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Fatty Acid Concentration of Farmed Tilapia (*Oreochromis niloticus*) in Selected Sites in the Philippinesa

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ABSTRACT

Background and objectives: *Farm-raised tilapia contains very low levels of the beneficial very long-chain omega-3 fatty acid and very high levels of omega-6 fatty acid. Excessive amounts of omega-6 fatty acids and a very high omega-6/omega-3 ratio, promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA (a low omega-6/omega-3 ratio) exert suppressive effects. Hence, the study determined the omega-3 and omega-6 fatty acid content of farmed tilapia and the omega-6/omega-3 ratio. Methods: Samples of farmed tilapia were taken from four sites namely: Batangas, Nueva Ecija, Pampanga and Isabela. The omega-3 and omega-6 fatty acid levels in farmed tilapia and the omega-6/ omega-3 ratio were analyzed using gas chromatography. Results: Tilapia from Isabela has the highest amount of fatty acids for both omega-6 (1.2 mg/100g) and omega-3 (EPA 1.5 mg/100 g; DHA 61.8 mg/100 g) respectively. On the other hand, tilapia from Batangas has the lowest amount of total fatty acids with omega-6 of 0.70 mg/100 g and omega-3 of EPA 4.2 mg/100 g and DHA 2.2 mg/100 g. For all the samples from each site, omega-3 has a higher amount of fatty acid compared to its omega-6 fatty acid while the omega-6 and omega-3 ratio in all the four sites was less than 1:1. Conclusion: The omega-3 (eicosapentaenoic acid and docosahexaenoic acid) fatty acid concentration of farmed tilapia taken from Isabela, Nueva Ecija, Pampanga and Batangas has higher concentration than omega-6 and the ratio between the two fatty acids met the values for the recommended ratio.*

Keywords: Arachidonic acid, Docosahexaenoic acid, Eicosapentaenoic acid, *Oreochromis niloticus*

INTRODUCTION

According to World Health Organization [1] coronary heart disease is one of the leading causes of mortality and morbidity worldwide. In the Philippines, mortality due to coronary heart disease reached 87,881 or 16.86% of total deaths. In terms of age adjusted death rate, it accounts to 161.43 per 100,000 population which ranks the Philippines as 29th in the world. This caused the dramatic change in the diet of the people worldwide over the past century especially the intake of fish. It was revealed that consumption of fish might reduce the risk of heart disease because of its rich source of omega-3 fatty acids [2].

Omega-3 and Omega-6 are Polyunsaturated fatty acids (PUFA) that are important in the body in regulating blood pressure and inflammatory response. Omega-3 fatty acid is also used as a protection from fatal heart disease and for prevention of diabetes and certain types of cancer. On the other hand, Omega-6 is essential for brain and muscle development and it is important in the nervous system as a transmitter of nerve impulses [3].

The recommended intakes of omega-3 fatty acid vary by country from 0.5 to 2% of energy: recommended intakes of alpha-linolenic acid (ALA) are between 0.6 and 1.2% of energy or 1-2 g/day [4]. However, the common fishes that

contain right amounts of these fatty acids are known to be expensive such as Salmon (*Salmo salar*) and Mackerel (*Scomber scombrus*). As an alternative, people choose cheaper fishes which can be found easily in markets such as Tilapia (*Oreochromis niloticus*).

Globally, tilapia production has increased by about 2.8 million metric tons in 2008 and is expected to increase even more to 8.9 million metric tons by the year 2020 [5]. In the United States, tilapia has shown the biggest gain in popularity among sea foods. The consumption of this fish increased from 1.5 million tons in 2003 to 2.5 million tons in 2010 [6].

In the Philippines, Tilapia is usually grown in fish ponds or fish pens. They have the capacity to grow under almost any condition that is why it is one of the most commonly consumed fishes by Filipinos. The national production of Tilapia had reached 321.08 thousand metric tons in 2018 [7]. Extraordinary growth production had been noted particularly from the late 70's up to 2006 [8].

However, according to the study conducted by Wake Forest University School of Medicine [9], farm-raised tilapia contains very low levels of the beneficial very long-chain omega-3 fatty acid and very high levels of omega-6 fatty acid. These fatty acid combinations are said to be harmful to human health, especially to those with heart disease, arthritis, asthma and other allergic and autoimmune diseases. Also, the amount of omega-6/omega-3 ratio present in tilapia is much higher compared to the normal amount recommended, much less than 1:1 to 4:1 [10]. There is a substantial evidence that decreasing omega-6/omega-3 ratio can also decrease inflammation [11,12]. Thus, increase amount of omega-6/omega-3 ratio can cause inflammation which can lead to an array of cardiovascular and metabolic disorders.

