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Fatty Acid and Free Amino Acid Composition of Muscles and Gonads from Wild and Captive Tilapia *Oreochromis niloticus* (L.) (Teleostei: *Perciformes*): An Approach to Development Broodstock Diets

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Abstract : The fatty acid and free amino acids compositions in muscles and gonads between wild and captive Nile tilapia *Oreochromis niloticus* (L.) (Teleostei: *perciformes*) broodstocks were compared to propose some guidelines of the fatty acid and amino acid requirements for developing specific diets of the broodstock. The total levels of ω3 and ω6 fatty acids were generally higher in polar lipids than in neutral lipids for all broodstock samples. In polar lipid, total ω3 fatty acid levels in wild broodstock was higher than that in captive fish, while an opposite trend was observed for ω-6 fatty acid. The results on captive broodstock showed that Linoleic Acid (LA) levels in muscle and gonads were higher compared to wild fish, especially in polar lipid. The LA level was higher than linolenic acid (LAN) level, irrespective of the lipid type and the tissue. Arachidonic acid (20: 4ω6, ARA) level in gonads and muscle, were higher in captive broodstock than the wild. All Essential Amino Acids (EAA) and most Non-Essential Amino Acids (NEAA) in ovaries were significantly higher ($p<0.05$) in wild fish than captive broodstock. Taurine and hydroxylproline recorded the significant higher ($p<0.05$) levels in captive broodstock than that in wild fish. In conclusion, LA and LAN level and the ratio should be considered when formulated diets for tilapia broodstocks are designed.

Key words: Broodstocks, fatty acids, free amino acids, *Nile tilapia*, neutral lipid, polar lipid

INTRODUCTION

Nile tilapia *Oreochromis niloticus* is an economically important species cultured in several areas of the world. It was the first cultured fish species in Egypt (GAFRD, 2005). In tilapia and other fishes culture, nutrition obviously plays an important role in the maintenance of a healthy and marketable product. Identifying appropriate broodstock and fry diets are a key for increasing the aquaculture potential for tilapia. Brood-fish nutrition is considered one of the major factors associated with egg and fry quality. In recent years, efforts have been made to development species-specific broodstock diets. As commercial diets for brood-fish are not formulated especially. Feed manufactures are not interested in formulating diets specifically for brood-fish due to the relatively small size of the market. However, in the case of tilapia, limited knowledge on nutrient requirements of brood-fish constrains the preparation of appropriate rations for brood-fish.

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

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(Sargent *et al*., 1989; Parpoura and Alexis, 2001). Freshwater fish differ significantly from marine fish with respect to fatty acid content and requirements. Guillou *et al*. (1995) revealed that in freshwater fish dietary linoleic acid (18: 2ω6) and linolenic acid (18: 3ω3) or both fatty acids are elongated and desaturated. The former fatty acid converted to arechidonic acid (20: 4ω6) and the latter to docosahexaenoic (22: 6ω3) in phospholipids fraction of fish. Whereas, in triglyceride fraction these fatty acids are deposited unaltered, when increasing the concentration of 18: 2ω6 and 18: 3ω3. However, the fatty acid composition of lipids from tissue and eggs of fish reflects the fatty acid content of the lipid in the diet supplied to the broodstock (Thrush *et al*., 1993; Harel *et al*., 1994; Ferna´ndez-Palacios *et al*., 1995; Rodrý´guez *et al*., 1998). Therefore, the fatty acid composition of tissue and egg lipids in farmed fish can differ from wild fish composition. So to get an optimal tissue and egg fatty acid composition, which relate to spawning quality and reproduction success (Ferna´ndez- Palacios *et al*., 1995), a correct dietary composition must be attained. No such data are available on the fatty acid composition of Nile tilapia. Therefore, information in this respect can be obtained through comparison of corporal fatty acid composition of captive fish with respect to wild fish. Knowledge of specific fatty acids requirements for tilapia broodstock is still rare. Santiago and Reyes (1993) found that cod liver oil (a source of ω3 fatty acid) resulted in highest weight gain associated with poor reproductive performance. Overall, seed production was found to be remarkably high for fish fed with soybean oil (source of 18: 2, ω6 fatty acid). Recently El-Sayed *et al*. (2005) found that Nile tilapia broodstock reared in freshwater could be fed only soybean oil (ω6 fatty acids) to obtain good spawning performance.

The concentration of Free Amino Acids (FAA) in animal tissues is a sensitive tool to determine the adequacy of dietary amino acids and to estimate the amino acid requirement of the animal (Pion, 1976). Since there is a slower rate of protein turnover in muscle than in other organs (Fauconneau, 1985), the influence of dietary amino acid profiles, may be more responsive in other tissues than muscle. Very little is known about the specific amino acid requirements of the brood-fish (Luquet and Watanabe, 1986) and data about free amino acids of freshwater fish ovaries is scare. So the FAA levels in gonads and muscles may be useful to know sufficiently levels of amino acids in practical diets.

The aim of the present study was compared the fatty acid and free amino acids compositions in muscles and gonads between wild and captive tilapia *Oreochromis niloticus* broodstocks to propose some guidelines of the fatty acid and amino acid requirements for developing specific diets of the broodstock.

MATERIALS AND METHODS

Experimental Fish and Samples Technique

Twenty-four wild tilapia *Oreochromis niloticus* broodstocks samples, with an average weight of 557.2±50.70 g were collected during the spawning period (September 2005) from Laguna Lake (freshwater), Philippines. Fish were sacrificed to collect their muscle and ovaries then stored at -80°C until further chemical analysis.

