fatty acids among total identified fatty acids) of diets (Mean ±S.E.)

Fatty acids	Diet-1	Diet-2	Diet-3
8:0	0.07^{b}±0.01	0.02^{a}±0.00	0.03^{a}±0.00
10:0	0.03^{a}±0.00	0.02^{a}±0.00	0.05^{a}±0.02
12:0	0.06^{a}±0.01	0.07^{a}±0.01	0.40^{b}±0.01
13:0	0.02^{a}±0.00	0.04^{a}±0.00	0.03^{a}±0.00
14:0	0.55^{a}±0.05	4.58^{c}±0.85	4.12^{b}±0.81
15:0	0.18^{a}±0.01	0.57^{c}±0.01	0.45^{b}±0.36
16:0	20.30^{c}±0.90	19.47^{b}±0.81	16.04^{a}±0.68
17:0	0.23^{a}±0.01	0.27^{b}±0.01	0.56^{c}±0.00
18:0	3.26^{a}±0.81	6.69^{b}±0.91	6.79^{b}±0.99
19:0	ND	0.26±0.01	ND
20:0	0.54^{a}±0.01	0.70^{c}±0.01	0.38^{b}±0.03
22:0	1.24±0.20	ND	ND
23:0	0.09^{a}±0.01	0.12^{b}±0.01	ND
24:0	1.61^{b}±0.24	0.97^{a}±0.01	ND
SAFA	**28.16^{a}±0.85**	**33.76^{b}±0.62**	**28.85^{a}±0.74**
16:1 n-9	0.18^{a}±0.01	0.35^{b}±0.01	5.45^{c}±0.05
16:1 n-7	0.57^{a}±0.01	5.37^{c}±0.15	1.29^{b}±0.27
18:1 n-9	26.37^{c}±0.80	20.11^{a}±0.55	21.50^{b}±0.26
18:1 n-7	1.85^{b}±0.05	2.59^{c}±0.10	0.23^{a}±0.02
20:1 n-9	0.68^{b}±0.05	0.31^{a}±0.05	3.90^{c}±0.05
21:1 n-9	0.13^{a}±0.01	0.04^{a}±0.00	4.91^{b}±0.05
MUFA	**29.77^{a}±0.62**	**28.13^{a}±0.65**	**37.28^{b}±0.36**
18:2 n-9	ND	0.08^{b}±0.00	0.02^{a}±0.00
18:2 n-6	38.21^{c}±0.46	25.00^{b}±0.31	14.51^{a}±0.35
18:3 n-3	3.32^{b}±0.06	1.98^{a}±0.01	1.82^{a}±0.10
20:2 n-9	ND	0.14^{a}±0.01	0.12^{a}±0.01
20:2n-7	0.07^{a}±0.00	0.15^{b}±0.00	0.22^{c}±0.00
20:3 n-7	ND	0.08±0.00	ND
20:4 n-6	0.05^{a}±0.00	1.18^{c}±0.02	0.76^{b}±0.00
20:4 n-3	ND	0.18^{b}±0.02	0.07^{a}±0.01
20:5 n-3	ND	4.42^{a}±0.06	6.39^{b}±0.07
22:4 n-6	ND	0.19^{b}±0.02	0.08^{a}±0.01
22:5 n-3	ND	0.38^{a}±0.01	0.30^{a}±0.00
22:6 n-3	0.43^{a}±0.02	3.70^{b}±0.15	9.60^{c}±0.05
PUFA	**42.07^{c}±0.41**	**38.11^{b}±0.53**	**33.87^{a}±0.42**
HUFA	**0.48^{a}±0.03**	**10.05^{b}±0.02**	**17.2^{c}±0.04**
n-3 PUFA	3.74^{a}±0.06	10.69^{b}±0.04	18.17^{c}±0.12
n-3 HUFA	0.43^{a}±0.01	8.71^{b}±0.03	16.36^{c}±0.04
n-6 PUFA	38.26^{c}±0.06	26.36^{b}±0.00	15.35^{a}±0.35
n-6 HUFA	0.05^{a}±0.00	1.37^{c}±0.01	0.84^{b}±0.01
n-3/n-6 PUFA	0.10^{a}±0.00	0.41^{b}±0.01	1.19^{c}±0.04

ND=Not Detected. Values within a row with different superscript letters are significantly different ($p<0.05$)

Table 4. Fatty acid profile (individual fatty acids as a percentage of total identified fatty acids) of initial fry and muscle of guppy after maturation (Mean ±S.E).

