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Full Length Research Paper

Effects of fish oil substitution with vegetable oils in diets of juvenile Nile tilapia, *Oreochromis niloticus (L.)* on growth performance, nutrients utilization and muscle fatty acids contents

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The present study was conducted at Shakshouk fish research station at Fayoum Governorate, Egypt to evaluate the effects of substitution of some vegetable oils (sunflower seed oil ,SO and cotton seed oil, CO) in juvenile Nile tilapia (*Oreochromis niloticus*) in growth performance, nutrient utilization and muscle fatty acids contents. Four isontrogenous and isocaloric diets (30.22±0.02% CP and 19.007±0.015 MJ kg^{-1}diet) were formulated to represent four dietary treatments. The first treatment (control) contained 6% of fish oil (FO), the second contained 6% (SO), the third contained 6%(CO) and the fourth diet contained a mixture (MX) 6% FO,SO and CO at a ratio of 1:1:1. Each dietary treatment was performed in three replicates and the experimental lasted 120 days after start. The experimental treatments were performed in 12 fiber glass tanks of (1m^3) volume each. Fish were fed diets at a rate 3% of the biomass daily divided into two equal portions. Results obtain are summarizes in the following: 1-the highest (P<0.05) growth performance parameters (finial weight, weight gain, daily gain and specific growth rate were recorded with FO and MX groups compared to other treatment groups; 2-the best nutrient utilization parameters (feed conversion ratio, protein efficiency ratio and net protein utilization) were recorded by FO and MX groups compared to other treatment groups; 3-the applied treatments showed insignificant effects on nutrients digestibility coefficient among dietary groups and ranged from 85.1-85.6, 89.6-90.2, 93.3-93.8,94.1-94.6 and 71.0-71.4% for Dry matter, Energy, CP, Fat and nitrogen free extract, respectively; 4-the applied dietary treatments had no significant effects of dry matter, crude protein and ash contents of whole fish body, however, fat contents in the whole fish body tended to increase in SO and CO and significantly between diets; 5-tilapia juvenile fed on FO diets showed the highest muscle fatty acids contents (P<0.05) of saturated, monounsaturated and polyunsaturated fatty acids compared to MX, SO and CO groups. Based on the obtained results it is to recommended the substitution of 66% fish oil (MX diet) with SO and CO oils in juvenile Nile tilapia diets without any diverse effects on growth performance, nutrients utilization and digestibility coefficient.

Keywords*:* Nile tilapia, vegetable oils, sunflower oil, cotton seed oil, digestibility coefficient, growth performance, fatty acid profile.

INTRODUCTION

Tilapias are widely cultured in many tropical and subtropical regions of the world. Tilapias are known as “aquatic chicken” because of their fast growth, good quality of flesh, disease resistance, adaptability to wide range of environmental conditions, ability to grow and reproduce in captivity and feed on low trophic levels. Therefore, they have become an excellent choice for aquaculture, especially in tropical and subtropical environments (more than 22 tilapia species are cultured worldwide) and they constitute the third largest group of farmed finfish “after carps and salmonids” (El-Sayed, 1999 and 2006). Global tilapia production was around 3.500.000 metric tons in 2011 and should exceed 3.8000.000 metric ton in 2012 and by 2015; world production tilapia is forecast to reach 4.6-5.0 million metric tons (Burden, 2012).

Fish meal and fish oil have been used for decades as the main dietary components of fish feeds, but they have became scarce and expensive, leading to search for alternatives. These alternatives should not only fulfil fish dietary needs, but also be cheap and available (Watanabe, 2002). The fish oil production in 2012 is forecast 340.000Mt as recorded by Nolte (2012).

The lipid nutrition and fatty acid metabolism of fish is of significant interest to the global aquaculture sector because of environmental and economic issues pertaining to the use of fish oil in aquaculture feed. The challenge for fish nutritionists is to reduce the utilization of fish oil in aquafeed formulations while ensuring that appropriate amounts of n-3LCPUFA are present in the final product (Turchini *et al.,* 2009).

Limited marine resources dictate the increased use of terrestrial plant-derived proteins and oils in formulated diets for farmed fish species (Pickova and Mokone, 2007). However, in opposition to mammals EPA (20:5n-3) predominates over arachidonic acid AA (20:4n-6) in membrane phosphatidylinositol. Fish are theoretically capable of biosynthesizing 22:6n-3 via the desaturation and elongation of α-linolenic acid (18:3n-3) found in some vegetable oils. However, the lipid metabolism of fish has adapted to an abundance of dietary 22:6n-3 and as a result, the effective utilization of the n-3 biosynthetic capability has been rendered dormant (Sargent *et al.,* 2002).

