www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 12: 743-749 (2012) DOI: 10.4194/1303-2712-v12_4_02

Lipid and Fatty Acid Composition of Commercially Important Tropical Freshwater Fish Gonads: Guidelines for Specific Broodstock Diet

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Received 20 January 2012
Accepted 13 September 2012

Abstract

Fatty acids compositions were analyzed in neutral lipids (NL) and polar lipid (PL) of gonads of Nile tilapia, ayungin and African catfish to elucidate some guesses for the fatty acids requirements for broodstock. The high value detected for both C16:0, C18:1 n-9 in all samples reflects a requirement for energy metabolism during the course of gonad development. The Lower proportion of polyunsaturated fatty acids (PUFA) was found in the NL of all gonads samples compared to PL. The higher percentages of n-3 HUFA in PL with respect to NL, suggests the importance of HUFA in the reproductive processes. In PL and NL, arachidonic acid (ARA) was the most abundant n-6 PUFA (ranged from 2.59 to 11.33% and from 0.16 to 3.19%, respectively). A relatively higher particularly eicosapentaenoic acid (EPA)/ docosahexaenoic acid (DHA) ratio was obtained in both NL and PL. All wild species studied are characterized by high ARA/EPA ratio in PL ranged from 1.72 to 5.47. Therefore, it is necessary to take into consideration not only the individual levels of HUFA but also the correct ratio among them (ARA/EPA/ DHA) through controlling LA and LNA level and ratio in the diets of tropical freshwater broodstocks.

Keywords: Broodstocks; gonads; neutral lipids; polar lipids.

Introduction

Most developing countries are located in tropical or sub-tropical areas, and fish is a vital component of food security for these countries. Rivers and lakes in these countries were more accessible and kinder sources of fish, and also carry over 40% of the world's known fish species (Zenebe *et al*., 1998). Moreover, the production and consumption of freshwater fish, has increased during recent years. Therefore effort is needed to improve the output performances and quality of the most important tropical freshwater fish. Currently, there is a high demand for stockable fry of these preferred species due to its faster growth rate and amenable to culture in different freshwater ecosystems (Mukhopadhyay and Kaushik, 2001).

So far, information on the effects of broodstock nutrition with regard to reproductive performances and the egg quality of fish species of economic importance like commercially important tropical freshwater fish is scarce. Despite relative paucity of work on broodstock nutrition, the nutritional status of broodstock is known to have a profound effect on the reproductive performance and quality of offspring in several species. Studies performed on Nile tilapia (Gunasekara *et al*., 1996), turbot (Mourente *et al*., 1991), lake trout (Lahnsteiner *et al*., 1999), goldfish (Mercure and Der Kraak, 1996) and yellow tail (Watanabe and Kiron, 1997), have demonstrated that incorporation of essential nutrients into the developing eggs depends on the availability of these nutrients in the female broodstock and consequently on the dietary input in the period preceding gonadal maturity.

Lipids are an important component of diet, both as energy and essential fatty acids sources, which fish need for basic functions, including growth, reproductive and maintenance of healthy tissues (Sargent *et al*., 1989). Significant changes and mobilizations of lipids take place during embryonic development; therefore, the importance of lipids in broodfish nutrition has been emphasized (Sargent, 1995). The fatty acid composition of lipids from gonads of fish reflects the fatty acid content of the lipid in the diet fed by the broodstock (Fernandez-Palacios *et al*., 1995). No such data are available on the fatty acid composition of gonads of commercially important tropical freshwater fish. Therefore, information in this respect can be used as a guideline

for developing appropriate broodstocks diets of commercially important tropical freshwater fish. Lipids can be divided into two main classes, i.e. neutral lipids (NL) and polar lipids (PL). PL are important constituents of membranes and they function as precursors in eicosanoid metabolism (structural fat), whereas the NL serve mainly as a depot of lipids used as an energy source (depot fat) (Henderson and Tocher, 1987). Therefore, for comparison between some species, fatty acid composition in both NL and polar lipids PL must be investigated.

