Fatty Acid Profile of Nile and Red Hybrid Tilapias Reared in Intensive and Extensive Systems

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Abstract

Long chain n-3 and n-6 polyunsaturated fatty acids which are present in many marine fish have long been found to confer beneficial effects on human health. As the world fish stock declines due to over fishing and depletion in the quality of marine environment, fish proteins are increasingly being sourced from fish farms using many water bodies. Information on fatty acid profile of farmed fish especially from aquaculture industry aids consumers to make pertinent dietary decisions. A study was conducted to examine the fatty acid composition of Nile and Red tilapia harvested from intensive culture system. Twenty two samples of Nile tilapia and 16 samples of Red tilapia cultured intensively were used in this study. All samples were subjected to total fatty acid extraction and their fatty acid composition was determined using gas liquid chromatography. Results showed that no significant difference in the total concentration of saturated fatty acids as a percentage of total fatty acids present between intensively (46.14%) and extensively (48.13%) cultured Nile tilapias and between Nile (46.14%) and Red tilapias (46.42%) reared in the intensive system. For monounsaturated fatty acids, the total concentration was lower ($p<0.05$) in extensively (19.76%) cultured compared with intensively (31.17%) cultured Nile tilapias. For n-3 polyunsaturated fatty acids, the concentration was higher ($p<0.05$) in tilapias from extensive system (19.57%) compared with those from intensive system (8.14%). But n-6 polyunsaturated fatty acids concentration of intensively cultured Nile (14.35%) and Red (16.32%) tilapias were higher ($p<0.05$) compared with extensively cultured tilapias (12.55%). For n-6:n-3 polyunsaturated fatty acids ratio intensively cultured Nile tilapias had higher ($p<0.05$, 1.88 to 2.24) than extensively cultured Nile tilapias (0.74). Different concentration of fatty acid composition of Nile tilapias cultured in the different systems could be due to the different nutrient composition of the feed consumed by these fish. Manipulation of the sources of poly unsaturated fatty acids in the feed of farmed tilapias could be a novel approach to enhance the n-6:n-3 polyunsaturated fatty acids ratio of intensively cultured tilapias.

Keywords: Tilapias, culture system, polyunsaturated fatty acids

Introduction

Tilapias are mainly lacustrine fish and well adapted to enclosed water. They are fast- growing, resistant to disease, easy to reproduce in captivity and able to tolerate a wide range of environmental conditions. They are widely cultured in tropical and subtropical regions of the world and constitute the third largest group of farmed finfish, with an annual production growth rate of about 11.5% globally (El-Sayyed, 1999). Tilapia aquaculture is rapidly expanding with a global production of about 2.8 million metric tons in 2008 and estimated to increase to 8.9 million metric tons by the year 2020 (Teoh *et al.*, 2011).

The rapid growth in tilapia production globally is mainly due to increasing use of commercial pelleted feeds and the introduction of improved strains of Nile tilapia (*Oreochromis niloticus*) which is the major farmed tilapia species. Nile tilapia is also the ninth most important species of *Oreochromis spp* produced in the world. The Genetically Improved Farmed Tilapia (GIFT) strain developed by the World Fish Center is one of the more successfully introduced farmed Nile tilapias. Despite the improved growth performance of the GIFT strain, the Red hybrid tilapia, a cross between *Oreochromis mossambicus x Oreochromis niloticus,* is still the dominant species farmed in Malaysia due to its preferred red coloration by local consumers (Teoh *et al.,* 2011) and increased marketability (Karapanagiotidis *et al.,* 2006).

Fish and marine mammals are among the richest sources of long–chain n-3 polyunsaturated fatty acids (PUFA) in nature. At present, fish products comprise an important part of the human diet, and their demand is expected to increase. Supply from marine source is declining due to uncontrolled harvesting, deterioration of habitat and reduction in fish stock, the effect of these is the inadequate supply from marine fish to meet consumer demand. Hence, the aquaculture has a significant role in ensuring fish supplies. Aquaculture is farming of aquatic organisms, including fish, mollusks, crustaceans and aquatic plants. Farming implies some form intervention in the rearing process to enhance production, such as regular stocking, feeding, breeding and protection from predator.

