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A COMPARISON OF LIPID CONTENT AND FATTY ACIDS PROFILE OF HYBRID TILAPIA, *OREOCHROMIS NILOTICUS* CULTURED IN FRESHWATER AND MARINE CONDITIONS

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Abstract: The paper discusses thoroughly the lipid content and fatty acids profiles of freshwater and marine-cultured hybrid tilapia, *Oreochromis niloticus*. Thirty pairs of matured broodstocks hybrid tilapia at size of 600±50 g body weight (BW) were selected and bred in both freshwater (0 ppt) and marine (30 ppt) conditions. Larvae were grown to 170±20 g in 4 months and 85 fishes were selected for qualitative and quantitative lipid and fatty acids analysis. Results have clearly shown that the content, degree of unsaturation and oxidation of lipids between freshwater and marine-cultured tilapia were not significantly different at 8.40±1.13%, 87.92±3.53%, 1.28±0.19 mEq/kg in freshwater tilapia and 8.12±0.16%, 94.15±11.64%, 1.50±0.68 mEq/kg in marine tilapia respectively. The qualitative results showed that the fatty acids content in both freshwater and marine tilapia had no significant difference. The strong finding of five health-beneficial fatty acids at sufficient level in marine-cultured tilapia, namely lauric acid, pentanedioic acid, heptadecanoic acid, erucic acid and arachidonic acid, have shown a high potential and benefit gained in marine-cultured tilapia.

KEYWORDS: Fatty acid, Lipid content, *Oreochromis niloticus*, Tilapia

Introduction

Tilapia *Oreochromis* sp. play an essential role as the second important freshwater species in the human food chain after the carp and tilapia is the second most important farmed fish after carp, and contributed a total global production at 3.5 million metric tonnes (mt) in year 2010 with sale value 5.7 billion US dollar (USD) and projected increased to 4.6 – 5.0 mt in 2015 (USD) (Burden, 2012). Asia, one of the major producers of tilapia and products, stands alone ensconced with about 70% of the total global market (Jusupeit, 2007). China remains as the main producer leading with the total production of 1.15 million mt followed by Egypt (290,000 mt), Philippines (241,000 mt), Indonesia (206,000 mt) and Thailand (180,000 mt) in 2009 (FAO Globefish, 2011). North America, Western Europe and Middle East countries are the active importers of tilapia products from Asian. The imports of tilapia products to United State of America have been increased tremendously from year 1997 to 2005 at an average increment of 56% annually with a total value of USD49.5 million to USD393 million, respectively (INFOFISH, 2007). Tilapia therefore has become a very important worldwide cultured species and serves as an important protein source in human dietary intake. In recent decades, the Hybrid-red tilapia has been studied extensively to produce a good strain for better productions.

Lipids, one of the most important macronutrients in fish play an essential role as both energy and fatty acids (FAs) sources for human health. N-3 and n-6 polyunsaturated fatty acids (PUFA) are the FAs which have been emphasised in recent decades to bring sufficient beneficial health benefits at the recommended level. Polyunsaturated omega-3 (n-3) fatty acids, eicosapentaenoic acid (EPA, C-20:5) and docosahexaenoic acid (DHA, C-22:6), are those of interest because they are claimed to decrease the risk of cardiovascular diseases. In addition, fatty acid composition data are needed by food scientists and nutritionists to aid them in dietary formulation, processing and products

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development (Ackman, 1999). Previous studies showed that the lipids in fish consisted of long-chain fatty acids, which are highly unsaturated with five or six double bonds and are essential for human health (Laakso *et al.*, 1990). The level of polyunsaturated fatty acids in marine fishes, specifically the eicosapentaenoic (EPA) and docosahexenoic (DHA) have been shown to be higher than the content of saturated fatty acids. However, the EPA content in fishes varies depending on the culture environments and food availability. Freshwater fish has a lesser EPA both on the content and its profile as compared to the marine finfish or other seafood (Castellucci and Torres, 2003).