The aim of the present study was to elucidate some guesses for the fatty acids requirements of tropical freshwater fish to develop specific diet for broodstock. In the authors' opinion, examined the fatty acid composition from PL and NL in mature gonads from wild tropical freshwater fish will help in recommend the dietary fatty acids requirement's for broodstocks of these species.

Materials and Methods

Eighteen samples of ripe gonads belonging to three species (3 samples per tissue: ovary and testis) of commercial importance tropical freshwater fish were obtained during the spawning period from Binangonan (having a long coast line facing the Laguna de Bay) in the province of Rizal, Philippines. These are Nile tilapia (*Oreochromis niloticus*), ayungin, (*Leiopotherapon plumbeus*) and African catfish (*Clarias gariepinus*). Samples of these species were introduced into crushed ice and transported into the laboratory. The samples were freeze-dried and stored at -80°C until lipid extraction.

Total lipid was extracted with chloroform/methanol (2:1 v/v) containing 0.01% of Butylated hydroxytoluene (BHT) as antioxidant (Folch *et al*., 1957). The organic solvent was evaporated under a stream of nitrogen and the lipid content was determined gravimetrically. PL and NL were separated by a silica cartridge (Sep-pak plus, Waters, Milford, MA, USA) as procedure described by Juaneda and Rocquelin (1985). Fatty acid methyl esters (FAME) were prepared by transesterification with borontrifluoride in methanol according to the procedure of Miyashita *et al*. (1999). The resultant fatty acids methyl esters were purified by thin-layer chromatography (Silicagel 70 Plate, Wako, Osaka, Japan; solvent system: petroleum ether/diethyl ether/ acetic acid = 90:10:1, v/v). The FAME was separated and quantified analyzed using GC-17A gas liquid chromatography (GC- 17A; Shimadzu, Kyoto, Japan) equipped with a hydrogen flame ionization detector (FID) and an Omegawax 320 fused silica capillary column (30 m_0.32 mm i.d.; Supelco, Bellefonte, PA, USA). Helium was used as carrier gas with pressure 80 kPa. The oven initial column temperature was 160°C for 5 min, followed by an increase at a rate of 4°C min^{-1} to a final temperature of 210°C. Individual FAME were identified by a reference to authentic standards (Funakoshi, Tokyo, Japan) and to a will characterized known fish oil FAME, and were quantified with an integrator (C-R7A plus; Shimadzu).

Result and Discussion

Lipids Classes of Gonads

No clear trend was observed for total lipid, polar lipid and neutral lipid. The total lipid (TL) and NL contents, for example, in tilapia ovaries (38.68% and 63.90%, respectively) were higher than testes (22.57% and 20.47%, respectively), while the opposite trend was observed in Silver perch (19.56% and 69.48% for ovaries and 33.74% and 86.15% for testes, respectively) which has hermaphroditic sex glands such that both sexes are in one individual (Table 1). The differences, however, were not observed for TL in the African catfish gonads which had fairly similar percentage for ovary and testis (19.06% and 19.45%, respectively) (Table 1). This may be explained by the variations in the different stages of gonad, because gonads samples were not examined individually for gonadal development and maturation through external symptoms.

Fatty Acid Profiles

Thirty two fatty acids in PL and thirty one fatty acids in NL were identified and compared between the three species. The fatty acids studied ranged from C14:0 to C24:1 and a few minor components of uncertain identity were omitted for calculation. In general the fatty acid profile of NL (Table 2) showed higher variation than that in PL (Table 3). Total saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) in NL (ranged from 42.63 to 55.83% and from 28.40 to 37.45%, respectively) were higher

Table 1. Composition of total lipid (%, dry basis), neutral lipid (NL, % of total lipid) and polar lipid (PL, % of total lipid) of wild tropical freshwater fish gonads studied