As farmed fish becomes a major contributor to world fish supplies, it is important to maintain the high lipid nutritional quality of the product and to continue to provide large amounts of the health-promoting n-3 PUFA for the consumers. Malaysians are now becoming more aware of the benefits of health food having high nutritional value. Studies showed that fish cultured in intensive system were characterized by increased fat deposition of mainly saturated and monosaturated fatty acid and 18:2 n-6 but fish reared in the extensive system benefited with higher proportions of 18:3 n-3, 20:5 n-3 and 22:6 n-3, higher n-3/n-6 PUFA ratios, and lower proportions of 18:2 n-6 which is the favorable fatty acid for human health. Therefore, a study was conducted to examine the nutritional content, mainly fatty acid composition, of different species of tilapia: black Nile tilapia (*Oreochromis niloticus*) and Red hybrid tilapia (*Oreochromis mossambicus x Oreochromis niloticus*) derived from intensive and extensive culture systems.

Materials and Methods

A total of 50 samples of tilapia fish were included in this study: 30 samples of Nile tilapias (*Oreochromis niloticus*) and 20 samples of Red hybrid tilapias (*Oreochromis mossambicus x Oreochromis niloticus*). The origin and sample statistics of the tilapia samples are presented in Table 1.

Table 1. Origin and mean body weight of tilapias sampled

Species	Culture systems	n	Mean (g)	SD	Range (g)
Nile tilapia	Extensive	10	225	48	200-300
	Intensive	22	407	51	350-450
Red hybrid tilapia	Intensive	16	417	43	350-450

For the present study 40 samples of tilapias from an intensive system were purchased from the Pasar Borong Selangor, that originated from farms that practised the intensive culture system, where the tilapias fed with commercial diets and harvested at about 4 months of age at average body weight of 350- 450 g. Another 10 samples of tilapias from an extensive system were obtained from a ex-mining pool, where the tilapias fed on natural foods found in the water body and the average body weight was 200-300 g. All factors during capture were not controlled and assessed. All tilapias were filleted at the left dorsal cranial part of the body and shaped into standard size of 5cm (long) x 3 cm (wide) x 1 cm (thick). Fillets were wrapped with the aluminum foil and stored at -4 ^{0}C until the samples were used. Fillets were cut and 1-g samples from the middle portion were taken for the total lipid extraction and placed in individual 50 ml Duran beakers.

Total lipid extraction (TLE)

Total fatty acids were extracted from fish meat samples using the chloroform-methanol 2:2 (v/v) solvent system according to the method of Folch *et al.* (1957) and modified by Rajion *et al.* (1985). Forty mℓ of chloroform – methanol (2:1, v/v) were added to 1.0 gram of minced meat sample in a 40-mℓ stoppered tube. The mixture was homogenized, shaken, flushed with nitrogen and sealed. The mixture was left to stand for at least twelve hours before been filtered through a No.1 Whatman filter paper into a separating flask. Five mℓ of chloroform-methanol (2:1, v/v) was used to wash the lipid residue on the tube and the filter paper. Ten mℓ of 0.9% NaCℓ was then added into a separating flask. The mixture was shaken vigorously for 1 minute and was left to stand for at least four hours. This procedure removed non-lipid contaminants which would be retained in the aqueous upper phase and the lower layer purified lipid (Rajion *et al*., 1985). After complete separation, the bottom phase was collected into a round-bottomed flask and evaporated by rotary evaporation at 70-75^{o}C.

The extracted lipids were then washed with 5 mℓ of chloroform-methanol (2:1, v/v) and transferred into methylation tubes with stoppers. One hundred µl of heneicosanoic acid ($C_{21:0}$) were added into the methylating tube as an Internal Standard (ISTD) prior to methylation.