Although vegetable oil VO contain negligible amounts of arachidonic acid, AA, the situation is complicated further due to them being rich in linoleic acid (18:2n-6) and linolenic acid (18:3n-3), which can be converted to dibono-y linolenic acid (DGLA, 20:3n-6) and 20:4n-3, respectively by ∆ 6 desaturase and elongase further to AA and EPA, respectively by ∆ 5 desaturase (Tocher *et al.,* 2010).

There are conflicting reports concerning the effects of FO substitution by other lipid sources on muscle proximal composition. Some results suggest that there are discernible effects of such substitution on fish edible parts (Francis *et al.,* 2006, Richard *et al.,*2006a, Huang *et al.,* 2007; Turchini *et al.,* 2007; Yildirim-Aksoy *et al.,* 2007). In mammals HUFAs have been shown to suppress fatty acid synthesis, increase fatty acid β-oxidation, and reduce triacylglycerol synthesis (Al-Hasani and Joost, 2005). In fish, n-3 HUFAs have been shown to reduce fat cell development and lipid accumulation in cultured preadipocytes (Todoreevie *et al.,*2009), reduce fat content in Atlantic salmon white adipose tissue and increase fatty acid β-oxidation activity in comparison with fish fed with rapeseed oil (Todoreeve *et al*., 2009).

Recently the benefits of increased EPA and DHA intake have been shown for a wide range of life style disorders with an associated inflammatory pathology as well as improving the symptoms of certain neurological disorders. Due to the proven benefits of n3-HUFA, numerous governmental and non-governmental organizations across the world currently advise increased fish intake as a means of improving the health of their citizeans (Kris-Etherton *et al.,* 2000; Gebauer *et al.,* 2006 and Kusunto *et al.,*2007).

The first review suggesting that fatty acids might be important in immune function was by Meade and Mertin (1978) and more recent reviews have confirmed the importance of PUFA, of both n-3 and n-6 series as modulators of immune function (Galder, 2001 and Yaqoob, 2004). In fish, dietary fatty acids and tissue fatty acid composition are closely correlated and changes in the dietary n-3/n-6 ratio can influence the composition of fish (Sargent *et al*., 2002).

As protein represents the most expensive component of aquafeeds (Cho *et al*., 2005), from an economic stand point it is vitally important that protein be utilized for the synthesis of muscle tissue and not for metabolic energy (Ozorio *et al*., 2006). The ability to utilize lipid rather than protein as an energy source can lead to a decrease loss of ingested protein by catabolism, thereby potentially reducing nitrrogenus waste input into culture system (Miller *et al.,*2005). In tilapia it has been suggested that protein sparing occurs when lipid levels are increased up to 60-100 g/ kg^{-1} (Jauncy and Ross, 1982).

Attempts have been made to replace fish oil in tilapia diets with various oil sources (Ribeiro *et al.,*2008 and Ferreira *et al*., 2011) and in other species such as rainbow trout (Alvarez *et al.,*2000 and Turchini and Francis, 2009), sea bream (Benedito-Palos *et al.,* 2007; 2008) and Merrry cod (Turchini *et al.,* 2011).

The present study aims to evaluate the effect of use some vegetable oils (single and blend) as an alternative

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source to replace fish oil on growth performance, nutrient digestibility and fatty acids composition of Nile tilapia reared in fiber glass tanks.

MATERIALS AND METHODS

Fish culture and experimental diets

The present study was conducted using the research facilities of the experimental station at Shakshouk, Fayoum Governorate, National Institute of Oceanography and Fisheries (NIOF). The system contained two water pumps and upstream sandy filter units at a point between the water source and tanks. Each pump was drowning the water from storage cement pond and forced it through storage units and then to the rearing tanks in open system. Physicochemical characteristics of water tanks were examined every week, (Table1) according to APHA (1992).

The experimental Nile tilapia fry used in the present study were obtained from a private hatchery near the station and transported through a plastic page to the rearing tanks in the station. The fry were acclimatized for two weeks in rearing tanks and fed on prepared powder diet contain 300 g kg^{-1} crude protein, formulated from the same ingredients use in the growth trial.,Nile tilapia fry with an initial average weight of 1.25 ± 0.05g were randomly distributed and stocked at 60 fry/tank in 12 fiber glass tanks with a water volume of ($1m^3$) each and the treatments were performed in triplicates. The diets were given at 3% of live body weight (BW) twice daily at 1000 and 1600 h for 120 days.

Four isonitrogenous diets were formulated to contain an average of 302.2 g kg^{-1} crude protein and meeting all known nutritional requirements of tilapia (NRC, 2011). The different lipid sources from fish oil (FO), sunflower oil (SO) and cotton seed oil diets (CO) were added to the diet at a level of 60 g kg^{-1}. However, the mixed diet (MX) contained a blend of fish oil and both vegetable oils at 60 g kg^{-1} inclusion level (20 g kg^{-1} of each fish oil, sunflower oil and cotton seed oil, respectively). The diet formulation and proximate analysis are shown in (Table 2). All diets were processed into dry sinking pellet form, using California pelleting machine with 2 mm diameter.