	Ayungin *Leiopotherapon plumbeus*		African catfish *Clarias gariepinus*		Tilapia *Oreochromis niloticus*	
	Ovary	Testis	Ovary	Testis	Ovary	Testis
TL	19.56±0.02	33.74±1.73	19.06±0.38	19.45±0.95	38.68±1.93	22.57±6.28
NL	69.48±1.80	86.15±2.89	38.58±1.77	58.68±1.32	63.90±0.12	20.47±4.23
PL	30.52±1.80	13.85±2.89	61.43±1.77	41.32±1.32	36.10±0.12	79.53±4.23

than those in PL (ranged from 30.71 to 54.40% and from 12.68 to 27.23%, respectively). The data agree with Ackman (1980) who reported that PL fraction contained lower SFA values, and much lower MUFA, while PUFA values were higher to NL fraction.

Saturated Fatty Acids (SFA)

SFA in both PL and NL constituted nearly half of the total fatty acids in fish gonads (Table 2). The most abundant SFA was C 16:0 (ranging from 16.60 to 28.97% in PL and from 29.90 to 39.96% in NL), which is noted for being a predominant source of potential metabolic energy in fish during growth and particularly during the egg formation stage in female fish (Henderson *et al*., 1984). Ackman and Eaton (1976) reported that palmitic acid was key metabolite in fish and that its level was not influenced by diet.

Monounsaturated fatty acids (MUFA)

PL contained lower C18:1 n-9 (ranged from 6.26 to 18.84%) than in the NL (ranged from 8.61 to 22.76%). Ostaszewska (2005) reported that the C16:0, C18:1 n-9, C20:1 n-9 and C22:1 n-11 fatty acids are mainly catabolic for energetic purposes. High amounts of such acids are consumed during fish growth and development, and they are easily

Table 2. Neutral lipid fatty acid composition (expressed as percentage of total fatty acids) of wild tropical freshwater fish gonads studied