Twenty four captive tilapia samples with an average body weight of 495.50±25.89 g were obtained from ponds of SEAFDEC AQD, Jalajala, Philippines. Captive broodstock were fed on a commercial diet (Fish Meal, Wheat, soybean meal, copra meal, Wheat bran, soybean oil, molasses, salt, limestone, mono-dicalcium phosphate, DL-methionine, L-Lysine, L-threonine, choline, chloride, anti-oxidant, mold inhibitor, vitamin and mineral premix) (Universal Robina Brand Co., Philippines). Selected sample breeders were obtained after fed a commercial broodstock diet for six months (from April to September 2005). Broodstock was fed diet to satiation twice a day at 9.00 h and 15.00 h. The proximate and fatty acid composition profile of commercial tilapia diet are shown in Table 1.

Table 1: Proximate chemical composition and fatty acid (wt. %) content of experimental commercial broodstock diet

Chemical compositions	Percentage
Moisture	13.00
Crude protein	28.00
Total lipid	6.42
Crude fiber	7.00
Ash	10.00
Nitrogen Free Extract (NFE)	35.58
Fatty acids (% of total fatty acids)	
14:0	6.75
16:0	16.57
16:1ω7	1.08
18:0	4.24
18:1ω9	21.86
18:1ω7	ND
18:2ω6	41.84
18:3ω3	5.72
20:0	0.24
20:1	0.29
20:2ω6	0.04
20:3ω6	ND
20:4ω6	0.08
20:5ω3	0.15
22:4ω6	ND
22:5ω6	ND
22:5ω3	ND
22:6ω3	0.09
Σ Saturates	28.27
Σ Monoenes	23.32
Σ ω6	42.02
Σ ω3	6.00
Σ sω-3HUFA	0.28
ARA/EPA	0.53
DHA/EPA	0.60
DHA/ARA	1.13
LA/LAN	7.31

ND: Not determine; HUFA: High polyunsaturated fatty acid; ARA: Arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; LA: linoleic acid; LAN: linolenic acid

Samples of stomach content were collected from 10 wild fish during the same periods to be used as an indictor for fatty acids composition in the natural food fed to wild fish. The chemical compositions of fatty acids profile for stomach natural food content of wild broodstock are shown in Table 2.

Chemical Analysis

All the samples were freeze-dried and pulverized. The dried samples were stored at -80°C until lipid extraction. Total lipid from original tissue 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. Total lipids classes, polar (PL) and neutral lipid (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

Table 2: The gastrointestinal fatty acid composition (wt. %) of experimental wild tilapia broodstock

Fatty acids (% of total fatty acids)	
14:0	2.07±0.28
16:0	28.26±1.00
16:1ω7	5.71±0.73
18:0	16.6±1.03
18:1ω9	9.16±1.10
18:1ω7	5.48±0.33
18:2ω6	4.28±0.37
18:3ω3	3.38±0.21
20:0	0.34±0.05
20:1	0.37±0.01
20:2ω6	0.40±0.05
20:3ω6	0.38±0.03
20:4ω6	3.12±0.39
20:5ω3	0.92±0.18
22:4ω6	0.43±0.04
22:5ω6	0.79±0.21
22:5ω3	0.91±0.09
22:6ω3	4.39±1.03
Total Saturates	50.86±1.87
Total Monoenes	20.68±1.79
Total ω6	10.11±0.67
Total ω3	11.01±1.38
Total ω3HUFA	6.39±1.26
ARA/EPA	3.72±0.45
DHA/EPA	4.85±0.84
DHA/ARA	1.34±0.23
LA/LAN	1.26±0.06

Values are mean±SEM; Annotation with different superscripts indicate statistically significant differenes, ND: Not Determine

320 fused silica capillary column (30 m 0.32 mm i.d.; Supelco, Bellefonte, PA, USA). Helium was used as carrier gas with a 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).

The Free Amino Acids (FAA) in muscles and gonads samples were extracted with a perchloric acid solution according to the method of Ogata and Murai (1994). The FAA levels were determined individually by an automatic amino acid analyzer (L-8500 Hitachi, Japan) with a ninhydrin reagent and lithium buffer system (Yamamoto *et al*., 1998).

Statistical Analysis

Data were statistically analyzed using a one-way analysis of variance ANOVA according to a procedure of SPSS (Version 11.0). Duncan's multiple range test (Duncan,1955) was used when significant ANOVA F values of FAA means were showed to compare differences among individual means at ($p \leq 0.05$) unless otherwise level was obtained. However, significant differences among the means of free amino acids FAA (FAA) were carried out by t-test.

RESULTS

Lipids and Fatty Acid

The present result showed that the total fatty acid contents was extremely higher differences between the commercial broodstock diet and the stomach broodstock contents , specially for higher level of linoleic acid (LA; 18: 2ω6) (Table 1 and 2). The same trend were observed for LAN; 18: 3ω3, Σω6 and LA/LAN ratios , while an opposite trend was showed for total saturates, total ω3, total ω3 HUFA fatty acids and ARA/EPA, DHA/ARA , DHA/ARA ratios fatty acids.