Apparent Digestibility Coefficient

The experimental test diets with addition of 5 g kg^{-1} chromic oxide (Cr_2O_3) were fed to fishes at the end of two weeks in order to study the apparent digestibility coefficient (ADC %) of nutrients. Any uneaten feed and fecal residues were siphoned out from the tank bottom two hours after first feeding (1000 h) and discarded. Fish fecal samples were collected every afternoon before the second feeding, new fecal materials were carefully siphoned and collected using the filtration system developed by Choubert *et al.* (1982). After freeze-drying of fecal samples, the feces were analyzed. Dry matter was calculated by gravimetric analysis at 105°C for 24h. Chromic oxide levels were determined spectrometry (Spectra, AA220FSNarin) based on the method described by Bolin *et al.,*(1952).

The apparent digestibility coefficients ADC for test diets were calculated according to the equation described by Cho (1993).

ADC (n) = 100- {100 (%Cr_2O_3d) / %Cr_2O_3f) × (% *N*f / % *N*d)}.

Where ADC(n) = apparent digestibility coefficients of a nutrient in the test diets; Cr_2O_3d = % chromic oxide of the diet ; Cr_2O_3f = % chromic oxide of the feces ; *N*d = nutrient in the test diet; *N*f = nutrients in feces.

Chemical analysis

The chemical composition of the experimental diets, feces and fish samples were performed via proximate composition analysis according to standard methods AOAC (1995). Briefly dry matter was determined gravimetrically in an oven dried samples at 105°C for 24h, protein (N×6.25) content was determined using Kjeldhal method and crude fat by chloroform-methanol extraction (2:1) using Soxhlet system. Ash was determined by incinerating samples in a muffle furnace at 550 °C for 18h. Nitrogen free extract (NFE) was calculated by the difference.

Fatty acids, total lipids were extracted from three samples (replicates) in each diet according to Folch *et al.,*(1957). Fatty acid methyl esters were prepared by acid-catalyzed transmethylation of total lipids (Shantha and Ackman 1990). They were analyzed in a Varian 3400 gas chromatograph, (equipped with a wax fused capillary Column (30 mx 0.25 mm i.d, film thickness, 0.25 Mm, Jw, USA), using helium as carrier gas (1.2 ml min^{-1}) and thermal gradient from 180 to 240°C/min. injector and flam ionization detector temperature were 260 and 250°C, respectively. Data were recorded on spectra physics 4270 integrator. Identification of individual fatty acid was made by comparison with known standard mixture.

Gross energy (MJ Kg^{-1} diet) was calculated according to Schulz *et al.,*(2007) using the following calorific values: 23.9, 39.8 and 17.6 MJ g^{-1} diet for protein, ether extract and nitrogen free extract, respectively. The metabolizable energy contents of the experimental diets were calculated as 18.9, 35.7 and 14.7 MJ g^{-1} diet for protein, lipid and nitrogen free extract, respectively according to Jobling (1994).

Growth performance and feed utilization

Standard formulae were used to assess growth-feed

Table 1. Averages of water physicochemical characteristics parameters during experimental period.

	Diets			
Parameters	**FO**	**SO**	**CO**	**MX**
Temperature ^{o}C	26.0 ± 0.12	26.1 ± 0.14	25.4 ± 0.15	25.23 ± 0.12
pH	7.4 ± 0.1	7.5 ± 0.11	7.6 ± 0.1	7.5 ± 0.1
Dissolved oxygen (mg l^{-1})	6.3 ± 0.11	6.3 ± 0.11	6.3 ± 0.12	6.2 ± 0.11
Salinity (g l^{-1})	1.22 ± 0.1	1.23 ± 0.1	1.22 ± 0.1	1.22 ± 0.1
Unionized ammonia (mg l^{-1})	0.02 ± 0.01	0.022 ± 0.01	0.021 ± 0.002	0.021 ± 0.001

utilization and other relevant parameters during the growth trial and these included, initial average weight, final average weight, total feed consumed, weight gain (g), average daily gain (g fish day^{-1}), specific growth rate (SGR% day^{-1}), feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU%).

Statistical analysis

One way Analysis of Variance (ANOVA) was applied to test the effect of different dietary oil sources on various growth parameters, chemical composition and apparent digestibility coefficients according to Snedecore and Cochran (1987). Duncan Multiple Range test was used to detect the significant differences between the means of treatments (Duncan, 1955). All analysis was performed using SAS (version 6, 1986 SAS Institute, Cary, NC, USA).