Fatty acids	Initial Fry	Mature Diet-1	Mature Diet-2	Mature Diet-3
12:0	$0.13^{a,b}$±0.03	$0.10^{a,b}$±0.01	0.08^{a}±0.01	0.17^{b}±0.02
14:0	2.38^{a}±0.21	2.93^{a}±0.29	2.79^{a}±0.25	3.70^{a}±0.54
15:0	1.48^{a}±0.18	3.74^{b}±0.39	$2.68^{a,b}$±0.58	1.55^{a}±0.35
16:0	15.90^{b}±1.45	26.92^{d}±1.24	21.18^{c}±1.59	13.18^{a}±0.86
17:0	0.91^{a}±0.12	$1.61^{a,b}$±0.24	1.15^{a}±0.18	2.09^{b}±0.26
18:0	14.04^{b}±1.52	11.91^{a}±0.84	$12.42^{a,b}$±1.50	12.13^{a}±0.45
20:0	0.58^{b}±0.05	0.24^{a}±0.04	0.45^{b}±0.05	0.60^{b}±0.05
22:0	$0.25^{a,b}$±0.03	0.16^{a}±0.04	$0.35^{b,c}$±0.03	0.48^{c}±0.06
SAFA	**35.65^{a}±1.67**	**47.58^{c}±1.56**	**41.09^{b}±2.51**	**33.68^{a}±1.05**
16:1 n-9	0.94^{c}±0.05	0.24^{a}±0.04	0.61^{b}±0.04	0.88^{c}±0.10
16:1 n-7	$6.69^{a,b}$±0.52	6.04^{a}±0.35	8.69^{b}±1.58	$7.45^{a,b}$±0.53
18:1 n-9	24.73^{b}±1.52	28.30^{b}±1.34	26.31^{b}±1.08	19.31^{a}±0.92
18:1 n-7	0.32^{a}±0.06	2.41^{b}±0.59	3.92^{c}±0.07	0.25^{a}±0.03
20:1 n-9	1.54^{a}±0.45	1.45^{a}±0.14	1.39^{a}±0.35	5.44^{b}±0.46
22:1 n-9	0.14^{a}±0.04	0.16^{a}±0.02	0.19^{a}±0.05	3.70^{b}±0.17
MUFA	**34.35^{a}±1.59**	**$38.59^{a,b}$±2.08**	**41.10^{b}±1.71**	**$37.04^{a,b}$±1.62**
18:2 n-9	0.30^{a}±0.02	1.06^{c}±0.07	0.61^{b}±0.04	0.55^{b}±0.04
18:2 n-6	11.24^{b}±0.75	7.82^{a}±0.26	$9.83^{a,b}$±0.60	$9.67^{a,b}$±0.55
18:3 n-6	0.62^{a}±0.05	0.55^{a}±0.11	0.67^{a}±0.06	0.66^{a}±0.02
18:3 n-3	0.92^{b}±0.07	0.26^{a}±0.03	0.42^{a}±0.04	0.90^{b}±0.04
18:4 n-3	0.38^{b}±0.04	0.15^{a}±0.04	0.26^{a}±0.03	0.57^{c}±0.01
20:2 n-9	0.03^{a}±0.02	0.37^{b}±0.02	0.34^{b}±0.02	0.35^{b}±0.05
20:2 n-7	$0.59^{a,b}$±0.05	0.29^{a}±0.04	$0.46^{a,b}$±0.04	1.04^{b}±0.31
20:3 n-6	0.81^{b}±0.06	0.52^{a}±0.05	0.41^{a}±0.03	0.47^{a}±0.03
20:4 n-6	4.43^{b}±0.51	1.64^{a}±0.35	1.39^{a}±0.33	1.86^{a}±0.80
20:3 n-3	0.05^{a}±0.01	0.04^{a}±0.01	0.04^{a}±0.02	0.38^{b}±0.03
20:4 n-3	0.026^{a}±0.01	0.03^{a}±0.01	0.02^{a}±0.01	0.26^{b}±0.05
20:5 n-3	1.02^{b}±0.04	0.04^{a}±0.01	0.25^{a}±0.04	1.83^{c}±0.13
22:4 n-6	1.37^{b}±0.21	0.36^{a}±0.04	1.56^{b}±0.40	0.39^{a}±0.03
22:5 n-3	1.19^{b}±0.08	0.06^{a}±0.01	0.23^{a}±0.02	2.46^{c}±0.06
22:6 n-3	7.06^{b}±0.42	0.69^{a}±0.03	1.34^{a}±0.22	7.91^{b}±0.39
PUFA	**30.00^{c}±1.26**	**13.83^{a}±0.52**	**17.81^{b}±0.23**	**29.28^{c}±0.57**
HUFA	**15.08^{b}±0.82**	**2.80^{a}±0.43**	**4.79^{a}±0.58**	**14.70^{b}±0.67**
n-3 PUFA	10.63^{c}±0.41	1.26^{a}±0.13	2.54^{b}±0.08	14.31^{d}±0.16
n-3 HUFA	9.28^{b}±0.52	0.81^{a}±0.05	1.84^{a}±0.16	12.46^{c}±0.16
n-6 PUFA	18.46^{c}±0.94	10.87^{a}±0.28	13.85^{b}±0.22	13.04^{b}±0.33
n-6 HUFA	5.80^{b}±0.3	1.99^{a}±0.38	2.95^{a}±0.73	2.25^{a}±0.83
n-3/n-6 PUFA	0.58^{c}±0.01	0.12^{a}±0.01	0.18^{b}±0.01	1.12^{d}±0.02

ND=Not Detected. SAFA, MUFA, PUFA and HUFA – see text. Values within a row with different superscript letters are significantly different ($p<0.05$)

Lipid content and fatty profile in eggs and embryos

The lipid content in the eggs and embryos is shown in Table 2. The percentage lipid content in the eggs and embryos of the guppies fed Diet-3 were significantly ($p<0.05$) higher than those fed the other two diets, with those fed diet-1 having the lowest. It was also observed that there was a little decrease in the lipid levels in embryos.