With all the presented information, the study was conducted in order to assess and compare the omega-3 (eicosapentaenoic acid and docosahexaenoic acid) and omega-6 (arachidonic acid) levels of farmed tilapia in selected sites in the Philippines.

MATERIALS AND METHODS

Collection of Samples

Samples of tilapia were taken from the chosen farms from each area located in Batangas, Isabela, Nueva Ecija and Pampanga. The inclusion criteria for the samples were the following: same species, fresh tilapia from fish farms and fish ponds and uniformity in the size of all the samples (medium sized, 15 cm-25 cm, 100 g-200 g) while the exclusion criteria were tilapia that grew freely on bodies of water or wild tilapia. The collected sample were preserved in crushed ice and was transported to the laboratory for fatty acid analysis and to the National Museum for specie validation. Samples from the fish gut were filleted and processed for fatty acid determination.

Preparation of Fatty Acid Methyl Esters

For the preparation of fatty acid methyl ester, fish fillet was added to 500 µL of a methyl tricosanoate solution in n-heptane at 1 mg/mL^{-1} and a total of 30 ± 1 mg of oil was extracted. Then, after the preparation of the solvent nitrogen flow, the lipids were saponified in a 0.5 mol/L^{-1} sodium hydroxide solution in methanol and esterified using a mixture of ammonium chloride, methanol, and sulfuric acid in the proportion of 1:30:1.5 (m/v/v). After the addition of 4 mL of a saturated sodium chloride solution, Fatty acid methyl esters (FAME) were extracted with 1 mL of n-heptane.

Determination of Fatty Acid Concentration

The lipid from the sample was extracted following the procedure of Folch, et al. [13] and alkaline based transesterification was used to prepare the Fatty acid methyl ester (FAME). The instrument used to analyzed fatty acid methyl esters was Shimadzu QP 2010 ULTRA Gas chromatography (GC) with Flame Ionization Detector (FID) and column of DB-23. The operating parameters were: column temperature 40° to 250°, temperature rise 20° per minute, detector temperature 260°, nitrogen carrier as, column rate 0.72 mL per minute, flow rate [14].

RESULTS

Levels of Fatty Acids of Farmed Tilapia

Fish samples from Isabela have the highest amount of omega-3, Eicosapentoic acid (EPA) and omega-6, arachidonic

acid (AA); 6.5 mg/100 g and 1.2 mg/100 g respectively. The lowest fatty acid contents were samples from Batangas, with AA level of 0.70 mg/100 g, and EPA of 4.6 mg/100 g (Table 1).

Table 2 depicts the omega-6/omega-3 ratio of samples taken from different sites. The lowest omega-6/omega-3 ratio, 0.03:1 was from samples taken from Nueva Ecija and Pampanga. Conversely, Batangas demonstrated the highest ratio with 0.21:1. All of the four sites met the recommended value for the omega-6/omega-3 fatty acid ratio, that is much less than 1:1 to 4:1 (Table 2).

Table 1 Fatty acid concentration of farmed tilapia of each sampling location

Specimen Site	Omega-3 Levels mg/100 g		Omega-6 Levels mg/100 g
	EPA	DHA	AA
Isabela	6.5	61.8	1.2
Batangas	4.6	2.2	0.7
Nueva Ecija	4.44	38.4	0.59
Pampanga	1.72	12.4	0.2

Table 2 Omega-6/Omega-3 ratio of samples taken from different sites

Site	Omega-6	Omega-3	Ratio
Isabela	1.2	34.15	0.04:1
Batangas	0.7	3.4	0.21:1
Nueva Ecija	0.59	21.42	0.03:1
Pampanga	0.2	7.06	0.03:1

DISCUSSION

Tilapia is one of the most consumed farmed fishes in the world. It is consumed across the globe and gaining popularity in Asian and American countries. It is inexpensive and mild in taste which makes it a good substitute for expensive alternatives such as salmon. The rapid expansion of aquaculture has catalyzed the growth of the tilapia industry globally. Tilapia, being an ideal choice for fish farming, has now become the second most cultured species after carps. Since the fish is omnivorous, hard and has good resistance to diseases, they are affordable and easy even for small farmers to grow. Currently, almost 6 million metric tons (MT) of tilapia are grown annually worldwide [15].