Table 3: Composition of total lipid, neutral lipid* and polar lipid** of muscles and gonads from wild and captive tilapia broodstock

	Muscles			
	Female		Male	
Lipid class (%)	Wild	Captive	Wild	Captive
TL	3.01±0.04	3.18±0.37	2.33±0.30	2.98±0.53
NL	27.6±2.011	25.92±1.38	53.59±32.2	18.89±1.24
PL	72.40±2.01	74.08±1.38	46.42±32.2	81.12±1.24
	Gonad			
	Ovary		Testis	
Lipid class (%)	Wild	Captive	Wild	Captive
TL	38.68±1.93	45.39±2.49	22.57±1.93	13.93±4.43
NL	63.90±1.13	73.73±2.14	20.47±1.18	24.65±1.44
PL	36.10±0.12	26.27±0.98	79.53±4.12	75.36±3.54

*(NL,% of total lipid), **(PL,% of total lipid)

Table 4: Fatty acid compositions of neutral lipid (% of fatty acids) in wild and captive tilapia broodstock muscles

	Captive		Wild	
Fatty acids	Male	Female	Male	Female
14:0	4.35±0.41	4.47±0.66	5.18±0.19	4.32±1.10
16:0	41.10±0.47	36.90±1.22	36.63±0.38	39.15±2.70
16:1ω7	5.58±1.25^{b}	7.10±1.77^{ab}	14.34±0.09^{a}	10.73±0.32^{ab}
18:0	15.23±3.20^{a}	9.88±1.13^{b}	7.85±0.16^{b}	13.82±1.41^{ab}
18:1ω9	21.87±3.60^{ab}	23.75±2.86^{a}	18.14±0.04^{ab}	13.60±1.21^{b}
18:1ω7	4.14±0.32	5.27±0.43^{ab}	4.97±0.03^{ab}	6.03±0.72^{a}
18:2ω6(LA)	0.90±0.09^{b}	3.04±1.13^{a}	1.08±0.05^{ab}	0.7±0.09^{b}
18:3ω3(LAN)	ND	0.27±0.08	0.52±0.08	0.35±0.08
20:0	0.23±0.02	0.46±0.19	0.30±0.08	0.44±0.18
20:1	0.29±0.01	0.39±0.05	0.83±0.05	0.46±0.09
20:2ω6	ND	ND	ND	ND
20:3ω6	ND	ND	ND	ND
20:4ω6(ARA)	2.20±1.81	0.79±0.20	1.09±0.05	0.81±0.01
20:5ω3(EPA)	0.17±0.02	0.42±0.08	1.38±0.09	1.16±0.05
22:4ω6	ND	0.19±0.08	0.28±0.05	0.19±0.09
22:5ω6	ND	0.26±0.08	0.53±0.09	0.37±0.05
22:5ω3	0.20±0.01	0.66±0.03	1.80±0.05	ND
22:6ω3(DHA)	ND	1.08±0.22	3.77±0.01	2.36±0.03
Total saturates	63.06±4.23	53.53±1.42^{bc}	52.12±0.68^{c}	60.75±0.90^{ab}
Total monoenes	31.88±5.15	36.51±2.79	37.98±0.63	30.91±0.36
Total ω6	3.46±2.08	4.31±1.46	2.40±0.93	1.94±0.61
Total ω3	0.36±0.02	1.52±0.80	4.25±3.81	2.87±2.65
Total ω3HUFA	0.36±0.02	1.43±0.73	3.60±3.60	2.48±2.48
ARA/EPA	12.41±9.81	2.49±0.69	0.79±0.03	0.70±0.03
DHA/EPA	ND	2.55±0.03	2.73±0.03	2.03±0.09
DHA/ARA	ND	0.74±0.41	3.46±0.09	2.91±0.05
LN/LNA	ND	12.90±0.03	2.10±0.20	2.40±0.01

Values are mean±SEM; Annotation with different superscripts indicate statistically significant differences; ND: Not determine; HUFA: High polyunsaturated fatty acid; ARA: Arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; LA: linoleic acid; LAN: linolenic acid

Total Lipids Level (TL) in gonads (ranged from 13.93 to 45.39) was extremely higher than that in muscles (ranged from 2.33 to 3.18) irrespective of the source. In muscles, the total and Polar Lipid (PL) contents were higher in captive male (2.98 and 81.12, respectively) than in wild male (2.33 and 46.42, respectively), while wild male had the highest value of neutral lipid (53.59) than captive male (18.89). The same trend was showed for tilapia female. The total and neutral lipid contents in broodstock ovary were showed higher than testes, either wild or captive broodstock, while an opposite trend was observed for polar lipid (Table 3).

Table 5: Fatty acid compositions of polar lipid (%) in wild and captive tilapia broodstock muscles