Although, fish lipids or fatty acids properties from fishes are important in human health, the off-flavour that occurred in fish had limited its acceptance for human consumption. As tilapia is the main freshwater cultured species in Malaysia, this off-flavour had certainly led to domestic market rejection (Personal communication with local tilapia farmers – East Cost and East Malaysia, 2008). The off-flavour phenomenon usually occurs in enclosed freshwater body with vegetation-rich area, high nutrient decomposition or run-off from potential pollution area or in an intensive culture system with insufficient water refreshment rate, as well as poor aquaculture practices. Ultimately, high nutrient density in water body is favourable with high light intensity and pH cause blooming of blue-green algae (Tucker 2010). These eutrophic environmental conditions are ideal for the development of heavy blue-green algae and actinomycete populations that contain off-flavour compounds that can be absorbed once they are present in pond-water body by aquatic animals. Fish absorb chemical compounds from these blue-green algae through their gill membranes as well as through their digestive tract by lipid oxidation catalysed by heme protein, ions and lipoxygenase (Fu *et al.*, 2009). The compounds are fat soluble and are stored in fatty tissues. Subsequently they promote the development of rancid and fishy odour during prolonged or improper storage caused by bacterial spoilage or by lipid oxidation (Miller, 2001).

The culture of tilapia in marine water has been initiated and lots of studies have been conducted to investigate salinity impact on tilapia previously. Different regions and water qualities may project different qualities of production. Additionally, tilapia farming in Malaysia still operates exclusively under a freshwater culture system. Therefore, there is a need to promote awareness of potentially culturing tilapia in marine water, either as open-sea cage culture or re-operation of abandoned marine shrimp farm or polyculture. Also, the culturing of marine tilapia can indirectly increase choice as marine food fish to fulfil increasing market demands of marine finfish for protein as well as fatty acids source. Therefore, this study was designed to investigate the characteristic of fatty acids profile distribution in marine-cultured tilapia as compared to freshwater-cultured tilapia.

Materials and Methods

Fish culture and management

A total of 30 matured and healthy tilapia *Oreochromis niloticus* broodstocks (10 male and 20 female) at size of 600±50 g (mean±standard deviation (SD)) of body weight (BW) were obtained from the local fish farm and cultured at the marine hatchery unit of Institute of Tropical Aquaculture, UMT. Premaxilla of all male tilapia were surgically removed prior the introduction into breeding tanks. Selected broodstock were distributed randomly into 10 rectangular fibreglass tanks (500 L; 5 tanks for fresh water and 5 tanks for marine water) filled with 300 L dechlorinated sea water for breeding purpose at the ratio of 1:2 (male to female) after 2 months of cultivation. 'L shape' ceramic substrates were located at corner of breeding tank as breeding substrate to avoid physical encounter for nesting territory. Salinity was increased gradually at 5 ppt weekly until 30 ppt as marine condition. Broodstock were fed twice a day with commercial tilapia pellet (28% crude protein) at 5% of their BW and 50% water exchange was done every four days (Deraman and Liew, 2009). Daily observation was done to evaluate the larvae production for all breeding tanks. Water quality was maintained at 27.5±0.5°

C of temperature, 8.12±0.17 of pH, and 6.52±0.23 mg/L of oxygen level with YSI instruments and salinity were monitored daily at 30±0.12 ppt by using refractormeter throughout the experimental period.

Larvae and juvenile grow-out

Collected free-schooling larvae were pooled in a new series of larvae tanks (300 L rectangular fibreglass tanks) according broodstock treatments and larvae were grown to fingerling size approximately 3 cm in body length. Larvae were fed with pre-starter pellet consisting of 34% crude protein at feeding rate of 10% BW and supplemented with *Moina macrocopa* every morning, whereas juvenile tilapia were fed with starter pellet (34% crude protein) at 8% BW 3 times a day and 80% of water exchange was done every 3 days. After a month of cultivation, larvae sized at approximately 2.5 cm in total length were transferred to grow-out tanks. Selected 120 juveniles were grow-out in 500 L fibreglass tank according to broodstock treatments for four months until sized at about 170 g for laboratory analysis. All grow-out and breeding tanks were equipped with external filter consisting of sponges, bio-ball, lava stone and activated carbon to provide continuous flow to the culture system and aeration provided to maintained oxygen level. A total of 85 fishes with size range of 170±20 g of BW were collected (local marketable size) from each culture conditions (fresh water and marine water). Selected fishes were preserved directly on ice and transferred to the laboratory for muscle sampling and analysis. Samples were cleaned and filleted. 200 g of tissue samples were pooled according to each freshwater and marine-cultured tilapia in triplicate for future analysis.

Lipid extraction

The fish lipid content was extracted by using the modified method of Bligh and Dyer (1959). 30 g fillets were cut into small pieces and homogenised with 60 mL of methanol mixed with 30 mL of chloroform in a warring blender (37BL6, USA) for 2 min. followed by adding 30 mL chloroform. After 30 s blending, 30 mL distilled water was added. The homogenate was stirred with a glass rod and vacuum-filtered through a Whatman No. 1 filter paper. The filtrate was transferred to a separator funnel. The lower clear-phase supernatant was drained into a 250 mL round-bottom flask and concentrated with a rotary evaporator (R-200 Switzerland) at a temperature of 40° C. The residue was then further dried in an oven (MMM Medeentar Enrichtungen GmbH MMM-Group, Germany) at a temperature of 40° C for 1h.