RESULTS

Physicochemical characteristics

Water physicochemical characteristics (Table 1) revealed that temperature, pH, dissolved oxygen, salinity and unionized ammonia are within the optimum ranges for rearing Nile tilapia according to (Wangead et al., 1988; El-Shafai et al., 2004 and Ferreira et al., 2011). Similar physicochemical conditions were found in all tanks.

Chemical composition of diets

As can be seen in (Table 2), the four experimental diets were almost similar in protein content (305.3-305.5 g kg^{-1}) and gross energy (19.0-19.01 MJ kg^{-1} diets). However, they differed in their fatty acid composition (Table 3). The principal fatty acid class of FO diet was PUFA, SFA and MUFA, accounting for 46.61, 32.7 and 20.69 %, respectively. The FO diet was characterized by an abundance of n-3 (31.48 vs. 15.13 for n-6). On the other hand, vegetable oil diets (SO, CO and MX) represented a decrease in each SFA and PUFA compared with FO diet. High level of n-3/n-6 ratio was recorded in FO diet, followed by MX, SO and CO, respectively.

Growth performance

As presented in (Table 4) average initial weight ranged between 1.2 to 1.3 g fish^{-1} with insignificant differences among the dietary groups indicating the random distribution of the experimental fish among treatment groups. Final weights of the fish fed FO recorded the highest value (44.4 g fish^{-1}) followed in an insignificant order by the MX group and in a significant ($P<0.05$) order by the SO and CO groups, respectively. The same trend was observed with total gain in weight and the daily gain, where the SO and CO groups recorded the lowest significant ($P<0.05$) values compared to the other treatments groups (FO and MX) among them differences in growth traits cited above were insignificant. Furthermore, the FO group recorded the highest value of SGR followed in an insignificant decreasing order by MX and significant ($P<0.05$) decreasing order by the SO and CO groups, respectively.

As shown in (Table 4) average amounts of feed consumed were found to be 64.0, 65.0, 67.0 and 68.0 g fish^{-1} for the FO, MX, SO and CO groups, respectively which indicate slight increases in feed consumption in the dietary VO groups compared to the FO group. On the other hand, the best FCR (lowest) values were obtained by the FO group followed in an insignificant increasing order by MX group and a significant ($P<0.05$) increasing (worth) order by the SO and CO groups, respectively. As presented in the same table the highest PER and NPU values (2.20 and 34.39) were recorded by the FO group followed in an insignificant decreasing order by MX group and significant decreasing order of SO and CO groups, respectively.

Apparent digestibility coefficient

Averages of nutrient apparent digestibility coefficients in present study for dry matter (DM), energy (E), crude protein (CP), fat and nitrogen free extract (NFE) are presented in (Table 5). Results revealed that apparent

Table 2. Ingredients and approximate composition of experimental diets (g kg^{-1}).

	Diets			
Ingredientes	**FO**	**SO**	**CO**	**MX**
Fish meal	100	100	100	100
Soybean meal	220	220	220	220
Corn gluten meal	250	250	250	250
Wheat bran	350	350	350	350
Fish oil	60	-	-	20
Sunflower oil	-	60	-	20
Cotton seed oil	-	-	60	20
Vitamin & mineral mix[1]	15	15	15	15
Chromic oxide	5	5	5	5
Proximate analysis (*n=3*)				
Dry matter	924	921	926	923
Crude protein	305.5	305.4	305.3	305.4
Ether extract	100.6	100.4	100.3	100.5
Nitrogen free extract	456.1	456.4	456.3	456.3
Crude fiber	53.2	53.3	53.4	53.3
Crude ash	84.6	84.5	84.7	84.5
Gross energy (MJ kg^{-1} diet)[2]	19.01	19.01	19.01	19.0
ME (MJ kg^{-1}diet)[3]	16.06	16.05	16.01	16.05

[1]Vitamin-mineral premix, mg Kg^{-1} dry diets: vitamin A (as acetate), 7500 Iu kg^{-1} dry diet, Vitamin D3 (as cholecalcipherol); 6000IU kg^{-1} dry diet, vitamine E (as DL- □-tocopheryl-acetate); 150 IU kg^{-1} dry diet, vitamin k (as menadione Na-bisulphate); 0.06 ascorbic acid (as ascorbyle polyphosphate), 150 D-biotin, 42 choline (as chloride) 3000; folic acid, 3 niacin (as nicotinic acid), 30 pantothenic acid,60 pyridoxine, 15; ribflavine, 0.06; manganese sulphate, 0.18 ;potassium iodide, 0.02 zinc sulphate.
[2] Schulz *et al.* (2007).
[3]Jobling (1994).

Table 3. Fatty acid composition of experimental diets (% of total fatty acids).