	Captive tilapia		Wild tilapia	
Fatty acids	Male	Female	Male	Female
14:0	0.52±0.02^{bc}	0.48±0.02^{c}	1.00±0.01^{a}	0.59±0.01^{b}
16:0	21.88±0.69^{b}	21.83±0.17^{b}	24.49±0.01^{a}	23.64±0.49^{a}
16:1ω7	0.61±0.05^{c}	0.89±0.10^{c}	3.01±0.01^{a}	1.81±0.39^{b}
18:0	10.52±0.54	10.45±0.71	8.53±0.05	9.56±0.77
18:1ω9	9.24±0.59^{b}	10.66±0.28^{a}	7.55±0.04^{c}	5.94±0.06^{d}
18:1ω7	2.91±0.17	2.92±0.11	2.86±0.14	3.64±0.46
18:2ω6 (LA)	10.44±0.51^{a}	12.51±1.07^{a}	1.59±0.06^{b}	1.38±0.13^{b}
18:3ω3 (LAN)	0.53±0.26	0.42±0.01	0.71±0.02	0.49±0.01
20:0	0.12±0.03	0.10±0.01	ND	ND
20:1	0.30±0.03	0.25±0.01	ND	ND
20:2ω6	0.80±0.06^{a}	0.63±0.06^{b}	0.19±0.03^{c}	0.19±0.03^{c}
20:3ω6	1.65±0.17^{a}	2.13±0.22^{a}	0.47±0.01^{b}	0.52±0.04^{b}
20:4ω6 (ARA)	10.39±1.20^{ab}	12.91±0.75^{a}	8.44±0.01^{b}	9.81±0.22^{b}
20:5ω3 (EPA)	1.31±0.58^{b}	0.93±0.36^{c}	4.38±0.13^{a}	4.53±0.69^{a}
22:4ω6	1.78±0.42^{ab}	2.01±0.20^{a}	0.95±0.07^{b}	0.93±0.13^{b}
22:5ω6	4.83±0.96	3.99±0.29	5.13±0.14	4.71±0.23
22:5ω3	2.15±0.45^{b}	1.48±0.06^{b}	5.12±0.13^{a}	4.96±0.72^{a}
22:6ω3 (DHA)	11.98±1.83^{ab}	8.29±1.43^{b}	15.85±0.17^{a}	16.57±0.06^{a}
Total saturates	34.17±0.84	33.75±0.82	35.92±0.02	35.55±0.35
Total monoenes	13.82±0.46^{b}	15.52±0.24^{a}	13.42±0.19^{b}	11.39±0.02^{c}
Total ω6	19.94±7.44	16.44±1.97	15.56±1.58	8.75±1.91
Total ω3	16.18±0.43^{b}	11.20±1.87^{b}	26.70±0.11^{a}	27.20±0.12^{a}
Total ω3 HUFA	15.65±0.68^{b}	10.78±1.87^{b}	26.00±0.10^{a}	26.71±0.11^{a}
ARA/EPA	9.37±3.22	18.52±6.20	1.93±0.05	2.21±0.29
DHA/EPA	12.15±6.76	10.42±2.07	3.63±0.15	3.75±0.59
DHA/ARA	1.19±0.31^{bc}	0.66±0.15^{c}	1.88±0.02^{a}	1.69±0.04^{ab}
LN/LNA	25.22±11.2^{a}	29.57±2.34^{a}	2.26±0.13^{b}	2.83±0.31^{b}

Values are mean±SEM. Annotation with different superscripts indicate statistically significant differences. ND: Not determine; HUFA: High polyunsaturated fatty acid; ARA: Arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; LA: linoleic acid; LAN: linolenic acid

Data of fatty acids profiles in lipid fractions for broodstock muscles and gonads are shown in Table 4-7. The fatty acid profile of neutral lipid (Table 4 and 6) generally, showed higher variation than that in polar lipid (Table 5 and 7). Total saturated and monosaturated fatty acid in neutral lipid were higher than those in polar lipid, irrespective of the tissues (gonads or muscles) and the sources (captive and wild). No clear trend was observed between different broodstock sexes. Oleic acid (18: 1ω9) levels in gonads and muscles were significantly higher ($p<0.05$) in captive broodstocks than wild.

The present data showed that compared with other saturated fatty acids , palmitic acid (16:0) recorded the highest significant ($p<0.05$) levels in wild broodstock (ranged from 21.52 to 39.63) and captive broodstock (ranged from 21.83 to 41.10%) and also, in gonads (ranged from 21.52 to 37.18%) and muscles (ranged from 21.83 to 41.10%). Moreover, palmitic acid was higher in the neutral lipid (ranged from 26.30 to 41.10%) than polar lipid (ranged from 21.52 to 28.97%). No significant differences ($p<0.05$) were observed for palmitic acid level in neutral lipid in ovary or testes broodstocks. The same trend was observed for polar lipid.

The ω3 and ω6 fatty acids values were generally higher in polar lipids than in neutral lipids for all broodstock samples. In polar lipid, total ω3 fatty acid levels in wild broodstock was higher than in captive fish, while an opposite trend was observed for ω6 fatty acid. The total level of ω3 in wild broodstock muscle showed the highest value compared to captive fish.

The Linoleic Acid (LA) levels in muscle and gonads for captive broodstock were showed significantly higher ($p<0.05$) compared to wild fish, especially in polar lipid (Table 5 and 7). The LA level was higher than Linolenic Acid (LAN) level, irrespective of the lipid type and the tissue of broodstock. This trend relation was much more prominent in captive broodstock than in wild fish and

Table 6: Fatty acids composition of neutral lipid (% of fatty acids) in wild and captive tilapia broodstock gonads