	Ayungin		African catfish		Tilapia	
	ovary	testis	ovary	testis	ovary	testis
14:0	3.70±0.13	3.55±0.20	6.72±2.72	1.77±0.23	7.46±2.58	2.63±0.10
14:1	0.59±0.07	0.34±0.06			0.81±0.06	0.42±0.02
15:0	1.04±0.13	0.66±0.15	1.47±0.27		1.30±0.05	0.64±0.14
16:0	34.25±0.75	36.81±2.56	39.96±3.07	29.19±1.18	37.18±2.04	30.46±2.63
16:1n-7	10.11±1.13	6.91±0.28	7.39±0.96	7.16±0.16	13.92±1.99	5.88±0.73
17:0	0.59±0.03	0.60±0.11	2.24±0.17	1.67±0.17	2.13±0.31	1.49±0.40
16:3n-6	1.54±0.15	1.35±0.39			1.67±0.31	1.66±0.16
16:3n-3	0.75±0.11	1.12±0.98			0.62±0.19	0.45±0.05
18:0	9.14±0.11	12.61±0.93	5.24±0.46	10.01±0.00	7.37±1.49	17.72±0.92
18:1n-9	12.71±0.87	15.28±2.22	15.34±2.12	22.67±0.67	8.61±0.58	14.15±6.13
18:1n-7	5.27±0.14	4.83±0.82	7.52±0.56	6.92±0.09	6.13±0.48	5.98±0.29
18:2n-6 (LA)	1.57±0.03	1.11±0.22	0.73±0.10	1.96±0.04	0.91±0.21	3.47±1.79
18:3n-6	0.21±0.10	0.20±0.10	0.13±0.02	0.00±0.00	0.24±0.07	0.50±0.10
18:3n-3 (LNA)	1.03±0.10	0.68±0.08	0.11±0.03	0.89±0.11	0.42±0.11	0.48±0.08
18:4n-3	0.20±0.05	0.26±0.06			0.09±0.00	0.05±0.02
20:0	0.22±0.01	0.37±0.04			0.28±0.01	0.12±0.01
20:1	0.69±0.01	0.98±0.18	0.30±0.07	0.71±0.11	0.76±0.30	1.39±0.10
20:2n-6	0.21±0.02	0.12±0.01		0.27±0.07	0.11±0.06	0.53±0.31
20:3n-6	0.41±0.00	0.23±0.06			0.11±0.06	0.45±0.01
20:4n-6 (ARA)	1.06±0.14	1.45±0.41	0.16±0.05	1.00±0.01	0.38±0.31	3.19±1.24
20:3n-3	0.30±0.01	0.23±0.04				
20:4n-3	0.52±0.03	0.31±0.06		0.21±0.01	0.22±0.02	0.35±0.05
20:5n-3 (EPA)	1.11±0.14	1.05±0.07		0.43±0.01	0.18±0.07	1.11±0.11
22:0	0.19±0.04	0.42±0.03			0.12±0.03	0.26±0.06
22:1	0.10±0.01	0.24±0.04				0.59±0.09
22:4n-6	0.43±0.09	0.35±0.02			0.25±0.05	0.47±0.07
22:5n-6	0.49±0.08	0.59±0.12			0.29±0.02	0.56±0.06
22:5n-3	1.73±0.18	1.37±0.11		0.48±0.03	0.62±0.51	1.83±0.13
22:6n-3 (DHA)	3.37±0.72	2.36±0.27		0.77±0.03	0.86±0.78	1.62±0.77
24:0	0.05±0.05	0.23±0.02				
ΣSaturates	49.16±1.05	55.26±1.60	55.62±0.54	42.63±1.12	55.83±2.81	53.31±1.22
ΣMonoenes	29.45±0.45	28.58±1.33	30.54±0.67	37.45±0.84	30.23±0.69	28.40±5.69
Σn-6	5.89±0.22	5.41±1.27	1.02±0.13	3.23±0.02	3.94±0.94	10.59±0.25
Σn-3	8.99±1.09	7.39±0.85	0.11±0.03	2.77±0.16	3.00±1.67	5.87±1.09
Σn-6/ Σn-3	0.66±0.20	0.73±0.11	9.23±1.80	1.16±0.23	1.32±0.09	1.80±0.13
Σn-3HUFA	7.02±1.05	5.32±0.22	0.00±0.00	1.88±0.05	1.87±1.37	4.90±1.05
ARA/EPA	0.96±0.01	1.35±0.31	0.00±0.00	2.35±0.01	1.74±1.10	2.80±0.85
DHA/EPA	3.01±0.28	2.29±0.41	0.00±0.00	1.81±0.05	3.78±3.05	1.42±0.57
DHA/ARA	3.14±0.26	1.93±0.56	0.00±0.00	0.78±0.03	1.78±0.64	0.49±0.05
LA/LAN	1.55±0.17	1.60±0.19	6.91±0.98	2.23±0.23	2.19±0.08	8.09±5.04

Table 3. Polar lipid fatty acid composition (expressed as percentage of total fatty acids) of wild tropical freshwater fish gonads studied