The fatty acid profiles in the lipids of the eggs and embryos are presented in Tables 5 and 6. The fatty acid profiles were dominated by a few of the fatty acids. The most abundant fatty acids in all treatments were 16:0, 18:0, 16:1 n-9, 18:1 n-9, 18:2 n-6, 18:3 n-3, 20:4 n-6 and 22:6 n-3. The guppy fed Diet-2 and Diet-3 had significantly ($p<0.05$) higher levels of PUFA and HUFA level in eggs and embryos. There was no difference of 18:2 n-6 levels between the eggs of the guppy fed the three different diets. The linoleic acid content, however, was higher in the embryos of the guppy fed Diet-1 than the other two diets. The18:3 n-3 content in the eggs of the fish fed Diet-3 was significantly higher than those fed the other two diets. In embryos, these levels were similar on Diets 2 and 3 but significantly ($p<0.05$) higher on Diet-3 when compared to Diet-1. There was no significant difference of 20:4 n-6 in the eggs of the fish fed three diets while levels in the embryos of the fish fed Diet-1 and 2 had significantly higher levels than the other. The EPA levels in the eggs of the guppy fed Diet-3 had significantly ($p<0.05$) higher levels than other two diets. In embryos, there was no significant ($p<0.05$) difference between Diets 2 and 3 while Diet-1 contained the lowest level. The DHA levels in the eggs and embryos of the guppy fed Diet-3 were found to be significantly higher than the fish fed Diet-1 and Diet-2. The n-3/n-6 levels in the eggs and embryos of the guppy fed Diet-3 were significantly higher than the guppy fed other diets.

Table 5. Fatty acid profile in eggs (individual fatty acids as a percentage of total Fatty acids identified) (Mean ±S.E.).

Fatty acid	Diet-1	Diet-2	Diet-3
14:0	2.28^{a}±0.1	3.16^{b}±0.10	3.26^{b}±0.23
15:0	1.90^{a}±0.23	2.85^{b}±0.14	$2.29^{a,b}$±0.18
16:0	19.75^{b}±1.16	11.44^{a}±0.58	9.94^{a}±0.98
17:0	0.98^{a}±0.15	1.55^{b}±0.10	0.91^{a}±0.08
18:0	12.29^{a}±1.09	11.55^{a}±1.52	10.16^{a}±0.84
20:0	0.08^{a}±0.01	0.54^{b}±0.06	0.46^{b}±0.06
22:0	0.07^{a}±0.01	0.41^{b}±0.09	0.49^{b}±0.08
SAFA	**37.34^{b}±1.58**	**31.48^{a}±1.53**	**27.49^{a}±1.55**
16:1 n-9	6.96^{b}±0.25	1.59^{a}±0.40	0.79^{a}±0.07
16:1 n-7	0.62^{a}±0.03	8.57^{b}±0.75	8.23^{b}±0.42
18:1 n-9	28.22^{b}±1.11	20.54^{a}±1.83	22.92^{a}±1.36
18:1 n-7	0.41^{a}±0.06	0.24^{a}±0.12	0.31^{a}±0.08
20:1 n-9	1.01^{a}±0.11	2.10^{b}±0.22	2.71^{b}±0.27
22:1 n-9	0.30^{a}±0.09	0.09^{a}±0.01	1.03^{b}±0.19
MUFA	**37.50^{a}±1.63**	**33.12^{a}±1.66**	**35.98^{a}±1.67**
18:2 n-9	$0.62^{a,b}$±0.34	0.47^{a}±0.06	0.68^{b}±0.04
18:2 n-6	9.56^{a}±0.21	7.92^{a}±0.81	8.25^{a}±0.94
18:3 n-6	3.71^{b}±0.50	$2.25^{a,b}$±0.27	2.11^{a}±0.10
18:3 n-3	0.16^{a}±0.06	0.40^{a}±0.06	1.44^{b}±0.24
18:4 n-3	0.14^{a}±0.02	0.29^{a}±0.06	1.15^{b}±0.11
20:2 n-9	0.14^{a}±0.05	0.60^{b}±0.02	0.14^{a}±0.04
20:2 n-7	0.19^{a}±0.06	0.68^{c}±0.05	0.44^{b}±0.04
20:3 n-6	0.72^{b}±0.05	1.05^{c}±0.08	0.35^{a}±0.04
20:4 n-6	2.96^{a}±0.75	5.67^{a}±0.42	3.27^{a}±0.71
20:3 n-3	0.20^{a}±0.01	0.73^{c}±0.06	0.48^{b}±0.02
20:4 n-3	0.31^{a}±0.01	0.85^{b}±0.13	0.97^{b}±0.15
20:5 n-3	0.17^{a}±0.02	0.20^{a}±0.04	1.28^{b}±0.28
22:4 n-6	2.84^{b}±0.50	2.26^{b}±0.14	0.80^{a}±0.10
22:5 n-3	0.38^{a}±0.04	2.37c±0.21	1.53b±0.13
22:6 n-3	3.10^{a}±0.45	9.69^{b}±0.58	13.69^{c}±0.53
PUFA	**25.16^{a}±1.06**	**35.40^{b}±1.19**	**36.53^{b}±1.08**
HUFA	**9.85^{a}±1.82**	**20.04^{b}±1.49**	**21.53^{b}±0.28**
n-3 PUFA	4.45^{a}±0.41	14.53^{b}±0.03	20.52^{c}±1.44
n-3 HUFA	3.95^{a}±0.47	13.11^{b}±0.20	17.46^{c}±1.08
n-6 PUFA	19.78^{b}±0.58	19.14^{b}±1.29	14.77^{a}±0.61
n-6 HUFA	5.80^{a}±1.25	7.93^{a}±0.29	4.07^{a}±0.81
n-3/n-6 PUFA	0.23^{a}±0.02	0.76^{b}±0.05	1.40^{c}±0.16