	Captive		Wild	
Fatty acids	Testis	Ovary	Testis	Ovary
14:0	1.95±0.54^{b}	2.98±0.08^{b}	2.63±0.09^{b}	7.46±2.58^{a}
16:0	26.30±7.60	31.95±1.85	30.46±2.63	37.18±2.04
16:1ω7	6.80±0.90^{b}	5.55±0.18^{b}	5.88±0.73^{b}	13.92±1.99^{a}
18:0	11.11±4.00^{ab}	7.02±0.76^{b}	17.72±0.92^{a}	7.37±1.49^{b}
18:1ω9	35.12±11.5^{a}	28.81±0.48^{ab}	14.15±6.13^{b}	8.61±0.58^{b}
18:1ω7	6.54±0.46^{a}	5.17±0.15^{b}	5.98±0.29^{ab}	6.13±0.48^{ab}
18:2ω6 (LA)	2.42±0.82	6.55±1.86	3.47±1.79	0.91±0.21
18:3ω3 (LAN)	0.17±0.06	0.29±0.14	0.39±0.16	0.42±0.11
20:0	0.31±0.19^{a}	0.18±0.02^{ab}	0.13±0.02^{b}	0.28±0.01^{a}
20:1	1.13±0.08^{a}	1.25±0.11	1.39±0.10	0.76±0.30
20:2ω6	0.15±0.04	0.29±0.07	0.53±0.31	0.11±0.06
20:3ω6	0.11±0.04^{b}	0.21±0.07^{ab}	0.40±0.5^{a}	0.11±0.0^{b}
20:4ω6 (ARA)	0.31±0.11^{b}	0.22±0.09^{b}	3.19±1.24^{a}	0.38±0.31^{b}
20:5ω3 (EPA)	0.15±0.04	0.05±0.01	1.21±0.02	0.18±0.06
22:4ω6	0.12±0.02	0.11±0.04	0.54±0.01	0.20±0.01
22:5ω6	ND	0.12±0.12	0.62±0.02	0.27±0.02
22:5ω3	0.15±0.01	0.18±0.01	1.96±0.04	0.62±0.51
22:6ω3(DHA)	0.21±0.02	0.40±0.23	1.62±0.77	0.86±0.78
Total Saturates	40.81±12.7	43.57±2.64	52.89±0.80	55.83±2.81
Total Monoenes	49.78±11.4^{a}	40.90±0.44^{ab}	27.95±5.24^{b}	30.23±0.70^{ab}
Totalω6	3.37±1.01	7.75±2.22	9.19±1.16	3.64±1.24
Totalω3	0.44±0.33	0.71±0.45	3.98±3.13	2.90±1.77
Totalω3HUFA	0.27±0.27	0.42±0.31	3.40±2.55	1.77±1.47
ARA/EPA	2.73±0.02	7.80±0.01	3.65±0.04	1.74±1.09
DHA/EPA	1.40±0.01	12.60±0.04	1.98±0.02	3.78±3.05
DHA/ARA	0.26±0.26	0.89±0.48	0.49±0.05	1.7±0.64
LN/LNA	14.64±0.09^{ab}	27.31±4.67^{a}	12.94±9.89^{ab}	2.19±0.08^{b}

Valuesare mean±SEM; Annotation with different superscripts indicate statistically significant differences; ND: Not determine; HUFA: High polyunsaturated fatty acid; ARA: Arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; LA: linoleic acid; LAN: linolenic acid

Table 7: Fatty acids composition of polar lipid (% of fatty acids) in wild and captive tilapia broodstock gonads

	Captive		Wild	
Fatty acids	Testis	Ovary	Testis	Ovary
14:0	0.87±0.35	1.51±0.14	0.91±0.37	2.14±1.03
16:0	24.95±6.80	24.58±1.84	21.52±2.69	28.97±0.57
16:1ω7	1.75±0.91	2.19±0.29	1.80±1.15	3.95±1.49
18:0	13.49±1.34^{ab}	16.82±1.27^{a}	11.52±0.04^{b}	12.87±1.68^{ab}
18:1ω9	11.44±2.09	13.39±1.77	8.65±2.74	6.70±1.31
18:1ω7	4.61±1.75	4.25±0.32	4.09±0.76	3.55±1.42
18:2ω6 (LA)	7.38±1.39^{a}	7.70±0.80^{a}	1.88±0.14^{b}	0.99±0.02b
18:3ω3 (LAN)	0.34±0.04	0.22±0.03	0.40±0.16	0.39±0.07
20:0	0.75±0.41	0.21±0.01	0.31±0.22	0.12±0.05
20:1	0.69±0.19	0.91±0.10	1.07±0.43	0.60±0.22
20:2ω6	1.12±0.84	0.68±0.07	0.94±0.56	0.26±0.03
20:3ω6	1.57±0.91	1.27±0.21	1.36±0.56	0.67±0.24
20:4ω6 (ARA)	6.51±1.05^{a}	4.14±0.51^{b}	7.47±0.41^{a}	3.82±0.97^{b}
20:5ω3 (EPA)	0.85±0.38	0.31±0.05	1.78±0.82	2.39±1.02
22:4ω6	1.70±0.74	1.13±0.14	1.81±0.19	1.12±0.39
22:5ω6	1.67±0.80	1.52±0.30	2.11±0.28	1.91±0.37
22:5ω3	3.06±0.97^{ab}	1.42±0.23^{b}	4.44±1.46^{a}	2.87±0.25^{b}
22:6ω3 (DHA)	7.97±5.15	11.81±1.91	9.56±2.94	13.27±2.07
TotalSaturates	42.24±9.53	43.90±1.53	36.04±3.77	46.59±4.06
TotalMonoenes	18.91±5.36	20.90±2.60	16.47±0.20	15.74±1.67
Totalω6	21.59±1.67^{b}	35.24±2.77^{a}	5.75±2.64^{c}	2.62±0.51^{c}
Totalω3	12.21±6.53	13.75±2.19	16.17±0.51	18.91±3.40
Totalω3HUFA	11.87±6.49	13.53±2.18	15.77±0.67	18.52±3.33
ARA/EPA	7.72±0.18^{b}	13.64±0.57^{a}	5.47±2.74^{bc}	1.75±0.34^{c}
DHA/EPA	8.31±2.33^{b}	38.49±1.38^{a}	7.79±5.23^{b}	6.35±1.84^{b}
DHA/ARA	1.09±0.33^{b}	2.83±0.10^{a}	1.27±0.33^{b}	3.57±0.36^{a}
LN/LNA	21.59±1.67^{b}	35.24±2.77^{a}	5.75±2.64^{c}	2.62±0.51^{c}

Values are mean±SEM; Annotation with different superscripts indicate statistically significant differences; ND: Not determine; HUFA: High polyunsaturated fatty acid; ARA: Arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; LA: linoleic acid; LAN: linolenic acid

Table 8: Free amino acid of ovary and muscle of captive and wild tilapia broodstock