Preparation of fatty acid methyl esters (FAME)

The lipid extract was dried under nitrogen stream. Two mℓ of 0.66n potassium hydroxide (KOH)/ methanol were added to the dry lipid extract and the tube was capped tightly with Teflon-lined caps. . The mixture was heated in a boiling water

bath (100^{o}C) for 10 min with occasional shaking, and then allowed to cool down at room temperature for 5 min.

Two mℓ of 14% boron triflouride (BF_3)/methanol were then added. The boron triflouride (BF_3)/methanol were used as a transesterification catalyst and in particular as a rapid esterifying reagent for free fatty acids (Christie, 1982). The mixture was capped and reheated in water bath for 20 min with occasional shaking. It was then cooled at room temperature. After the mixture had cooled down, 4mℓ of distilled deionised water was added to deactivate BF3 followed by adding 4mℓ of petroleum ether as fatty acid methyl esters (FAME) carrier. The mixture was vortexed for 1 min to ensure proper mixing and centrifuged with 3000 rpm for 10 min. The upper petroleum phase was transferred into a test tube and washed with 1mℓ of distilled water. The petroleum ether fraction was then transferred into another test tube with 0.5g of anhydrous sodium sulphate. In the final step, the petroleum ether containing FAME was transferred into a 4mℓ storage vial and stored at 4^{0}C until analysis by gas liquid chromatography.

Gas-liquid chromatography (GLC)

The methyl esters were quantified using gas chromatograph GC (Agilent 7890N) on a 100m x 0.25mm ID (0.20 μm film thickness) Supelco SP- 2560 capillary column (Supelco, Inc., Bellefonte, PA, USA). One microlitre of the sample was injected using an auto sample into the chromatograph, equipped with a flame ionization detector (FID) at a split ratio of (10:1). High purity nitrogen (99.999% from an atmospheric nitrogen gas generator) was the carrier gas at 40mℓ/min. High purity hydrogen (Dominick Hunter, Parker Hannifin ltd, UK) and compressed air (Malaysian Oxygen Bhd., Malaysia) were used to flame ionization detector in the gas-liquid chromatography. The injector temperature was programmed at 250^{0}C and the detector temperature was 300^{0}C. The column temperature programmed included an initial temperature of 150^{0}C held for 2 min, and then warmed to 158^{0}C at 1^{0}C/min which was then held at this temperature for 28 min, finally the column was warmed to 220^{0}C at 1^{0}C/ min, and maintained for 20 min to achieve the optimum separation. The peaks of samples were identified and concentrations calculated based on the retention time and peak area of known standards (Sigma Chemical Co., St. Louis, Missouri, USA). The fatty acid concentrations were expressed as the percentage of total fatty acids by weight of the total fatty acid content measured in each sample. Automatic expression of the peak areas as percentage of a detected fatty acid was obtained with a programmed PC under Microsoft Excel 2000 (Microsoft Corp., Redmond, USA).

Statistical analysis

Data of fatty acid profile of Tilapia fillets grouped by culture systems and species were analyzed for mean difference between groups using independent sample t-test, using SAS package version 19.0.

Results and Discussion

The fatty acid profiles of Nile tilapia in extensive and intensive culture systems and between species, Nile and Red hybrid tilapias, are presented in Table 2. The percentage of total saturated fatty acids (SFAs) showed non significant difference between tilapias raised in extensive and intensive culture systems. Significant differences between Nile and Red tilapias raised in the intensive culture system also were not detected. The result revealed that

the total SFAs were 48.13% for , 46.14% and 46.42% for Nile tilapia raised in the extensive culture system, Nile tilapia in the intensive culture system and Red hybrid tilapia in the intensive culture system, respectively, with palmatic acid (16:0) was the primary saturated fatty acids in all samples, followed by stearic acid (18:0). In addition, the result revealed that extensive Nile tilapias contained significantly higher proportion of 15:1, 18:0, 24:0 ($p< 0.05$) than intensive Nile tilapias, and for 14:0 showing significantly higher proportion ($p< 0.05$) in intensive Nile tilapias compared with extensive Nile tilapias. On the other hand, Nile tilapias reared in intensive system had significantly higher 14:0 than Nile tilapias from the extensive system and significantly higher proportion of 24:0 in Red tilapias rather than Nile tilapias when reared in the intensive system.