	Ayungin		African catfish		Tilapia	
	ovary	testis	ovary	testis	ovary	testis
14:0	0.95±0.32	2.62±1.47	0.42±0.08	0.49±0.02	2.14±1.03	0.91±0.37
14:1	0.19±0.08	0.43±0.12			0.46±0.21	0.23±0.03
15:0	0.41±0.17	0.66±0.16	0.38±0.03	0.38±0.03	0.78±0.25	0.33±0.21
16:0	28.76±0.27	33.10±2.74	18.19±1.12	16.60±0.40	28.97±0.57	21.52±2.69
16:1n-7	1.79±0.80	4.65±1.69	2.14±0.30	0.28±0.03	3.95±1.49	1.80±1.15
17:0	0.55±0.04	0.93±0.49	1.80±0.23	0.89±0.11	1.29±0.85	0.92±0.52
16:3n-6	1.15±0.06	1.96±0.21			1.42±0.40	1.12±0.17
16:3n-3	0.21±0.11	0.24±0.03			0.35±0.04	0.35±0.00
18:0	13.50±2.00	15.27±1.95	17.19±0.67	11.47±1.47	12.87±1.68	11.52±0.04
18:1n-9	6.47±1.50	6.26±0.56	18.84±1.17	14.01±0.51	6.70±1.31	8.65±2.74
18:1n-7	1.96±0.17	3.89±0.50	5.98±0.25	6.64±0.36	3.55±1.42	4.09±0.76
18:2n-6 (LA)	1.27±0.22	0.96±0.16	1.63±0.28	2.00±0.01	0.99±0.02	6.87±5.13
18:3n-6	0.21±0.00	0.40±0.06	0.34±0.06	0.38±0.02	0.35±0.20	0.46±0.04
18:3n-3 (LNA)	0.27±0.12	0.39±0.03	0.32±0.04	0.60±0.01	0.39±0.07	0.40±0.16
18:4n-3	0.17±0.09	0.06±0.01			0.13±0.04	0.05±0.02
20:0	0.17±0.12	0.10±0.03		0.36±0.04	0.12±0.05	0.31±0.22
20:1	0.49±0.08	0.96±0.14	0.27±0.01	0.58±0.03	0.60±0.22	1.07±0.43
20:2n-6	0.18±0.05	0.18±0.05	0.44±0.00	0.39±0.01	0.26±0.03	0.94±0.56
20:3n-6	0.76±0.16	0.38±0.12	0.64±0.11	0.49±0.09	0.67±0.24	1.36±0.56
20:4n-6 (ARA)	4.30±0.24	2.59±0.90	7.51±0.87	11.33±0.33	3.82±0.97	7.47±0.41
20:3n-3	0.20±0.04	0.22±0.06	0.21±0.01		0.10±0.07	0.22±0.02
20:4n-3	0.27±0.02	0.25±0.06	0.15±0.04	0.47±0.04	0.28±0.06	0.51±0.34
20:5n-3 (EPA)	2.65±0.71	1.53±0.59	3.84±0.29	2.88±0.12	2.39±1.02	1.78±0.82
22:0	0.71±0.44	0.92±0.39	0.14±0.01	0.53±0.03	0.26±0.15	0.42±0.12
22:1	0.29±0.17	0.21±0.10			0.14±0.02	0.34±0.04
22:4n-6	1.37±0.00	0.78±0.21	1.22±0.05	1.56±0.06	1.12±0.39	1.81±0.19
22:5n-6	2.33±0.40	1.86±0.56	1.45±0.05	3.39±0.12	1.91±0.37	2.11±0.28
22:5n-3	3.64±0.27	2.19±0.16	1.71±0.09	2.41±0.01	2.87±0.25	4.44±1.46
22:6n-3 (DHA)	18.66±2.20	8.16±0.92	7.85±1.29	7.60±0.60	13.27±2.07	9.56±2.94
24:0	0.56±0.37	0.80±0.38	0.11±0.01		0.18±0.13	0.22±0.02
24:1	1.50±1.45	0.96±0.39		0.84±0.17	0.37±0.33	0.55±0.12
ΣSaturates	45.59±2.14	54.40±4.09	38.23±0.09	30.71±0.96	46.59±4.06	36.14±3.67
ΣMonoenes	12.68±0.85	17.36±1.27	27.23±0.63	22.34±0.02	15.74±1.67	16.72±0.45
Σn-6	11.55±0.12	9.11±2.22	13.23±1.22	19.53±0.33	10.51±1.32	22.13±6.37
Σn-3	26.05±1.46	13.04±1.77	14.06±0.97	13.95±0.45	19.77±3.53	17.18±0.04
Σn-6/ Σn-3	0.44±0.03	0.70±0.10	0.94±0.21	1.40±0.13	0.53±0.12	1.29±0.12
Σn-3HUFA	25.41±1.78	12.35±1.74	13.75±0.94	13.35±0.45	18.90±3.46	16.39±0.21
ARA/EPA	1.72±0.37	1.74±0.07	1.95±0.08	3.95±0.28	1.75±0.34	5.47±2.74
DHA/EPA	7.83±2.93	6.48±1.48	2.08±0.49	2.65±0.32	6.35±1.84	7.79±5.23
DHA/ARA	4.39±0.76	3.67±0.74	1.08±0.30	0.67±0.03	3.57±0.36	1.27±0.33
LA/LAN	6.35±3.59	2.40±0.21	5.30±1.44	3.36±0.03	2.62±0.51	26.56±23.45