	Ovary			Muscles		
Free amino acids	Captive tilapia	Wild tilapia	t-test	Captive tilapia	Wild tilapia	t-test
Essential amino acids						
Arginine	0.36±0.06	2.14±0.14	**	0.24±0.07	0.2±0.05	NS
Histidine	0.095±0.03	0.66±0.05	*	0.575±0.06	0.675±0.07	NS
Isoleucine	0.275±0.06	1.49±0.04	**	0.035±0.01	0.085±0.01	*
Leucine	0.31±0.09	2.22±0.13	**	0.085±0.01	0.155±0.02	**
Lysine	0.21±0.05	1.785±0.21	*	0.305±0.08	0.315±0.06	NS
Methionine	0.045±0.01	0.23±0.03	*	0.03±0.01	0.045±0.01	NS
Phenylalanine	0.135±0.04	1.35±0.04	**	0.08±0.00	0.13±0.01	*
Threonine	0.285±0.06	1.47±0.16	*	0.31±0.04	0.335±0.08	NS
Tryptophan	ND	0.14±0.01		ND	ND	NS
Valine	0.335±0.06	1.48±0.01	**	0.1± 0.01	0.175±0.02	NS
Non-essential						
Phosphoserine	0.155±0.03	0.43±0.0	**	ND	ND	
Taurine	1.565±0.03	2.5±0.54	NS	16.81± 0.15	8.24±0.53	**
Phosphoethanolamine	0.21±0.02	0.185±0.04	NS	ND	ND	
Aspartic acid	0.32±0.02	1.365±0.24	*	0.075±0.02	0.1±0.03	NS
Serine	0.335±0.08	0.965±0.13	NS	0.195±0.02	0.255±0.03	NS
Asparagine	0.175±0.06	0.925±0.06	*	ND	ND	
Glutamic acid	0.78±0.10	2.8±0.35	*	0.555±0.08	1.53±0.26	NS
Glutamine	0.235±0.06	0.765±0.03	*	0.395±0.04	0.23±0.04	NS
Sarcosine	0.055±0.02	0.045±0.01	NS	0.03±0.0	ND	
á- aminoadipic acid	0.05 ±0.00	0.19±0.0	NS	ND	0.03±0.01	
Glycine	0.16 ±0.02	1.16±0.14	*	4.88±0.89	10.115±0.83	NS
Alanine	0.525±0.18	1.795±0.18	*	1.44±0.04	1.425±0.29	NS
Citrulline	0.045±0.02	0.11±0.02	NS	ND	0.01±0.01	
â-Alanine	0.035±0.03	ND		0.035±0.01	0.035±0.01	NS
Tyrosine	0.16±0.04	1.335±0.01	**	0.065±0.02	0.115±0.01	NS
ã-Aminobutyric acid	0.005±0.01	0.025±0.03	NS	0.005±0.01	0.015±0.01	NS
Ethanolamine	0.07±0.00	0.04±0.00	NS	0.01±0.00	0.005±0.01	NS
Ammonia	0.11±0.00	0.22±0.02	*	0.39±0.12	0.505±0.08	NS
Ornithine	0.015±0.01	0.305±0.23	NS	0.265±0.02	0.285±0.18	NS
Carnosine	ND	0.11±0.00		0.055±0.06	0.035±0.04	NS
Hydroxyproline	ND	ND		0.23±0.02	0.12±0.0	*
Proline	0.215±0.02	0.975±0.09	*	0.26±0.04	1.125±0.86	NS
TNRS	7.32±1.56	29.55±3.92	*	27.45±1.01	26.28±0.89	NS
TNEAA	2.74±0.73	10.76 ±1.51	*	8.03 ±1.51	14.90 ±1.54	*
EAA+T+C	2.26±0.69	14.59 ±1.22	**	1.83±0.18	2.22±0.18	NS
EAA/NEAA	0.82	1.36		0.23	0.15	

Values are mean±SEM. *p<0.05, **p<0.01. -EAA: Essential amino acid; EAA+C+T: total amount of 10 essential amino acids, cystine and tyrosine; NEAA: Total of non-essential amino acids including hydroxyproline. ND: Not determine

in polar lipid than in neutral lipid. Moreover, in broodstock gonads and muscle, arachidonic acid (20: 4ω6, ARA) level was showed higher in captive fish than the wild fish except for polar and neutral lipid in wild tilapia testes. The opposite trend was observed for both eicosapentaenoic acid (20: 5ω3, EPA) and docosahexaenoic acid (22: 6ω3, DHA). In polar lipid, the fatty acids ARA, EPA and DHA were higher than that in neutral lipid. The ARA/EPA ratio was showed paralleled with the same trend of ARA level, while LA/LAN ratio was showed paralleled with the same trend of LA level.

Free Amino Acids (FAA)

The Free Amino Acid (FAA) content of Nile tilapia broodstock are shown in Table 8. Generally, all Essential Amino Acids (EAA) and most non-essential amino acids (NEAA) in ovaries were significantly higher ($p<0.05$) in wild fish than that in captive broodstock. The total EAA levels in ovary of the wild tilapia (14.59%) were significantly higher ($p<0.01$) than the farmed fish (2.26%). In