Table 2: Fatty acid composition of tilapias in different species and culture systems (expressed as a percentage of the total fatty acids present)

Fatty Acids	Culture System		
	Intensive		Extensive
	Nile tilapia[1]	Red hybrid tilapia[1]	Nile tilapia[1]
14:0	4.48 ± 0.47[a]	2.54 ± 0.17[b]	3.02 ± 0.48[b]
15:0	0.46 ± 0.10[a]	0.47 ± 0.06[a]	0.53 ± 0.06[a]
15:1	0.41 ± 0.05[b]	0.60 ± 0.06[b]	1.08 ± 0.18[a]
16:0	23.31 ± 0.49[a]	24.74 ± 0.85[a]	24.92 ± 0.75[a]
17:0	7.03 ± 0.84[a]	9.95 ± 1.36[a]	8.69 ± 1.19[a]
18:0	7.47 ± 0.42[b]	6.72 ± 0.27[b]	10.82 ± 0.64[a]
20:0	0.84 ± 0.08[a]	0.77 ± 0.13[a]	0.68 ± 0.10[a]
22.0	0.96 ± 0.09[a]	1.25 ± 0.13[a]	0.99 ± 0.10[a]
24:0	1.13 ± 0.22[b]	2.18 ± 0.20[a]	2.75 ± 0.42[a]
18:1 n-9	29.45 ± 1.28[a]	26.47 ± 1.41[a]	15.93 ± 2.46[b]
24:1	0.79 ± 0.07[b]	0.59 ± 0.10[b]	2.56 ± 0.36[a]
18:3 n-3 (ALA)[2]	0.89 ± 0.08[b]	1.34 ± 0.11[c]	2.69 ± 0.29[a]
20:5 n-3 (EPA)[2]	1.56 ± 0.13[b]	1.21 ± 0.09[b]	2.41 ± 0.27[a]
22:6 n-3 (DHA)[2]	5.02 ± 0.50[b]	4.86 ± 0.50[b]	11.90 ± 1.54[a]
18:2 n-6 (LA)[2]	10.11 ± 0.46[a]	11.68 ± 1.18[a]	5.51 ± 0.65[b]
18:3 n-6 (GLA)[2]	0.41 ± 0.05[a]	0.62 ± 0.06[a]	0.52 ± 0.14[a]
20:2 n-6 (AA)[2]	3.71 ± 0.62[b]	4.03 ± 0.36[b]	6.58 ± 0.64[a]
22:5 n-6 (DPA)[2]	1.56 ± 0.13[b]	1.21 ± 0.09[b]	2.41 ± 0.27[a]

[abc]Different superscripts in the same row indicate significant differences at $p<0.05$.

[1]Black tilapia (extensive): n=10, Black tilapia (intensive): n=22, Red tilapia (extensive): n= 16

[2]LA= Linoleic acid, GLA= γ- Linolenic acid, AA= Arachidonic acid DPA= Docosapentaenoic Acid, ALA=α -linoleic acid, EPA=Eicosapentaenoic acid, DHA= Docosahexaenoic acid

Intensively cultured Nile tilapias (31.17%) contained higher total MUFAs compared with extensively cultured Nile tilapias (19.76%), but between Nile and Red tilapias cultured intensively no significant difference was observed (Table 3). Oleic acid (18:1 n-9) was the primary MUFA in

intensive and extensive cultured Nile tilapisa and also for Red hybrid tilapias.