catabolized by the mitochondrial, *β*-oxidation (Henderson, 1996). Therefore, the high value detected for both C16:0, C18:1 n-9 in all samples reflects a requirement for energy metabolism during the course of gonad development.

Polyunsaturated Fatty Acids (PUFA)

Our data showed higher variation for PUFA profile in NL than that in PL (Table 3). The Lower proportion of PUFA was found in the NL of all gonads samples compared to PL. The primary source of total PUFA found in gonads samples was the highly unsaturated fatty acids (HUFA), namely n-3 fatty acids EPA and DHA. The higher percentages of n-3 HUFA in PL with respect to NL, suggests the importance of these fatty acids in the reproductive processes. The sum of n-3 HUFA in PL of ovaries and testis, being 25.41% and 12.35%, respectively, in the Ayungin and 13.75% and 13.35%, respectively for African catfish and in tilapia, being 18.90% and 16.39%, respectively, were shown to be more than twice that found in the muscles of the same species in our previous studies (Suloma *et al*., 2008). This result emphasized the importance of dietary HUFA for the reproductive processes, which should be kept mind

when developing specific diets for tropical freshwater fish. These results agree with Bell *et al*. (1997) who reported that HUFA levels in eggs and newly hatched larvae from eight species of marine teleost were several folds higher than in the normal body lipids of these fish. Because of the specific role of (n-3) HUFA, especially DHA, in maintaining the structural and functional integrity in cell membranes, especially in the neural cell, the relative percentage of this HUFA is expected to increase during the gonad development stage (Mourente *et al*., 1991). HUFA are also utilized for energy, DHA and EPA are relatively conserved in comparison with MUFA during the gonad development (Henderson *et al*., 1984). Tocher and Sargent (1984) reported 31.4% DHA in Atlantic herring roe and 2 8.6% DHA in cod roe from the total phospholipid fraction. Kaitaranta (1980) also reported average contents of 32.6% and 25.6% of DHA in the PL of whitefish flesh and roe, respectively.

In PL and NL, ARA was the most abundant n-6 PUFA (ranged from 2.59 to 11.33 % and from 0.16 to 3.19%, respectively). ARA is always found more in PL than NL of all the tissues, probably due to its functionality in cell membrane (Alexis and Nengas, 1996; Bessonart *et al*., 1999; Fountoulaki *et al*., 2003; Furuita *et al*., 2003). ARA has similar biologically importance as EPA and DHA and considered as the precursor of several eicosanoids which are produced by the ovarian tissues and play an important role in the ovulation process (Venkatesh *et al*., 1992; Knight *et al*., 1995; Goetz *et al*., 1987; Murdoch *et al*., 1993; Suloma and Ogata, 2011) and cholesterol accumulation in tissues (Norambuena *et al*., 2012). However, EPA plays an important role in the function of eicosanoids derived from ARA as it competes with the enzyme systems producing eicosanoids from ARA, thus exerting a modulating influence over the quantity and efficacy of ARA-derived eicosanoids (Bruce *et al*., 1999).

Our results also demonstrate a relatively low concentration of the other essential PUFA in both PL and NL, Linoleic acid (LA) (ranged from 0.96 to 6.87% and from 0.73 to 3.47%, respectively) and linolenic acid (LNA) (ranged from 0.27 to 0.60% and from 0.11 to 1.03%, respectively), in all gonads samples, which reflect the low level of these fatty acids in the natural food. Moreover, due to capable of freshwater fish to convert these fatty acid to the higher homologues such as EPA, DHA and ARA, the absolute amounts of LA and LNA in the flesh and gonads fish will decrease (Takeuchi *et al*., 1983; Teshima *et al*., 1992).