ND=Not Detected SAFA, MUFA, PUFA and HUFA – see text

Values within a row with different superscripts are significantly different ($p<0.05$)

Table 6. Fatty acid profile of embryos (individual fatty acids as a percentage of total fatty acids) (Mean ±S.E.).

Fatty acid	Diet-1	Diet-2	Diet-3
14:0	2.00^{a}±0.10	2.98^{b}±0.24	3.44^{b}±0.13
15:0	1.59^{a}±0.06	2.51^{b}±0.27	2.75^{b}±0.14
16:0	19.70^{c}±1.51	13.25^{a}±0.66	15.76^{b}±1.48
17:0	1.82^{a}±0.08	2.93^{a}±0.66	1.67^{a}±0.23
18:0	13.51^{b}±0.84	12.32^{b}±0.67	9.36^{a}±0.81
20:0	0.19^{a}±0.03	0.55^{c}±0.05	0.34^{b}±0.01
22:0	0.51^{a}±0.03	0.37^{a}±0.05	0.40^{a}±0.01
SAFA	**39.31^{b}±1.46**	**36.00^{a}±1.27**	**33.22^{a}±1.30**
16:1 n-9	6.30^{a}±0.65	6.83^{a}±0.50	7.36^{a}±0.38
16:1 n-7	0.54^{a}±0.09	$0.78^{a,b}$±0.06	0.90^{b}±0.08
18:1 n-9	28.54^{b}±0.10	21.24^{a}±1.12	23.07^{a}±0.83
18:1 n-5	0.33^{a}±0.05	0.23^{a}±0.02	0.23^{a}±0.04
20:1 n-9	0.25^{a}±0.04	1.76^{b}±0.33	2.79^{c}±0.03
22:1 n-9	1.22^{b}±0.01	0.08^{a}±0.01	1.19^{b}±0.05
MUFA	**37.17^{b}±1.75**	**30.91^{a}±1.35**	**35.53^{b}±1.14**
18:2 n-9	0.78^{c}±0.04	0.47^{b}±0.05	0.15^{a}±0.02
18:2 n-6	10.24^{b}±1.35	9.12^{a}±0.21	8.35^{a}±0.15
18:3 n-6	1.26^{a}±0.14	1.15^{a}±0.13	0.82^{a}±0.13
18:3 n-3	0.29^{a}±0.04	$0.43^{a,b}$±0.04	0.73^{b}±0.14
18:4 n-3	1.13^{b}±0.11	0.22^{a}±0.01	1.73^{c}±0.17
20:2 n-9	0.27^{a}±0.03	0.67^{b}±0.05	0.18^{a}±0.06
20:2 n-7	0.43^{a}±0.02	0.62^{a}±0.07	0.49^{a}±0.05
20:3 n-6	0.68^{b}±0.04	0.87^{b}±0.06	0.36^{a}±0.04
20:4 n-6	4.17^{b}±0.16	4.91^{b}±0.30	2.88^{a}±0.02
20:3 n-3	0.15^{a}±0.04	0.27^{a}±0.06	0.39^{a}±0.07
20:4 n-3	0.13^{a}±0.03	0.16^{a}±0.04	0.44^{a}±0.12
20:5 n-3	0.19^{a}±0.01	0.55^{b}±0.05	0.58^{b}±0.08
22:4 n-6	1.17^{b}±0.10	1.46^{b}±0.19	0.61^{a}±0.03
22:5 n-3	0.23^{b}±1.0	2.59^{a}±0.31	1.54^{a}±0.36
22:6 n-3	3.56^{a}±0.30	9.64^{b}±0.32	12.04^{c}±0.18
PUFA	**27.52^{a}±1.55**	**33.09^{b}±1.21**	**31.25^{b}±0.94**
HUFA	**9.43^{a}±0.09**	**19.29^{b}±0.55**	**17.92^{b}±0.88**
n-3 PUFA	5.65^{a}±0.01	13.84^{b}±0.17	17.43^{c}±1.10
n-3 HUFA	4.09^{a}±0.17	12.93^{b}±0.07	14.59^{b}±0.73
n-6 PUFA	17.51^{b}±0.08	17.50^{b}±0.88	13.01^{a}±0.24
n-6 HUFA	5.34^{b}±0.26	6.37^{b}±0.49	3.49^{a}±0.01
n-3/n-6 PUFA	0.55^{a}±0.00	0.79^{a}±0.03	1.34^{b}±0.11

ND=Not Detected SAFA, MUFA, PUFA and HUFA – see text

Values within a row with different superscript letters are significantly different ($p<0.05$)

The comparison of SAFA, MUFA, PUFA and HUFA levels in the different stages are presented in Fig. 1. SAFA levels were higher in Diet-2 while PUFA levels were higher in Diet-1. MUFA and HUFA levels were higher in Diet-3 than the other two diets. Comparison of the total n-3 and n-6 PUFA, HUFA and n-3/n-6 PUFA levels in different stages are presented in Fig. 2. Comparison of total PUFA and HUFA levels in eggs and embryos eggs are presented in Fig. 3 and 4. All figures demonstrate that the fatty acid patterns in muscle, eggs and embryos of the fish reflect those present in the three diets.