Fatty acid (%)	FO	SO	CO	MX	Fatty Acid (%)	FO	SO	CO	MX
14:0	7.1	6.41	5.8	6.45	20:4n-3	0.61	0.14	0.15	0.3
16:0	19.1	15.4	14.51	16.35	20:5n-3	10.41	0.71	2.4	3.88
16:1n-7	8.11	3.2	1.2	3.1	22:	0.4	0.48	0.5	0.55
18:0	5.5	7.2	7.1	6.8	22:1n-11	0.9	0.6	0.56	0.66
18:1n-9	9.88	20.46	24.8	19.84	22:1n-9	0.4	0.22	0.18	0.25
18:2n-6	11.47	32.4	31.6	25.12	22:5n-3	0.5	0.41	0.38	0.31[ab]
18:3n-3	7.16	1.8	1.6	3.5	22:6n-3	9.8	4.6	4.4	6.28
18:4n-3	2.1	1.1	0.9	1.4	∑ SAF[1]	32.7	29.9	28.27	30.61
20:0	0.6	0.41	0.36	0.46	∑ MUFA[2]	20.69	25.0	27.28	24.65
20:1n-9	1.4	0.52	0.54	0.8	∑ PUFA[3]	46.61	45.1	44.45	44.74
20:2-n-6	0.46	1.4	0.9	0.94	∑ n-3	31.48	9.21	10.27	16.26
20:3n-6	1.1	0.89	0.78	0.93	∑ n-6	15.13	35.89	34.18	28.49
20:4n-6	2.1	1.2	0.9	1.5	n-3/ n-6	2.05	0.25	0.30	0.57
20:3n-3	0.9	0.45	0.44	0.58					

[1]SFA= refers to the sum of C14:0, C16:0, C:18, C20:0 and C22:0 percentages.
[2]MUFA= refers to the sum of C16 :1n-7, C18:1n-9, C20: 1n-9, C22:1n-11 and C22:1n-9 percentages.
[3]PUFA=C18:2n-6, C18:3n-3, C18:4n-3, C20:2n-6, C20:3n-6, C20:4n-6, C20: 3n-3, C20: 4n-3 C20:5n-3, C22:5n-3 and C22:6n-3.

Table 4. Growth performance of Nile tilapia fed the experimental diets for 120 days.

	Diets			
Parameters	**FO**	**SO**	**CO**	**MX**
Initial average weight (g fish^{-1})	1.2±0.05	1.3±0.06	1.3±0.5	1.2±0.05
Final average weight (g fish^{-1})	44.4^{a}±1.4	40.3 ^{c}±1.6	38.3 ^{d}±1.8	44.1^{ab}±1.3
Gain in weight (g fish^{-1})	43.2^{a}±1.1	39.0^{c}±1.5	37.5 ^{d}±1.6	42.9^{ab}±1.4
Average daily gain (g fish day^{-1})	0.36^{a}±0.15	0.32^{c}±0.12	0.31 ^{d}±0.16	0.35^{ab}±0.11
Specific growth rate (% day^{-1})[1]	3.0 ^{a}±0.18	2.85^{b}±0.14	2.80 ^{c}±0.13	3.0^{a}±0.16
Feed consumed (g fish^{-1})	64.0±1.8	67.0±1.6	68.0±1.5	65.0±1.4
Feed conversion ratio[2]	1.48^{a}±0.15	1.71^{c}±0.11	1.81 ^{d}±0.12	1.51^{ab}±0.1
Protein efficiency ratio[3]	2.20 ^{a}±0.2	1.89^{c}±0.15	1.80 ^{d}±0.18	2.16^{ab}±0.16
Net Protein Utilization (NPU%) [4]	34.39^{a}±2.15	28.80^{c}±1.12	27.45^{d}±1.11	33.29^{ab}±2.1

Values represent mean and standard deviation. Means in the same row with different superscript letters are significantly different (P<0.05).
[1]Specific growth rate = 100 X (Ln final weight- Ln initial weight)/ 120 day.
[2]Feed conversion= (feed given per fish)/ (weight gain per fish).
[3]Protein efficiency ratio = (weight gain per fish)/ (protein intake per fish).
[4]Net protein utilization (%) = (final body protein - initial body protein)/(protein intake) ×100.

Table 5. Apparent digestibility coefficients (%) for the experimental diets.

	Diets			
Nutrients	**FO**	**SO**	**CO**	**MX**
Dry matter	85.6	85.4	85.1	85.5
Energy	90.2	89.8	89.6	90.1
Protein	93.8	93.5	93.3	93.6
Fat	94.5	94.2	94.1	94.6
Nitrogen free extract	71.4	71.2	71.0	71.1

Table 6. Carcass analysis of Nile tilapia fed the experimental diets (g kg^{-1} wet basis).