Fatty Acids Ratios

Balance in the diet of both of n-3 and n-6 which are critical during organogenesis in embryos and larvae is required in the broodstock diet for optimum reproductive success of fish (Acharia *et al*., 2000; Bell *et al*., 1997; Nandi *et al*., 1999). Our results showed that n-3/n-6 ratios of all samples in PL and NL within a narrow range (0.44–1.80), with one exception in NL for African catfish ovary which had (9.23) value. This suggests that a proper balance in the diet of both of these PUFA which are critical during organogenesis in embryos and larvae is required in the broodstock diet for optimum reproductive success of fish (Bell *et al*., 1997). Bell *et al*. (1990) and Bromage (1995) reported that diets with an over high ratio of n-6/n-3 PUFA could exaggerate stress response in fish broodstock leading to cardiac pathologies. The involvement of essential fatty acids in broodstock fish and developing eggs and larvae and their fundamental involvement in stress reactions demands consideration of what constitutes an optimal or even desirable dietary ratio of n-6/ n-3 PUFA in broodstock.

A relatively higher DHA/EPA ratio was obtained in both NL and PL (ranged from 1.42 to 3.78% and from 2.08 to 7.83%, respectively). Similar findings on relative proportions of DHA and EPA have been reported in capelin roe (Henderson *et al*., 1984), and in fish roe in general (Tocher and Sargent, 1984). therefore, DHA must be superior to EPA in the spesiefic diets for the broodstocks of tropical freshwater fish. The same trend was observed for LN/LNA ratio in gonad either in NL and PL (ranged from 1.55 to 8.09% and from 2.40 to 26.56%, respectively). All wild species studied are characterized by high ARA/EPA ratio in PL ranged from 1.72 to 5.47. ARA and EPA, precursors for biosynthesis of eicosanoids (prostaglandins, thromboxanes and leukotrienes) which exercise important functions (Schacky, 2000). Moreover, the resulting ARA-derived eicosanoids have a considerably higher biological activity than the eicosanoids derived from EPA. EPA competes for the prostaglandin synthesis enzyme binding site with ARA and can reduce the production and efficacy of ARA derivatives, and thus exerts a modulating influence over the quantity and efficacy of ARA acidderived eicosanoids (Weber, 1990). It therefore seems that both these fatty acids, ARA and APA are required in sufficient quantities for an increased production of eicosanoids with a consequence of greater response in ovulation. As general it seems to be that n-6 family's play an important role in reproduction process of tropical freshwater fish broodstock more than n-3 fatty acid families. When formulated for the broodstocks under captivity system, the ARA/EPA ratio may be controlled by the LN/LNA ratio in the feeds. Some studies have pointed out the physiological importance of maintaining correct proportions of EPA, ARA and DHA fatty acid in the phospholipids of the cell membrane bilayer (Bruce *et al*., 1999; Sargent *et al*., 1999). According to these studies, which have defined the critical role played by eicosanoids in numerous physiological functions, the possible interactions between their precursors, like ARA and EPA, support the hypothesis

that a suitable ARA, EPA and DHA profile in the diet must be supplied.

Conclusions

From the above results and discussion, it may be concluded that it is necessary to take into consideration not only the individual levels of HUFAs but also the correct ratio among them (ARA/EPA/ DHA) through controlling LA and LNA level and ratio in the diets of tropical freshwater broodstocks. More studies need to be conducted to determine the minimum and maximum value of (ARA/EPA/ DHA) ratio needed for broodstock diets. Moreover, the result showed that ARA in male is more than female especially in neutral lipid with the expetion of Silver perch which has hermaphroditic sex glands. These data may be an indicator to the importance of ARA for reproductive process in male. The study suggest that the PUFA requirement may differ between male and female. Therefore, further work is needed to develop mechanisms by which it can deliver specific diets separately to male and female which may occur naturally.

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