Degree of unsaturation

The degree of fatty acids unsaturation was analysed by using the iodine value method (AOAC, 2000). Sample of 0.2 g was added into 15 mL cyclohexane and 25 mL Wijs solution in a dry 500 mL conical flask. Mixture was kept in dark at 25° C. After 30 min, 20 mL of KI and 150 mL distilled water were added. Titration was carried out with 0.1 M $Na_2S_2O_3$ until the yellowish colour disappeared. Titration was continued by slowly adding 1 mL of starch until the blue colour emigrated. The volume of solution used in titration was used to calculate the iodine value of the oil sample.

Peroxide level determination

The peroxide level was determined by using the peroxide-value titration methods (EDQM, 2005). A 5 g oil sample was added with 30 mL mixture of acetic acid and chloroform at ratio 3:2 in a conical flask and shaken until the oil was dissolved. Subsequently, 5 mL of saturated potassium iodide were then added and left for 1 min for reaction prior to addition of 30 mL of distilled water. The mixed solution was then titrated. Titration was conducted with 0.01 M sodium trisulphide until the yellowish colour absconded. A few drops of starch indicator were added and titration was carried continuously until the blue colour changed to colourless. Similar titration was also carried out for control solution which had no oil sample.

Fatty acid methyl ester analysis (FAME)

The fatty acid methyl esters (FAME) were prepared by the methylation of the tracylglycerols as described by Joseph and Ackman (1992). The content and composition of the FAME were

separated by gas chromatography (Shimadzu GC 2010) fitted with a flame ionisation detector and a fused-silica DB-WAX capillary column (30 m length × 0.25 mm). The replacement of hydrogen bonds by methyl esters was used to ensure sufficient volatility analysis. The operation temperatures were set at 250° C for detector and 230° C for injection port. Temperatures were programmed to increase to 100° C and held for 1 min, continued to be heated up to 180° C with an increase of 8° C per min and held for 2 min. Next, it was heated up again at 5° C per min up to 230° C with the final holding time of 2 min. The retention times and peaks area percentages were computed automatically by the Varian 4290 integrator.

Data analysis

Results were presented as the mean value ± standard deviation (SD). The percentages of extracted lipid, fatty-acid composition, peroxide value and iodine value were compared by using a simple student's *t*-test with MINITAB 14.0. The normality were checked prior to the analysis and the level of significance was taken as $p<0.05$.

Results and Discussion

Lipid content

Lipid content in freshwater tilapia was 8.40%, slightly higher than marine tilapia at 8.12%, however, there were no significant different observed ($p>0.05$; Table 1). The percentage of lipid content in freshwater tilapia were shown between 1.1 to 1.2 g/100 g of tilapia fillet, which can be classified as the lean fish (>2% of total lipid) (Ackman, 1999). Lipid contents are varied according to their species, diets, geographical origin, season, sex maturity and age factors.

Degree of unsaturation

The degree of unsaturation between freshwater and marine tilapia were shown to be of no significant difference ($p>0.05$). The result showed a higher degree of unsaturation on the lipid in marine tilapia as compared to freshwater tilapia (Table 1). It has also been indicated that marine tilapia contains a higher PUFA than the freshwater tilapia feeding with the same commercial pellet. Hence, both linoleic and linolenic acid profiles are found to be higher in marine tilapia than freshwater tilapia. It has been supported from the previous study that the marine fishes generally contain 56-92% of unsaturation fatty acids higher than saturated fatty acids of 3.6-11.4% (Suriah *et al*., 1995). This might be due to the fact that freshwater fishes fed mainly on vegetation feed, whereas the main food sources of marine fish diet consisted of zooplanktons which are rich in unsaturated fatty acid (Suriah *et al*., 1995).

Level of oxidation

The results of the present study clearly showed that the oxidation level between marine and freshwater-cultured tilapia have no significant differences ($p>0.05$; Table 1). The oxidation of lipids is one of the major causes of the quality deterioration in fish, particularly on the flavour, colour, texture and nutrition value (Frankel, 2004). Basically, the primary end products of lipid oxidation are hydroperoxides, also known as peroxides, which are unstable organic compounds that are formed from triglycerides (Lawson and Hughes, 1988). The main cause of lipid peroxidation has relation with its high percentage of PUFA in tilapia fish. There was an insignificant difference of lipid oxidation rate between marine and freshwater-cultured tilapia found in the present study, although more fatty acids were found in marine-cultured tilapia. This indicated a more stable fatty profile in marine tilapia than freshwater-cultured tilapia.