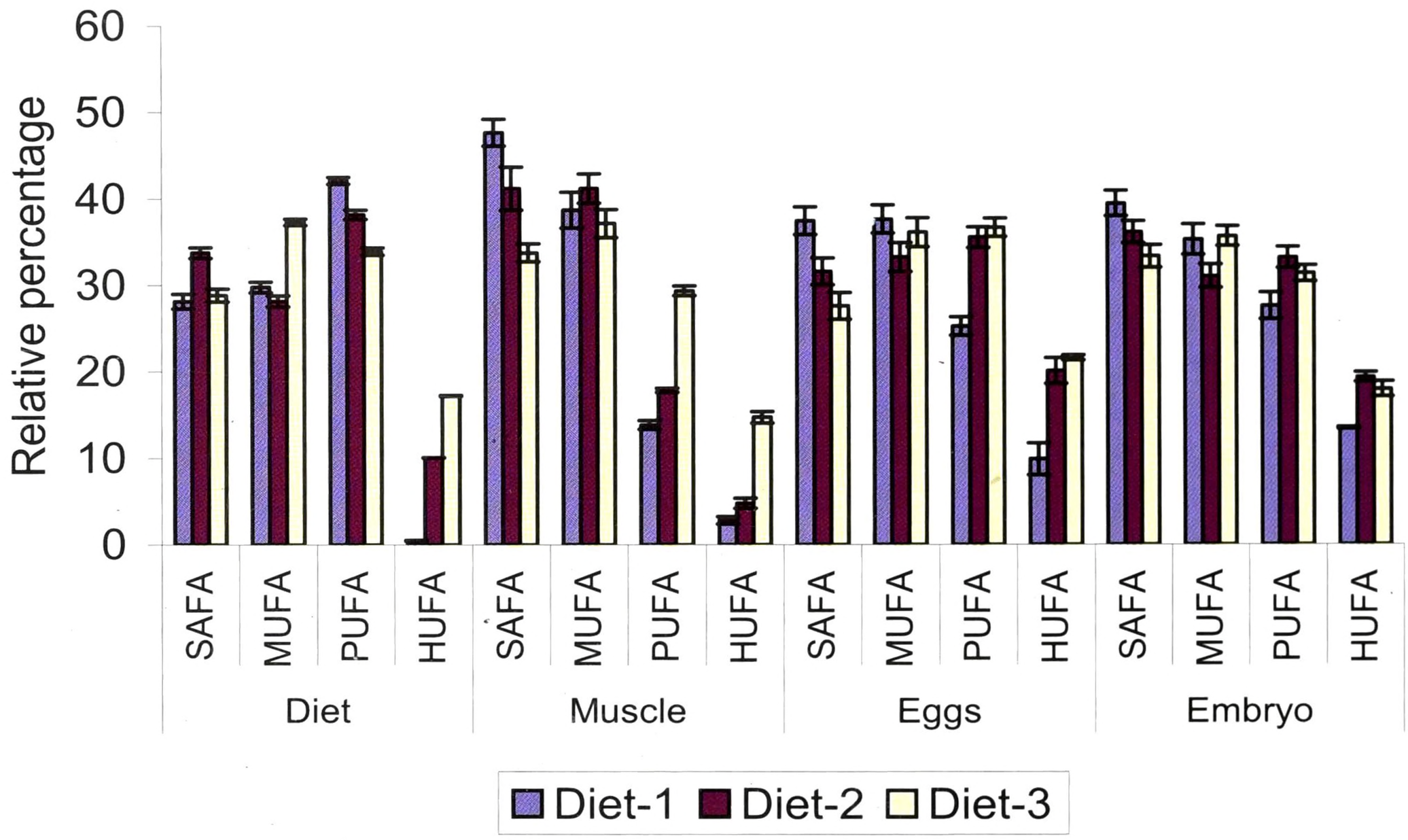

Fig. 1. Comparison of SAFA, MUFA, PUFA and HUFA levels in different stages. Vertical bars ± SE.