For total n-3 PUFA percentage, it was higher ($p<0.05$) in extensive Nile tilapia (19.57%) compared with intensive Nile tilapias (8.27%), but no tsignificantly different between Nile tilapias and Red hybrid tilapias cultured in an intensive system. For total n-6 PUFA percentage, tilapias harvested from the intensive system (14-35 – 16.32%) had significantly higher ($p<0.05$) compared with those reared extensively (12.55%). In the present study, among n-3 series of fatty acids, the percentage of ALA (18:3 n-3), EPA (20:5 n-3) and DHA (22:6 n-3) in extensively cultured Nile tilapias were significantly higher ($p<0.05$) than those in intensively cultured Nile tilapias but no significant difference was found between genotypes of tilapias. The results of this study revealed that among n-6 series of the fatty acids, the primary fatty acids were arachidonic acid (20:4 n-6) for Nile tilapia in extensive system, and linoleic acid (18:2 n-6) for Nile tilapia in intensive system. But there was no significant difference between Nile and Red hybrid tilapias on both types of fatty acids.

Table 3: Total fatty acid composition of tilapias in different species and from culture systems (expressed as a percentage of the total fatty acids present)

Fatty Acids	Culture System		
	Intensive		Extensive
	Nile tilapia[2]	Red hybrid tilapia	Nile tilapia
∑ SFA[1]	46.1 ± 0.78[a]	46.4 ± 1.16[a]	48.1 ±1.18 [a]
∑ MUFA[1]	31.17 ± 1.08[a]	29.21 ± 1.25[a]	19.76 ± 2.03[b]
∑ n-3 PUFA[1]	8.27 ± 0.65[b]	8.01 ± 0.74[b]	19.57 ± 0.65[a]
∑ n-6 PUFA	14.35±0.60[a]	16.32 ± 1.23[a]	12.55 ± 0.77[b]
∑ Total UFA[1]	53.80 ± 0.78[a]	53.55 ± 1.16[a]	51.896 ± 1.18[a]
n-6 : n-3 ratio	1.88 ± 0.10[a]	2.24 ± 0.20[a]	0.74 ± 0.10[b]
USFA/SFA	1.17 ± 0.03[a]	1.17 ± 0.06[a]	1.09 ± 0.05[a]
PUFA/SFA	0.49 ± 0.02[b]	0.54 ± 0.05[b]	0.68 ± 0.07[a]

[ab]Different superscripts in the same row indicate significant differences at $p<0.05$.
[1]SFA= Saturated fatty acid, MUFA= Monounsaturated fatty acid, PUFA= Polyunsaturated fatty acid UFA= Unsaturated fatty acid

The ratio of n-3 to n-6 fatty acids was significantly higher ($p<0.05$) in intensively cultured tilapias (1.88 to 2.24) than in extensively cultured tilapias (0.74) which showed that the nutritional quality in the lipid components of intensively cultured Nile tilapias was higher than extensively cultured Nile tilapias. Culture system did not affect the USFA/SFA ratio. PUFA/SFA ratio of extensively cultured Nile tilapias was significantly higher ($p<0.05$) compared with intensively cultured Nile tilapias with the values of 0.68 and 0.49, respectively.

Fatty acids composition of Nile tilapias from extensive and intensive culture systems were similar to those reported for tilapias and other fish species (Karapanagiotidis *et al.*, 2006; Gonzalez *et al.*, 2006; Fuentes *et al.*, 2010; Gul Harlioglu *et al.*, 2011). For the different genotypes of Nile and Red hybrid tilapias, only 3 types of fatty acids: 14:0, 24:0 and 18:3 n-3, were found to be significantly

different. This finding is in agreement with the studies by Teoh *et al.* (2011) who also reported on the significant effect of tilapia genotype for 24:0, 18:3 n-3 and 22:1 n-9. From the present study Nile tilapia showed significantly higher value for 14:0 and significantly lower value for 18:3 n-3 than Red hybrid tilapia. But Teoh *et al.* (2011) showed Nile tilapias had lower 14:0 and significant higher 18:3 n-3 compared to Red hybrid tilapias and dietary lipid sources had significantly affected the fatty composition. This was because of similar composition fatty acids of the diet in commercial pellets used by the farm.