		Diets			
Items	**Initial**	**FO**	**SO**	**CO**	**MX**
Dry matter	242±1.6	271^{a}±3.18	270^{a}±3.6	276^{a}±3.14	274^{a}±1.16
Crude protein	152±1.8	158^{a}±2.16	152^{a}±2.2	56^{a}±2.2	154^{a} ±2.11
Crude lipid	32±1.5	55^{b}±1.8	62^{a}±1.6	61^{a}±1.4	58^{b}±1.16
Crude ash	58±1.2	58^{a}±1.4	56^{a}±1.6	59^{a}±1.2	54^{a}±1. 6

Values represent mean and standard deviation. Means in the same row with different superscript letters are significantly different (P<0.05).

digestibility coefficients DM, E, CP, fat and NFE were not significantly affected with the dietary treatments.

Carcass analysis

Results of whole fish body chemical analysis showed that the applied dietary treatments had no significant effects on whole body dry mater, crude protein and ash contents, although the total lipid was affected and significance (P<0.05) between groups (Table 6). Fish fed with CO, SO and MX showed high lipid content (62, 61 and 58 g kg^{-1}, respectively) compared with those fed FO diet (55 g kg^{-1}). As illustrated in (Table 7), the muscle fatty acid composition were affected by dietary oil source (P<0.05). Fish fed FO diet had the highest (P<0.05) PUFA among the experimental groups. The same trend was observed with the n-3 HUFA, which showed a high level (P<0.05) compared with the VO groups. In contrast, the VO groups recorded high values of n-6 HUFA and lower n-3HUFA. Consequently, high n-3/n-6 ratio was obtained with FO group (1.08) compared with 0.63, 0.47 and 0.43 for MX,

Table 7. Fatty acid composition of tilapia muscle fed experimental diets (% of total fatty acids).

Fatty acid (%)	FO	SO	CO	MX	Fatty acid (%)	FO	SO	CO	MX
14:0	4.24^{b}	3.88^{c}	4.6^{a}	4.25^{b}	20:4n-3	0.8^{a}	0.26^{c}	0.24^{c}	0.42^{b}
	±0.3	±0.2	±0.23	±0.32		±0.1	±0.1	±0.1	±0.15
16:0	19.45^{c}	21.4^{b}	22.52^{a}	21.5^{ab}	20:5n-3	2.85^{a}	1.38^{c}	1.26^{c}±	1.84^{b}
	±1.14	±1.11	±1.16	±1.2		±0.5	±0.2	0.18	±0.2
16:1n-7	4.5^{a}	3.5^{b}	4.65^{a}	4.18^{b}	22:0	0.06^{a}	0.15^{a}	0.12^{a}	0.12^{a}
	±0.13	±0.11	±0.16	±0.12		±0.01	±0.05	±0.04	±0.02
18:0	12.1^{a}	11.61^{a}	11.2^{a}	11.5^{a}	22:1n-11	0.26^{a}	0.16^{a}	0.14^{a}	0.17^{a}
	±1.11	±1.16	±1.14	±1.12		±0.02	±0.01	±0.01	±0.02
18:1n-9	12.4^{b}	14.28^{a}	13.43^{ab}	11.83^{c}	22:1n-9	0.05^{b}	0.4^{a}	0.3^{a}	0.26^{a}
	±1.4	±1.3	±1.16	±1.2		±0.01	±0.1	±0.1	±0.1
18:2n-6	11.1^{d}	19.64^{a}	18.8^{ab}	17.4^{c}	22:5n-3	6.6^{a}	1.7^{c}	1.4^{c}	3.31^{b}
	±1.25	±1.5	±1.3	±1.2		±1.15	±0.5	±0.4	±0.8
18:3n-3	1.45^{a}	1.21^{b}	1.28^{b}	1.5^{a}	22:6n-3	10.65^{a}	5.56^{c}	4.91^{c}	7.11^{b}
	±0.14	±0.11	±0.12	±0.15		±1.8	±1.15	±0.5	±.12
18:4n-3	1.2^{b}	3.1^{a}	2.9^{a}	2.6^{a}	∑ SAF[1]	35.95^{b}	37.28^{a}	38.65^{a}	37.58^{a}
	±0.11	±0.13	±0.15	±0.18		±2.12	±1.18	±1.5	±1.6
20:0	0.1^{a}	0.24^{a}	0.21^{a}	0.21^{a}	∑ MUFA[2]	18.65^{a}	18.96^{a}	20.23^{a}	17.75^{b}
	±0.08	±0.06	±0.04	±0.05		±1.4	±1.5	±1.6	±1.2
20:1n-9	1.44^{a}	0.62^{b}	1.71^{a}	1.31^{a}	∑ PUFA[3]	45.4^{a}	43.76^{b}	41.12^{d}	44.67^{ab}
	±0.11	±0.1	±0.12	±0.11		±1.8	±1.5	±1.4	±1.6
20:2-n-6	1.46^{a}	1.85^{a}	1.64^{a}	1.62^{a}	∑ n-3	24.15^{a}	13.91^{c}	12.59^{c}	17.42^{b}
	±0.1	±0.12	±0.15	±0.13		±2.1	±1.5	±1.2	±1.8
20:3n-6	1.88^{a}	1.78^{a}	1.81^{a}	1.81^{a}	∑ n-6	22.25^{c}	29.55^{a}	28.68^{ab}	27.25^{ab}
	±0.2	±0.27	±0.18	±0.22		±1.11	±1.5	±1.4	±1.6
20:4n-6	5.81^{a}	6.58^{a}	6.28^{a}	6.42^{a}	n-3/ n-6	1.08^{a}	0.47^{b}	0.43^{b}	0.63^{b}
	±0.5	±0.4	±0.6	±0.4		±0.16	±0.12	±0.11	±0.14
20:3n-3	0.6^{a}	0.7^{a}	0.6^{a}	0.64^{a}					