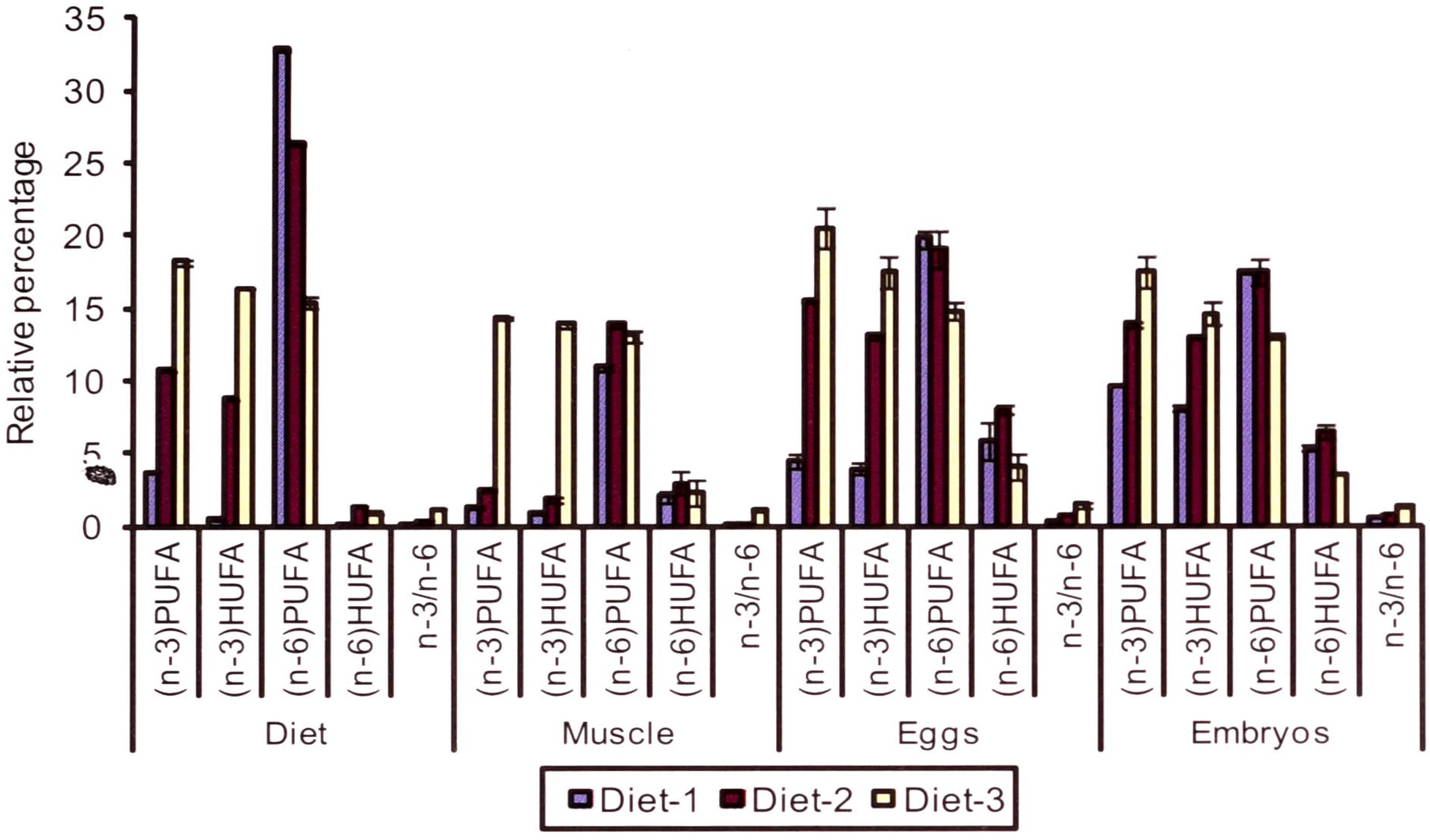

Fig. 2. Comparison of (n-3) and (n-6) PUFA and HUFA and (n-3)/(n-6) PUFA levels in the different stages. Vertical bars ± SE.