The total percentage of SFAs and n-3 PUFAs were higher in extensively cultured Nile tilapias than those of intensively cultured Nile tilapia, whereas Nile tilapias from intensive culture system showed higher content of MUFAs than Nile tilapias from extensive culture system. This was probably due to the high content of MUFAs in the diet of intensively cultured Nile tilapias. Oleic acid (18:1 n-6) was identified as the major MUFAs in Nile tilapias from intensive and extensive systems. The higher value of oleic acid in intensively cultured fish could be due to the high content of oleic acid in the commercial feed as was similarly found by Fuentes *et al.* (2010) in sea bass and Gonzalez *et al.* (2006) in yellow perch.

In this study it was determined that the total SFA was higher ($p>0.05$) in extensively cultured Nile tilapias than intensively cultured Nile tilapias. Palmatic acid (16:0) was the major fatty acids Nile tilapias caught from extensive and intensive culture systems, followed by stearic acid (18:0). Stearic acid content was found to be high in other fish species (Chen *et al*., 1995; Fuentes *et a*l., 2010)

As for PUFA content, extensively cultured Nile tilapias had higher percentage of n-3 PUFA and n-6 PUFA compared with intensively cultured Nile tilapias. Arachidonic acid (20:4 n-6) was the major fatty acid in n-6 PUFA. Freshwater fish were reported to contain higher concentration of arachidonic acid and linoleic acid when compared to marine fish which could be due to dietary effect and saturation and/or elongation mechanism (Ackman *et al.,* 2002). The higher concentration of arachidonic acid in Nile tilapias harvested from extensive culture system could be attributed to the type of diet of the Nile tilapias in the extensive environment comprising of insect larvae, freshwater algae and crustaceans which had high content of linoleic acid and linolenic acid (Gonzalez *et al.,* 2006). Besides, for n-3 PUFA, DHA (22:6 n-3) was the major fatty acid in this group, with the DHA and EPA (20:5 n-3) were higher in the extensively cultured Nile tilapias compared with intensively cultured Nile tilapias. This finding is in agreement with Karapanagiotidis *et al.* (2006) for tilapias and Fuentes *et al.,* (2010) for sea bass. For n-3/n-6 ratio, the values for extensively cultured Nile tilapias were significantly lower compared with intensively cultured Nile tilapias. This finding is contrary to previous reports for fish with n-3/n-6 ratio was found to be significantly higher ($p<0.05$) in fish from extensive system (Alasalvar *et al.,* 2002; Jankowska *et al.,* 2010; Fuentes *et al.,* 2010). Although the n-3 PUFA: n-6 PUFA ratio was low but the proportion of n-3 PUFA and n-6 PUFA was still significantly higher in extensively cultured Nile tilapias than in extensively cultured Nile tilapias.

Fatty acid composition of the fish tissue reflected the fatty acid composition of the diet, besides other factors which might influence the fatty acid composition; endogenous factors such as the conditions within the fish including age, sex and life cycle which affected the chemical

composition of the whole body of fish, as well as the composition of organs and tissues (Rassamusen, 2001) and exogenous factors including external environmental conditions such as water temperature, salinity and the type and level of feed ingredients (Lie, 2000).

With regards to PUFA/SFA ratio, higher level was detected in extensively cultured Nile tilapias compared with intensively cultured Nile tilapias. According to the nutritional guidelines of the Department of Health (1994) of the UK, a ratio of 0.45 or more is recommended as a balanced fatty acid intake in healthy diet. Hence, it can be concluded that from the finding of this study that extensively cultured and intensively cultured Nile tilapias and also intensively cultured Red hybrid tilapias have high PUFA/SFA ratio beneficial to human health.

Conclusions

The total saturated fatty acids and n-3 polyunsaturated fatty acids were higher in extensively cultured Nile tilapias than intensively cultured Nile tilapias. But the n-6:n-3 PUFA ratio was higher in tilapias from intensive culture system than that from extensive culture system. These differences may be attributed to the diet content of the Nile tilapias. Thus enhancing n-3 PUFA (especially DHA and EPA) content in the diets of tilapias could result in higher n-3:n-6 PUFA ratio. This is because fatty acid proportions of fish are very much dependent on the type of fish diet.

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