Values represent mean and standard deviation. Means in the same row with different superscript letters are significantly different (P<0.05).
[1]SFA= refers to the sum of C14:0,C16:0, C:18, C20:0 and C22:0 percentages.
[2]MUFA= refers to the sum of C16 :1n-7, C18:1n-9, C20: 1n-9, C22:1n-11 and C22:1n-9 percentages.
[3]PUFA= C18:2n-6, C18:3n-3, C18:4n-3, C20:2n-6, C20:3n-6, C20:4n-6, C20: 3n-3, C20: 4n-3 C20:5n-3, C22:5n-3 and C22:6n-3.

SO and CO, respectively. The dominant fatty acids in the muscle of FO fed fish were 16:0, 18:0, 18:1n-9,18:2n-6, 22:6n-3 and 20: 5n-3. On the other hand, the dominate fatty acids in muscle of fish fed MX, SO and CO were 16:0, 18:0, 18:1n-9, 18:2n-6, 20:4n-6 and 22:6n-3. Amongst the fish receiving FO diet its recorded high DHA (10.65%) compared to the other experimental groups (7.11, 5.56 and 4.91% for MX, SO and CO, respectively).

DISCUSSION

The replacement of dietary FO in aquafeeds with readily available and more economical alternatives sources, such as vegetable oils (VO) is consequently a highly investigated research topic and an approach increasingly being adopted by feed - manufacture companies.

The growth performance of tilapia in the present study was agree with previously reported findings for this species (Ferreira *et al.,* 2011) and similar to the results obtained by (Matsushita *et al.,* 2006; Yones and Abdel-Hakim, 2010), the decrease of any different treatments was likely attributable to the subsequent presence of secondary levels of dietary EPA,20:5n-3 and DHA,22:6n-3 as reported previously when similar diets were implemented for this species (Ribeiro *et al.,* 2008 and Ferreira *et al.,* 2011). However, during the growth trial some interesting, statistically significant results (P<0.05) were observed in parameters related to nutrient efficiency from FCR, PER and NPU among diets.

As can be seen from (Table 4), the FCR, PER and NPU were not significantly different (P<0.05) between FO and MX diets and the best value was recorded by FO. On the other hand, fish fed SO and CO diets showed the

worst value for these parameters. Similar and comparable results of FCR and PER were recorded with tilapia (Bahurmiz and Ng, 2007; Gao *et al.,* 2011 and Yones and Abdel-Hakim, 2010).

Amongst the VO-based diets in the present trial, fish receiving the MX diet showed high growth performance and net protein utilization. This result is in agreement with the other several warm water finfish species obtained by (Ng and Gibon, 2010). They recorded that VO has been shown to be responsible for enhanced growth performance and an apparent protein-sparing effect in tested diets.

The experimental diets in the present study showed a good digestibility coefficient for the tested diets. These results are in agreement with the results of (Mamun *et al.,*2004 and Yones and Abdel-Hakim 2010). Similar and comparable ADC values of feed dry matter, protein, lipid and energy were also observed by several authors in digestibility studies with tilapia (Shiau *et al.,*1987; Koprucu and Ozdemir 2005; Gay-Sliessegger *et al.,* 2005, Yones, 2010 and Yones and Abdel-Hakim 2010).

The carcass proximate composition of tilapia indicated that the dry matter, protein and ash were not affected by the dietary oil sources. Similar results have been reported for tilapia by (Borgeson *et al.,* 2006; Yue and Zhou 2008 and Ferreira *et al.,* 2011) and Murray cod (Turchini *et al.,* 2011). In the present study, the modification of parameters relative to fat utilization in FO and MX fed fish did not result in any significant modification to the total lipid content of analyzed tissues, although the lipid content of whole body samples of fish fed MX was numerically smaller than that SO and CO treatments.