Table 1: Comparison on percentage of lipid, unsaturation degree and oxidation level of lipid content between freshwater and marine tilapia.

Composition content	Tilapia Culture Environment	
	Freshwater	Marine
Lipid (%)	8.40±1.13	8.12±0.16
Unsaturation degree (%)	87.92±3.53	94.15±11.64
Oxidation level (mEq/kg)	1.28±0.19	1.50±0.68

Data were presented as mean ± standard deviation. No significantly difference detected ($p>0.05$).

Fatty acid profile

Fatty acid analysis showed that the marine tilapia contained five additional fatty acids, namely lauric acid, pentanedioc acid, heptadecanoic acid, arachidonic acid (ARA) and erucic acid which were not found in freshwater tilapia (Table 2). This indirectly indicated that the consumption of marine tilapia promotes a higher value in beneficial health effect to human beings than the freshwater tilapia. Arachidonic acid was the most abundant n-6 PUFA followed by linoleic acid. This was in agreement with Steffens (1997) who reported that tropical water fish generally contain high proportions of ARA. From a human health point of view, ARA is the principal n-6 fatty acid in the brain. Together with DHA, they play a very important role in brain development, particularly to young infants (De Uequiza, 2000). It has been also reported that ARA provides numerous neurological benefits for children during the growing stage (Osibona *et al*., 2006).

The linoleic acid and linolenic acid are known to be important in human nutrition due to their benefits for preventing skin disease (Osibona *et al*., 2006). However, the content of linoleic acid and linolenic acid in the present study showed no significant differences between the freshwater and marine tilapia ($p>0.05$). This is because marine tilapia was not cultured under natural marine environment and the fish were not exposed to the natural food chains and were only fed with commercial pellets. The variation of PUFAs in fish is not restricted to intra-species specific but extend to growing environments. Respectively, the results showed were both FAs were not inspired by the culture conditions.

Freshwater fish contain lower fatty-acid quality compared to marine fish. These differences can be attributed to the fact that the freshwater fish are fed largely by vegetation and plant materials whereas the marine fish are fed widely on fatty-acids-rich planktons which can easily be absorbed into their tissue (Simopoulos, 1996). In the study done by Dale (2001), the common fatty-acid compositions which were found in the hybrid red tilapia were myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), arachidonic (20:1) and omega-3 fatty acids. Oleic acid had the highest percentage (35.4%) followed by palmitic acid (25.7%) and linoleic acid (11.9%). However, no arachidonic was found in the freshwater-cultured tilapia in the present study.

Table 2: Comparison of fatty acids profile between freshwater and marinewater cultured tilapia.

Fatty acid content	Tilapia Culture Environment	
	Freshwater (%)	Marine (%)
Octanoic acid	0.72±0.02	0.61±0.02
Lauric acid		0.47±0.05
Myristic acid	0.96±0.04	1.50±0.02
Pentanedioic acid		0.49±0.05
Palmitic acid	14.09±0.14	13.2±0.34
Palmitoleic acid	2.50±0.26	3.99±0.09
Heptadecanoic acid		1.47±0.07
Stearic acid	5.12±0.15	2.33±0.08
Cis-9-oleic acid	39.46±0.52	52.36±1.53
Linoleic acid	22.63±0.58	22.66±0.86
Linolenic acid	4.45±0.03	4.45±0.15
Arachidonic acid		0.44±0.05
Eicosapentanoic acid	1.05±0.04	0.99±0.08
Behenic acid	6.01±0.07	2.14±0.04
Erucic acid		0.39±0.02

Data were presented in mean ± standard deviation. No significantly difference detected ($p>0.05$).

The present study showed that higher numbers of fatty acids were found in marine-cultured tilapia than freshwater tilapia (Table 3). As the freshwater tilapia were cultured in dechlorinated seawater with regular water replacement, it restricted any possibilities of plankton blooming in the cultured tank to be served as an additional food source. Furthermore, it is not surprising that lower fatty-acids profiles were indicated through this finding. On the other hand, the marine tilapia were cultured mainly by the seawater pumped directly from the South China Sea through the channel and filtered by using general marine-filtration system. It provided numerous planktons which are favoured as the additional good sources of PUFA intake which enhanced the valuable fatty-acid contents. This is in agreement with the study done by Dale (2001), whose results indicate that freshwater tilapia contains lower fatty acids, particularly with reference to omega-3 FA. Hence, the lower FA in the study might also be due to the

Table 3: Group of fatty acids and number of n-3/n-6 ratio between freshwater and marinewater cultured tilapia.