The American Heart Association has recommended that people eat fish rich in unsaturated fats at least twice a week. Consumption of fatty fish has been suggested to reduce the risk of CVD, which is mainly due to its high level of omega-3 polyunsaturated fatty acids [16]. This leads to considerable interest in evaluation of fatty acid contents of fish that are most commonly consumed globally. Fish has been known as a source of polyunsaturated fatty acids omega-3 and omega-6. Omega-3 fatty acid plays a vital role in the brain function and for the normal growth and development. They are an integral part of cell membranes throughout the body and affect the function of the cell receptors in these membranes. They provide the starting point for making hormones that regulate blood clotting, contraction and relaxation of artery walls, and inflammation. They also bind to receptors in cells that regulate genetic function. Likely due to these effects, omega-3 fats have been shown to help prevent heart disease and stroke, may help control lupus, eczema, and rheumatoid arthritis, and may play protective roles in cancer and other conditions [17].

Along with omega-3 fatty acids, omega-6 fatty acids play a crucial role in brain function, and normal growth and development. As a type of Polyunsaturated fatty acid (PUFA), omega-6 helps stimulate skin and hair growth, maintain bone health, regulate metabolism, and maintain the reproductive system. Unfortunately, high omega-6 PUFA dietary intake has been linked to inflammation, mainly because eicosanoids derived from omega-6 PUFA are proinflammatory. Arachidonic acid belongs to the omega-6 (n-6) Polyunsaturated fatty acids (PUFA) and is a precursor to a number of potent pro-inflammatory mediators including well described prostaglandins and leukotriene. Recommendations for

increasing omega-3 and reducing omega-6 have been proposed by nutritionist. Optimal dietary intakes of the n-6: n-3 ratio should be around 1-4:1.

An article published by Wake Forest University Baptist Medical Center [9] alleged that tilapia contains potentially dangerous fatty acid combination. The results of their study showed that tilapia has very low levels of beneficial omega-3 fatty acids and very high levels of omega-6 fatty acids. The researchers say that the combination could be a potentially dangerous food source for some patients with heart disease, arthritis, asthma and other allergic and auto-immune diseases that are particularly vulnerable to an "exaggerated inflammatory response." The result of this study has been controversial and dissuades consumers from buying tilapia. Although this study was disputed by Harvard Medical School, the article has reached most fish consumers across the globe and it still affect public opinion.

In view of this, our paper analyzed the fatty acids of farmed grown tilapia in the Philippines in an effort to approximate its omega-3 and omega-6 content. The Philippines is one of the top producers of tilapia and approximately 60 metric ton was exported according to the Philippine Statistics Authority [7] thus analysis of the fatty acid content of tilapia from the Philippines would benefit the local farmers and consumers.

Our results showed that omega-3 were higher than omega-6 fatty acids in all four sites sampled. The samples with the highest total omega-3 fatty acid (34.15 mg/100 g) was from a farm in Isabela province. The highest DHA and EPA levels from the samples were 61.6 mg/100 g and 6.5 mg/100 g respectively. As to the omega-6 omega-3 fatty acid ratio, all the samples from the selected sites met the recommended value of the World Health Organization. Conversely, in several published researches, total omega-6 is higher than omega-3 fatty acids in samples taken from several sites such as farm and lakes and ranges from 11.1 to 38.7, whereas total omega-3 ranges from 3.64-8.0 [6,18]. The disparity in fatty acid composition could be attributed to diet, location, species and environmental condition as suggested by Justin et al., in 2003. He stated that diet is the main factor affecting the n-6 and n-3 FA content in tilapia and whether the diet is natural (i.e. wild tilapia) or compounded (i.e. farmed tilapia).

In the Philippines farm made feeds are not commonly used because of erratic supplies of raw materials, and high capital requirements. Tilapia feeds in the Philippines contain the ingredients: corn, soybean meal, fishmeal, poultry by-product meal, brewer's yeast, corn gluten, rice bran, copra meal, brewer's grain, wheat pollard, molasses, vegetable oil, salt, limestone, dicalcium phosphate, ethoxyquin, L-lysine, DL-methionine, binder, mould inhibitor, virginiamycin [19].

This study showed that farmed tilapia in the Philippines has a good combination of omega-3 and omega-6 fatty acids and could be attributed to feed used. However, because of limited samples it is recommended to explore fatty acids composition in different sites such as farmed and lakes and correlate with feeds given.

CONCLUSION

From the results, it was found that fish samples in all sites has higher fatty concentration of omega-3 (eicosapentaenoic acid and docosahexaenoic acid) than omega-6 and the ratio between the two fatty acids met the recommended values. Thus, the fish samples from all the sites can be a good source of omega-3 and omega-6 fatty acids.

DECLARATIONS

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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