In a previous trial in sea bream (Penedito-Palos *et al.,* 2007; 2008), no determined effects on growth performance were found with the replacement of up to 60% of the added FO, where a slight but significant reduction in feed intake and weight gain was found with total replacement. In the same manner, Regost *et al.*(2003) also reported the feasibility of total replacement of FO by vegetable oils in turbot fish meal-based diets. However, in the present study we reported that the use of well balanced plant protein diets with a low inclusion level fish meal (100 g kg^{-1}), just to cover EFA needs, VO can replace up to 66% of fish oil without adverse effects on growth performance of tilapia. This is in accordance with the previously reported results for tilapia fed low-fish meal content diets which the dietary FO was replaced by VO alternatives (Ferreira *et al*., 2011).

It is well documented that in fish, the substitution of dietary FO with alternative lipid sources lacking in n-3 LCPUFA is responsible for increased elongase and desaturase activity and transcription rate (Francis *et al.,* 2007, Turchini *et al.,* 2006). However, it has also been shown that metabolic effort is insufficient to compensate for the decreased n-3 LCPUFA intake, resulting in a significant reduction in n-3 LCPUFA tissue level (Turchini and Francis, 2009). In the same manner, the present study demonstrate that tilapia fed a vegetable oil based diet had a marked enhancement in the accretion and desaturated of FO. However, this was insufficient in preserving the 20:5 n-3 and 22:6n-3contents of the whole body, which were lower than the fish fed FO based diet.

As evident in the literature (see review by Robin *et al.,*2003 and Turchini *et al.,*2009) and in agreement with the present study, the fillet fatty acid make-up at the end of the grow-out period was affected by the dietary lipid source. These deviations from the overall fatty acid composition of fish fed each FO and VO diets were recorded, although large differences remained apparent. However, by observing individual fatty acids in diets and the resultant fillets, it was evident that the fatty acid composition of fish fillets was not a simple reflection of the diet, but a clear indication of an active *in vivo* fatty acid metabolism in this species. Specifically, at the end of the grow-out period the content of 18:1n-9 was reduced (from diet to fillet) into 14.28 and 11.43 in the fillet of fish fed each SO and CO, respectively, this is in agreement with previous studies in which it was demonstrated that fatty acids provided in dietary surplus are the object of intensive β-oxidation for energy production (Stubhaug *et al.,* 2006; 2007). Therefore, the results of the present study are in agreement with other published studies (Stubhaug *et al.,* 2006), clearly suggest that some dietary fatty acids, particularly MUFA and to a leaser extent SFA, can be preferentially β-oxidation when provided in dietary surplus. Therefore, it is feasible to suggest that some dietary n-3 LC-PUFA, DHA in particular, can be spared from catabolism and thereby significantly improve its deposition into the fillet.

The fatty acid composition of tissue in fish is known to be affected by the fatty acid composition of dietary lipids. Such differences in lipid profile presumably reflect different rates of assimilations of dietary fatty acids together with different rates of catabolism of fatty acids once assimilated. Furthermore, interaction between the different fatty acids determine the final composition of muscle lipid (Torstensen *et al.,*2000; Bell *et al.,*2003a; 2003b; Fonseca-Madrigal *et al.,* 2005 and Matsushita *et al.,* 2006). In general, the substitution of FO (rich in n-3 HUFA) by vegetable oils (rich in n-6PUFA in diets) results in a reduction in n-3 long-chain fatty acids, EPA and DHA, and an increase in the levels of 18-carbon fatty acids such as oleic, linoleic and linolenic acids in tissues of several species (Greene and Selivonchick 1990; Yildirim-Aksoy *et al.,* 2007; Pettersson *et al.,* 2009). Similar observations were made in this study, particularly regarding n-3 and n-6 PUFAs. The fish fed on SO and CO diets showed increased linoleic acid (18:2n-6) levels, whereas fish fed with the FO diet had significantly higher levels of docosahexionc acid DHA (22:6n-3). The arachidonic acid (ARA, 20:4n-6) concentration found in tilapia muscle in this study ranged from (5.81 to 6.58%) are markedly higher than the ones reported by Matsushita *et al. (*2006), ranging from (1.2 to 2.0%) using

different vegetable oils, while its comparable to the previous results of Ferreira *et al.,* 2011 (5.8 to 8.12%) and Ribeiro *et al.* (2008) who found levels from (5.8 to 9.2%) in tilapia muscle.

Results of the present study suggest that potential exists for replacing FO with a blend of VO in the feed of Nile tilapia, *Oreochromis niloticcus*, without compromising growth performance. It is important to establish that alternative dietary lipids to FO are not only supplied in the correct quantities and balance for optimal growth and feed efficiency, but can maintain optimal flesh chemical composition. The present study suggests that the growth performance can be more successfully attained if dietary FO is replaced by a blend of 66% VO and that provides a more balance fatty acids composition in comparison to replacement with a single VO.

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