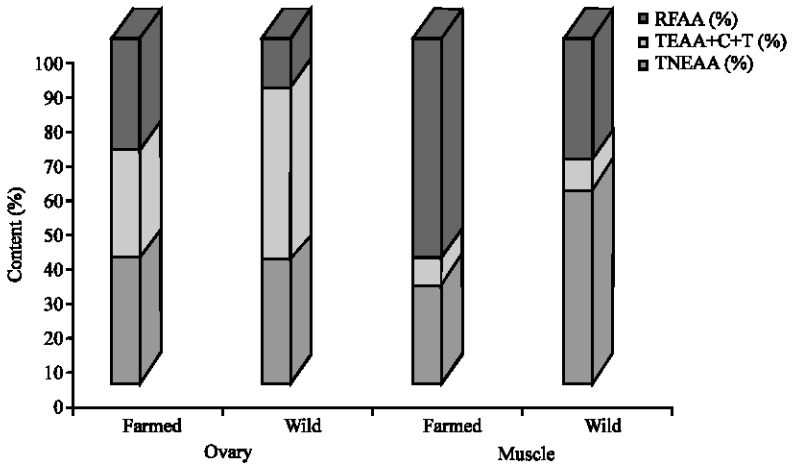

Fig. 1: Effect of dietary amino acid on free amino acid content (%) of cascass Nile tilapia wild and capitive

ovary the TNEAA and trinucleotide repeats (TNRS) levels (10.76 and 29.55%, respectively) were significantly higher ($p<0.05$) in wild broodstock than that in captive muscles (2.74 and 7.32, respectively).

The isoleucine and leucine as branched-chain amino acids were significant higher ($p<0.05$) levels in wild than in captive broodstocks for both muscles and ovaries. Taurine and hydroxylproline recorded the significant higher ($p<0.05$) levels in broodstock captive than wild fish.

Data of free amino acid profile in Fig. 1 showed that, the essential free amino acids were higher significant ($p<0.05$) in gonads compared to muscles broodstock. In gonads, the total level of free essential amino acid was higher in wild broodstock (49.56%) than that in captive (30.51%), meanwhile in muscles the total level of non-essential free amino acids were showed higher in wild broodstock (56.62%) than in captive fish (29.16%).

DISCUSSION

Chancing in Fatty Acid Composition of Muscles and Gonads

In the present study, the fatty acid composition of Total Lipid (TL), Neutral Lipid (NL) and Polar Lipid (PL) in mature gonads and muscles from wild and captive tilapia broodstocks were examined. The results were compared with the fatty acid profile of commercial broodstocks diet and profile for stomach natural food content of wild broodstocks. The higher neutral lipid (NL) level observed in ovaries than in testis (Table 6), revealed the importance of this lipid class in female gonads and eggs. Generally, NL is considered as reserve lipid and does not necessarily imply as energy reserve. Neutral lipid would probably play a double role: first by storing large amounts of saturated and monounsaturated fatty acid for energy purposes and second, act as a temporary reservoir of important physiological polyunsaturated fatty acid (Tocher *et al*., 1985; Napolitano *et al*., 1988). The data agree with Ackman (1980) who reported that polar lipid fraction was content lower saturated fatty acids values and much lower monounsaturated fatty acid values than the neutral lipid. Ackman and Eaton (1976) reported that palmitic acid was key metabolite in fish and that its level was not influenced by diet. When tissues lipid were contained high levels of fatty acids 16: 0 and 18: 1ω9 indicates that these fatty acids are not only the main source of energy in these tissues, but also, together with ω3 PUFA,

the primary fatty acids selectively incorporated into membrane phospholipids (Ibeas *et al*., 1996). In the present study the neutral lipid fraction palmitic acid was showed higher in ovary more than testes, which may be indicator for female need more supplying of energy requirements for reproductive more than male. Ostaszewska (2005) reported that the C16: 0, C18: 1 ω9, C20: 1 ω9 and C22: 1 ω11 fatty acids are mainly catabolic for energetic purposes. High amounts of all these acids are consumed during fish growth and development and they are easily catabolic by the mitochondrial, β-oxidation (Henderson, 1996).

Differences in fatty acid composition of gonads between captive and wild broodstocks have been attributed to the sources of broodstock diet. As general it seems to be that ω6 family's play an important role in reproduction process of tilapia broodstock more than ω3 fatty acid families. Scare data are available for ω3 and ω6 levels and ratios for broodstock gonad. In gonads, the total ω6 fatty acid families in polar lipid showed greater for captive broodstock than that of wild fish. A same trend was observed for LN/LAN ratios in gonad either neutral or polar lipids. Moreover, higher ARA/ EPA ratio was recorded in polar lipid of captive broodstock gonads compared to wild fish. These differences in fatty acid composition are probably due to the different levels of LN, LAN , EPA and ARA in wild natural food with respect to captive broodstock diet (Table 1 and 2). However our results agree with the finding of Ross (2003) who reported that polyunsaturated fatty acids, in neutral lipids and polar lipids were higher in the ovaries of wild fish compared to ovaries of tropical red snapper *Lutjanus campechanus* fed complete diet.