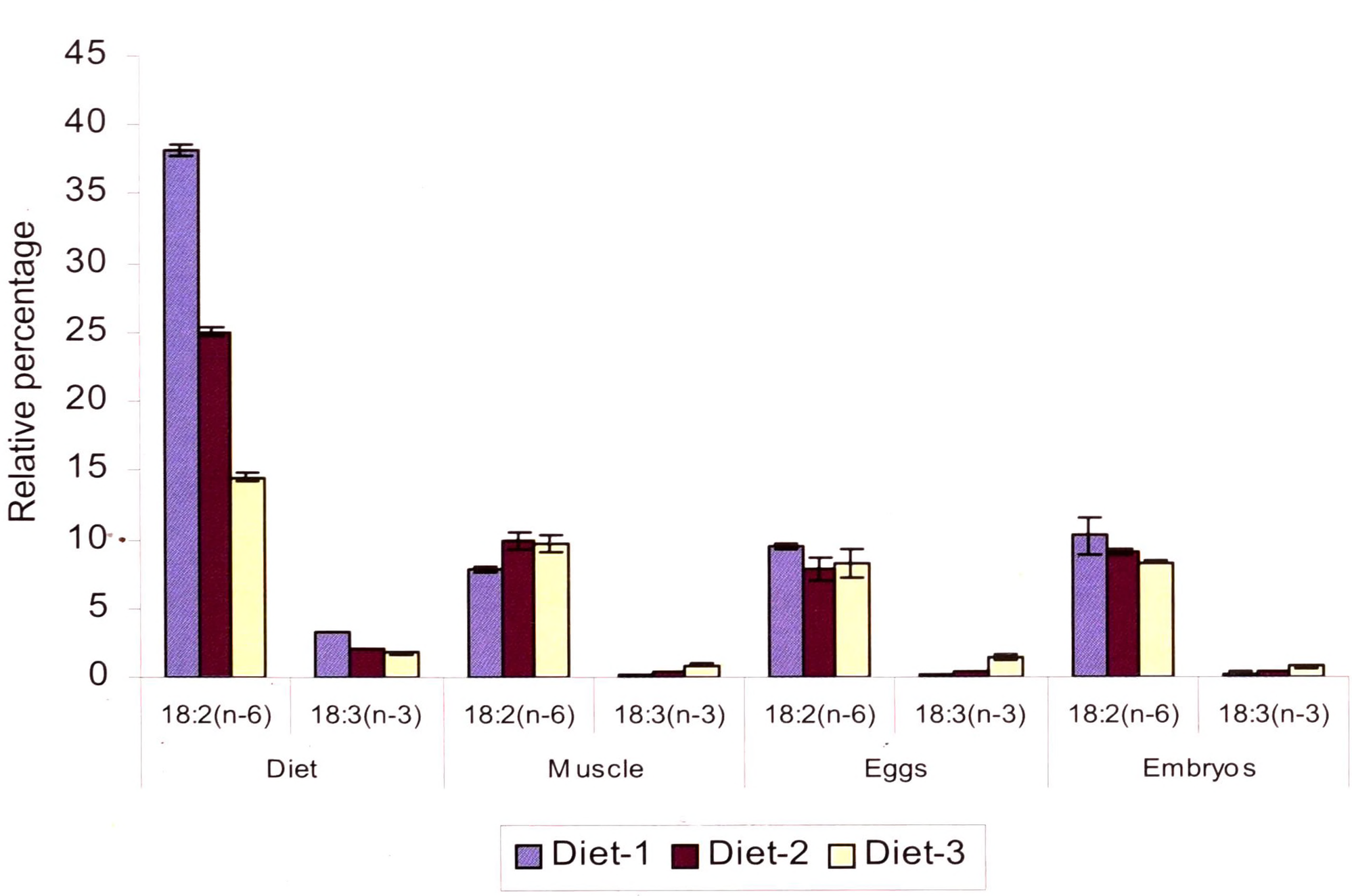

Fig. 3. Comparison of important PUFA levels in different stages. Vertical bars ±SE.

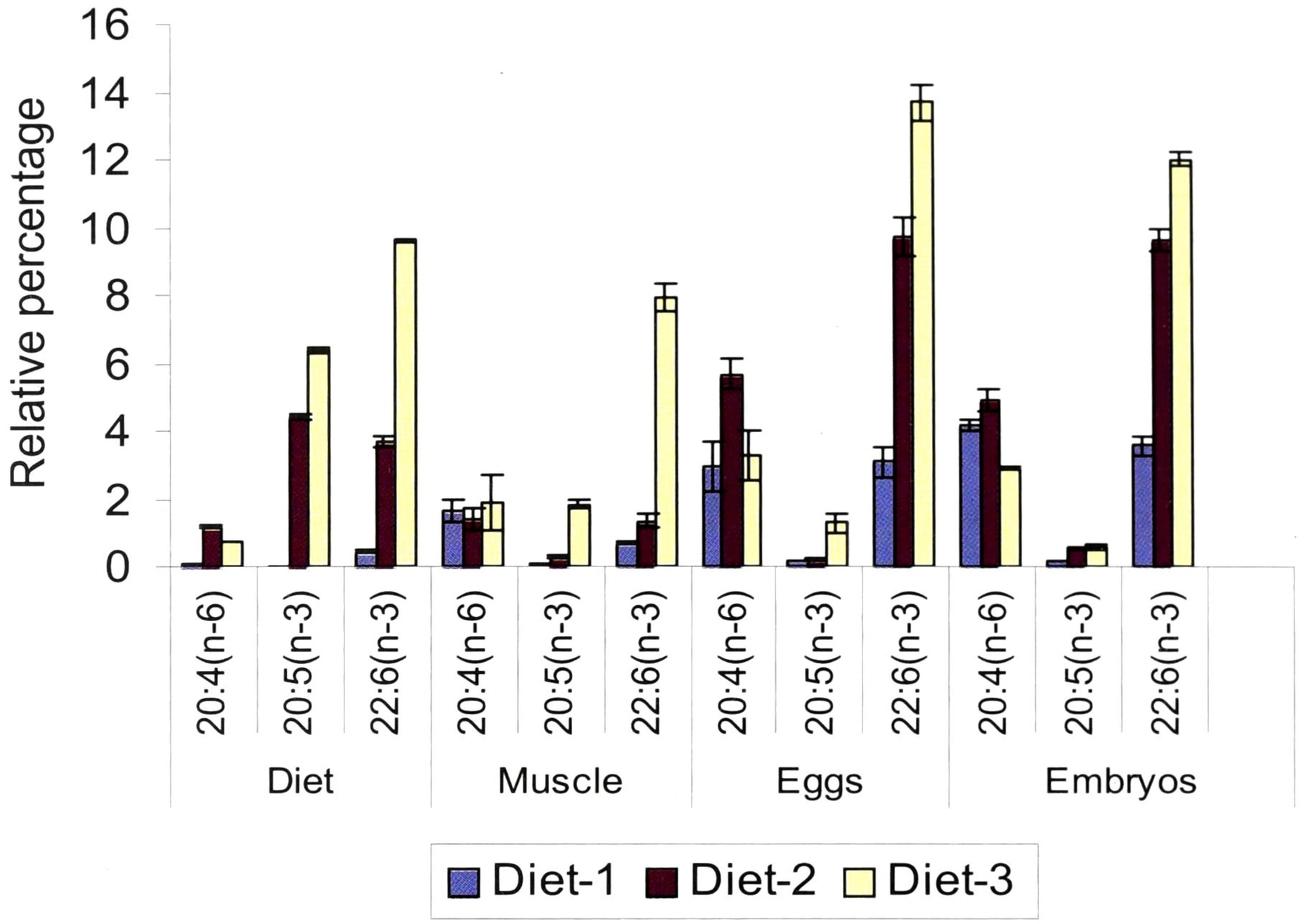

Fig. 4. Comparison of important HUFA levels in different stages. Vertical bars ± SE.