Group of fatty acid	Tilapia Culture Environment	
	Freshwater (no.)	Marine (no.)
Saturated, SFA	5	8
Monounsaturated, MUFA	1	2
Polyunsaturated, PUFA	2	3
Omega 3, n-3	2	2
Omega 6, n-6	1	2
Omega 9, n-9	1	2
n-3/n-6	2:1	1:1

limitations of maturation and spawning processes inbetween the study periods. This is supported by the study of Osibona *et al*. (2006) on African catfish where reserved lipids were used during spawning activities, particularly for the gonad formation.

The percentages of linoleic acid, n-6, obtained were significantly higher than linolenic acid, n-3 in marine tilapia as compared to freshwater tilapia. Tilapia requires a greater amount of n-6 linoleic acid as compared to n-3 linolenic acid for their maximal growth. Based on studies done by Petenuci *et al*. (2008), lower concentration of n-6/n-3 were in freshwater-cultured tilapia due to low concentration of LNA (n-3 series precursor) and lack of n-3 PUFA in commercial feeds. Nutritionists have consolidated the benefits of MUFA and PUFA in diets to prevent atherosclerosis and reduce the risk of cardiovascular disease with a ratio PUFA:MUFA:SFA at 1.0:1.5:1.0 (Moreira *et al*., 2001) which is very near to the results in present study at 1:1 to 2:1 (Table 3). The ratio of n-6/n-3 found by Petenuci *et al*. (2008) was 2.8 as in other freshwater-farmed species. The recommended ratio was 2:1 to 3:1 (Simopoulos *et al*., 1999) which is close to present result obtained.

Notwithstanding, many previous studies have found the strong relationship between fatty acids and off-flavour development. Jenschke *et al*. (2007) reported that individual fatty acids and long-chain unsaturated fatty acids play a significant role in the development of the off-flavour, where 18:1(n-7) and 20:2(n-6) were related to development of off-flavour in meat. Yancey *et al*. (2006) noted that trans 18:1(n- 9), 16:1, 17:1, linoleic acid (18:2) and unsaturated fatty acids [(20:3(n-6) and 20:2(n-6) played a pivotal role in the development of the off-flavour in meat products. Additionally, Hodgen (2006) also found a strong correlation between off-flavour and primary by-products of oleic, linoleic, and arachidonic acids identified by mass spectrometry. According to Campo *et al*. (2003) oleic, linoleic and a-linolenic acids were the main mono, di and tri-enoic fatty acids in adipose tissue that are susceptible to thermal oxidation in accordance with the number of double bonds and the oxidation of the long chain n-3 PUFA, especially in fish produced by 2-trans, 4-cis, 7-cis-decatrienal which were related to fishy off-flavour. Overall, high lipid content easily lead to oxidation which could increase susceptibility to off-flavour development. Although our study found that marine-cultured tilapia contained more fatty acids then freshwater-cultured tilapia (14:10 in number, Table 2), the oxidation degree between the two tilapias were not significantly different (Table 1). As oxidation is the main cause of off-flavour, this result highlighted the stable fatty acids content in marine tilapia toward off-flavour development. Nevertheless, off-flavour can be avoided with good aquaculture practise prior to harvest and proper storage procedures at post-harvest stage.

Conclusion

As a conclusion, both qualitative and quantitative lipid contents between the freshwater and marine-cultured tilapia were homogeneous. Marine tilapia can be therefore introduced and promoted as the new variety of aquaculture species in marine fish-culture industry. Their high quality of nutritional value will produce a better sensory taste. Marine-cultured tilapia contain a quality fatty-acid profile similar to freshwater-cultured tilapia, but with several beneficial fatty-acid profiles and favoured sensory taste. The suggestions for the future studies can be focused more on providing a marine food source to improve n-6 to n-3 ratio as well as improving taste and palatability. Lastly, the study has showed the potential nutritional values of culturing tilapia in marine environment.

Although this is not new in aquaculture, the findings in this study at least could encourage domestic aquaculturists to re-operate abandoned marine shrimp farm or sea-cage culture to increase domestic food-fish selection.

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