In muscles, the fatty acid composition of broodstock was clearly influenced by their sources of diet. The LA and LAN levels and their ratios in captive broodstock fed commercial diet were the major influences, directly on the same captive broodstock fatty acid muscles levels. The same trend was observed for ARA, EPA and DHA levels and its ratios. These observations agree with the fact that freshwater fish had ability to convert dietary 18:3 ω3 and 18:2ω6 to their longer-chain HUFA products, including 20:5ω3, 22:6ω3 and 20:4ω6. Freshwater fish have higher levels of ω6 fatty acids than the marine species (NRC, 1993). However, fatty acid composition of body lipids is most clearly reflected by the dietary lipids. Stickney and McGeachin (1984) and Stickney and Hardy (1989) reported that even higher amounts of ω6 fatty acids were needed when *T. aurea* fingerlings were reared with various diets, but they also observed that this requirement could be reduced when ω3 fatty acids were present. Huang *et al*. (1998) found that the growth rates of hybrid tilapia fed soybean oil and fish oil were similar and were both better than that of fish fed lard or HUFA. They also suggested that ω3 HUFA, such as linolenic, EPA and DHA are important for these fish. In contrast to the earlier findings with tilapia, studies by Chou and Shiau (1996) and Chou *et al*. (2001) have demonstrated that hybrid tilapia, in addition to their requirement for LA, have a requirement for EPA and/or DHA. Recently, El-Sayed *et al*. (2005) found that Nile tilapia broodstock reared in freshwater can be fed only soybean oil (ω6 fatty acids) to obtain good spawning performance, while broodstock reared at salinity rate of 7 and 14‰ would need an exogenous source of dietary ω3 HUFA. The data agreed with the finding of Cejas *et al*. (2003) who reported that the higher percentages of DHA, EPA and ARA in PL with respect to NL, where there is a predominance of saturated and monounsaturated fatty acids, suggest the importance of polyunsaturated fatty acids as components of membrane phospholipids. Arachidonic acid was more concentrated in the Polar Lipid (PL) fractions than in the Neutral Lipid fractions (NL) for gonad. ARA has been described as one of the main components of certain phospholipids, specifically phosphatidylinositol (Bell *et al*., 1997; Bruce *et al*., 1999). Arachidonic acid is always found more in polar lipids than neutral lipids 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). Thus, any elevation in ARA will seems to affect polar lipids more than neutral lipids fraction. Therefore, it is necessary to take into consideration not only the individual levels of these fatty acids but also the correct ratio among them (AA/EPA/ DHA) through control LA and LAN level and ratio in the diets of tilapia broodstocks.

Moreover, the result showed that Arachidonic acid in male is more than female especially in neutral lipid. Those data may be indicator for more important for reproductive process on male. This may imply that this class of fatty acid is important in testis function and previous studies have shown the steroidogenic effects of arachidonic acid and its metabolites in fish (Wade *et al*., 1994).

Despite the lack of growth effects when using vegetable oils to replace fish oils in fish diets, fillet fatty acid profile is known to be markedly influenced by dietary fatty acid compositions. The use of vegetable oils (lacking in EPA and DHA) in tilapia feeds will decrease the concentrations of beneficial omega-3 HUFA in fish fillets destined for the human consumer. EPA and DHA are known to provide positive health benefits such as decreasing the risks of degenerative diseases such as cardiovascular diseases, cancer and many others. Ng and Chang (2004) showed that the fatty acid composition of fillets of hybrid tilapia raised to marketable size (after 5 months) reflected that of dietary oils used with a marked decrease in omega-3 HUFAs in fish fed 100% added soybean oil (SBO) or Crude Palm Oil (CPO). Reduction in omega-3 HUFA in the fillets of tilapia fed diets with CPO blended with Fish Oil (FO) or Linseed Oil (LSO) were not as drastic.

Chancing in Free Amino Acid Composition of Muscles and Gonads

It's well known that the whole body amino acid composition of fish fed dietary compositions did not show marked differences. This is, in a way, expected because body proteins are synthesized based on the genetic information of DNA, so that amino acid composition of specific body proteins is the same irrespective of dietary protein levels. On other hand tissue free amino acids, is the result of dietary amino acid supply and metabolism of protein and amino acids within the body, so that it does not directly reflect the amino acid pattern of the diet (Pion, 1976; Cowey and Walton 1989). The influence of dietary protein levels on tissue FAA levels has been examined in rainbow trout (Cowey *et al*., 1977; Ogata *et al*., 1985; Yokoyama and Nakazoe, 1991; Ogata and Murai 1994; Yokoyama *et al*., 1994). These studies have shown, in general, that the tissue EAA levels tended to increase as the dietary protein level increased.

Determination of protein requirements in animals depend in the maximum economically performance, while in wild there is no limit for protein level because its dependence on the availability of food in the around environment which are almost had highly protein content. The previews facts explain why the ovary of the wild fish had highly significant level of essential and non-essential free amino acid compared to farmed gonad samples.

The low level of taurine in present study of gonad broodstock compared to muscle confirm the fact that, taurine has not been recorded in the Free Amino Acid (FAA) pool in the eggs and/or pre-feeding larvae of freshwater species that have been investigated, for example, rainbow trout (Zeitoun *et al*., 1977), coregonid species (Dabrowski *et al*., 1985), Nile tilapia (Gunasekera *et al*., 1996). However, taurine is found in significant quantities in marine species throughout development (Conçeicão, 1997). Also the finding of taurine in high percent in muscle in both wild and farmed samples agree with the suggestion that taurine is synthesized by larval stages from precursors to taurine present in micro-algae feeds and its absence in adult suggest that this acid is a dietary essential for adults (Welborn and Manahan, 1995).

A high correlation between NEAA patterns in the diet and tissues have not been reported previously except for Atlantic cod plasma in the study of (Lyndon *et al*., 1993). These findings may clear, that not only the amino acid content of a diet, but also the amino acid balance in the diet (which reflects the quality of broodstock nutrition strongly) can affect the tissue free EAA.

From nutrition standing point of view, the present result has raised many important issues for tilapia broodstocks farming:

- It is widely accepted that fatty acid composition of fish tissues reflects dietary fatty acid composition.

- The dietary precursors 18: 3ω3 and 18: 2ω6 PUFA must now be considered as well as end-product of 20: 5ω3, 22: 6ω3 and 20: 4ω6.
- It is also obvious that ARA level in tissues including gonads is affected by dietary LA level.
- The importance of the dietary ratio of ARA: EPA for determining eicosanoids actions.
- The tissue free amino acids, is the result of dietary amino acid supply.
- Further experiments are necessary to determine the correct profile of fatty acids requirements in the diet of Nile tilapia broodstocks. More research is still needed to understand the activities of the various desaturation and elongation fatty acids pathways in tilapia.

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