Changes in fatty acid composition during embryogenesis

Levels of the fatty acids considered important in eggs and embryos are shown in Table 7, where it can be seen that there was no significant difference between diets in most of these fatty acids. The PUFA levels in the eggs of the guppies fed Diet-2 and 3 were, however, found to be significantly ($p<0.05$) higher than that in embryos. The 18:2 n-6 fatty acid levels were found to be significantly ($p<0.05$) higher in embryos of the fish fed Diet-1 than in eggs. All the fatty acids except 18:2 n-6 and SAFA levels found to be higher in eggs than embryos, while there were slightly higher levels of all the fatty acids except SAFA, 18:2 n-6, 18:3 n-3 and EPA in the eggs of the guppy fed Diet-2 than embryos. There were no significantly different in DHA levels in the eggs and embryos of the fish fed Diet-1 and Diet-2. The ratios of n-3/n-6 were significantly ($p<0.05$) higher in embryos of the fish fed Diet-1 than in their eggs while there was no significant difference of this ratio in the eggs and embryos of the guppy fed Diet-2 and Diet-3.

Table 7. Comparison of important fatty acid levels in eggs and embryos of guppy (Mean ±S.E.).

Fatty acid	**Diet-1**		**Diet-2**		**Diet-3**	
	Eggs	Embryos	Eggs	Embryos	Eggs	Embryos
18:2 n-6	9.56^{a}±0.21	10.24^{a}±0.35	7.92^{a}±0.81	9.12^{a}±0.21	8.25^{a}±0.34	8.35^{a}±0.15
18:3 n-3	0.16^{a}±0.06	0.29^{a}±0.04	0.40^{a}±0.06	0.43^{a}±0.04	1.44^{b}±0.24	0.73^{a} ±0.14
20:4 n-6	2.96^{a}±0.75	4.17^{a}±0.16	5.67^{b}±0.42	4.91^{a}±0.30	3.27^{a}±0.71	2.88^{a}±0.02
20:5 n-3	0.17^{a}±0.02	0.19^{a}±0.01	0.20^{a}±0.04	0.55^{a}±0.05	1.28^{b}±0.28	0.58^{a}±0.08
22:6 n-3	3.10^{a}±0.45	3.56^{a}±0.30	9.69^{a}±0.58	9.64^{a}±0.32	13.69^{b}±0.53	12.04^{a}±0.18
SAFA	37.34^{a}±1.58	39.31^{b}±1.43	31.48^{a}±1.53	36.00^{b}±1.27	27.49^{a}±1.55	33.22^{b}±1.30
MUFA	37.50^{a}±1.63	37.17^{a}±1.75	33.12^{a}±1.66	30.91^{a}±1.35	35.98^{a}±1.67	35.53^{a}±1.14
PUFA	25.16^{a}±1.06	27.52^{a}±1.55	35.40^{b}±1.19	33.09^{a}±1.21	36.53^{b}±1.08	31.25^{a}±0.94
HUFA	9.84^{a}±1.82	8.71^{b}±0.09	20.04^{a}±1.49	19.29^{a}±0.55	21.53^{a}±0.28	17.92^{a}±0.88
n-3 PUFA	4.45^{a}±0.41	5.65^{a}±0.01	14.53^{a}±0.03	13.84^{a}±0.17	20.52^{b}±1.44	17.43^{a}±1.10
n-3 HUFA	3.95^{a}±0.47	4.09^{a}±0.17	13.11^{a}±0.20	12.93^{a}±0.07	17.46^{b}±1.08	14.59^{a}±0.73
n-6 PUFA	19.78^{a}±0.58	17.51^{a}±0.08	19.14^{a}±1.29	17.50^{a}±0.88	14.77^{a}±0.61	13.01^{a}±0.24
n-6 HUFA	5.80^{a}±1.25	5.34^{a}±0.26	7.93^{a}±0.29	6.37^{a}±0.49	4.07^{a}±0.81	3.49^{a}±0.01
n-3/n-6 PUFA	0.23^{a}±0.02	0.55^{b}±0.00	0.76^{a}±0.05	0.79^{a}±0.03	1.40^{a}±0.16	1.34^{a}±0.11

Values of the same feed within a row with different superscript letters are significantly different ($p<0.05$)

Conclusion

It was concluded from the results of the present study that levels of fatty acids in the muscle, eggs and embryos of the guppy, reflect the levels in their diets. HUFA, n-3 PUFA, n-6HUFA and n-3HUFA, especially DHA, which play a crucial role in development and survival of larvae, were highly concentrated in eggs and embryos. It is clear that providing those important fatty acids in their diets to guppy broodstock will help in embryonic development as has already been shown in the case of food fish.

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