Mediterranean Fisheries and Aquaculture Research

homepage: www.dergipark/medfar.gov.tr

ORIGINAL RESEARCH PAPER

Received: 7 July 2018 | Accepted: 7 Sept 2018

Comparing the Effects of Feeding a Fish Oil- or a Cod Liver Oil -Based Diet on Growth, Feed Utilization and Muscle Fatty Acid Composition Nile Tilapia (*Oreochromis niloticus)*

Arzu Özlüer Hunt[1*], Ferbal Özkan Yılmaz[2], Zeynep Erçen[3]

[1]Faculty of Fisheries, Aquaculture Department, Mersin University, Mersin/Turkey
[2]Faculty of Fisheries, Basic Sciences Department, Mersin University, Mersin/TURKEY
[3]Vocational School of Adana, Food Processing Department, Cukurova University, Adana/ TURKEY
*:email: ahunt@mersin.edu.tr

ABSTRACT

The present study was conducted to study the effect of fish oil (FO) and cod liver oil (CLO) as the dietary lipid sources on the growth performance, feed utilization and fatty acid (FA) composition of *Oreochromis niloticus*. Two isonitrogenous (38% crude protein), isocaloric (18.9 MJ GE/kg) diets containing 8% lipid were formulated. Each diet was fed to triplicate groups of 17 fish with a mean initial body weight of 18.18 ± 0.02 g. Fish were fed with %3 of their body weight two twice daily. The fish were kept at 28±1 °C in 6 square experimental cages (1×1×1.25 m) for 60 days. Results revealed that the source of lipid significantly affect (P<0.05) final body weight, live body weight and daily growth rate but did not affect specific growth rate, feed conversion ratio and survival rate of tilapia. Significant difference in the fillet proximate composition of fish fed FO or CLO diets were observed. Fish fed FO diet showed lower lipid deposition and higher protein amount in muscle tissues (P<0.05). The deposition of fatty acids in fish tissues was mostly affected by the fatty acid profile of the diets. When compared to fish fed FO diet, fatty acid profiles of fillets in fish fed CLO-based diet had significantly higher concentration of saturated and monounsaturated fatty acids, but lower levels of polyunsaturated fatty acids (PUFA). Fillet of fish fed the FO diet had significantly higher concentrations of DHA (docosahexaenoic acid) compared with fish fed CLO-based diet (P<0.05). EPA (eicosapentaenoic acid) was not significantly different between diets (P>0.05). In fish fed FO diet, both $n-3$ and $n-6$ PUFA were the highest when compared with fish fed with CLO diet. The lipid source did not influence (P<0.05) hepatosomatic index (HSI) and viscerosomatic index (VSI). However, fish fed CLO contained diet showed significantly higher liver fat (20.20±0.22) than fish fed FO diet (13.88±0.22) (P<0.001).

KEYWORD: Cod liver oil, Fatty acid composition, Fish oil, Growth oil, EPA/DHA, *Oreochromis niloticus*

How to cite this article: Ozluer Hunt, A., Özkan Yılmaz, F., Erçen, Z. (2018). Comparing the Effects of Feeding a Fish Oil- or a Cod Liver Oil -Based Diet on Growth, Feed Utilization and Muscle Fatty Acid Composition Nile Tilapia (*Oreochromis niloticus)*. *MedFAR*., 1(3):130–142.

1. Introduction

It has been widely acknowledged that fisheries play a crucial role in improving food security in developing countries. It is a vital source of protein, essential fatty acids, vitamins and minerals for people in low income and food-deficient countries. An estimated 520 million people, nearly 8% of the world population, derive their nutrition from fisheries and fish-related economic activities. Furthermore, with the continued increase in the awareness of health benefits, the global demand for aquatic foods, even in the developed countries, is expected to continue to rise. The world's population is expected to grow by more than 30 % by 2050, resulting in an estimated 2.3 billion more mouths to feed, with the major growth expected in the developing countries where fish is the main source of food protein (UN, 2010).

With the understanding that the natural world resources have started to become incapable to respond to the needs of the world population which is estimated to have increased 3 times in the last 1000 years, the production of fisheries products obtained through capture fisheries has started to be rapidly moved to aquaculture so that the aquaculture in the world has started to show a rapid progress. Since the 1950s, aquaculture has gained a rapid momentum and has become source of food rich in protein and fatty acids, and at the same time production of aquatic products has increased from 34.6 million tons per year to 70.2 million tons per year (FAO, 2015). The intensive aquaculture of tilapia, *Oreochromis spp*. is rapidly developing and tilapias are the second most widely cultured fish in the world. Tilapia started to be produced in Asia in the early 1950s, with production of 112.000 tons in 1970s, 1.100.000 tons in 2001 and 4.9 million tons in 201. Tilapia farming is well developed in Latin America, Australia and some European countries (FAO, 2015). Tilapia (including all species) is the second most widely farmed fish after carps. Nile tilapia is the most widely cultured tilapia species with significant contribution to overall tilapia production. In terms of value, Nile tilapia is the sixth most important farmed species in the world and third in terms of international trade (Kumar and Engle, 2006).

Tilapia farming enjoys growing influence on global fish supplies. As aquaculture is turning to become a major supplier of aquatic products in the world, sustaining the nutritional quality of farmed products and source of health promoting *n*-3 PUFA for the human consumption is gaining more and more importance (Ioannis et al., 2007). Commercial fish feeds, especially marine fish feed, contain high amounts of marine fish oil (FO) obtained from anchovy, sardine, capelin, menhaden and herring (Grant et al., 2008) and FO is used as the main dietary lipid source in many commercial fish feeds. The trend to increase lipid content of fish feeds for marine fish to optimize growth, feed conversion and protein utilization has caused an increase in demand for fish oil. The global production of fish oil (FO) based on fisheries landing is stable, and it is estimated that by 2020 the fish feed industry will require at least 50% of total world production of fish oil (Barlow, 2000; Montero et al., 2005). Aquafeeds are made using fish oil as the main source of lipids, since it has been rapidly available and enjoys a high content of *n*-3 HUFA (highly unsaturated fatty acids), which is essential fatty acidy for fish. On the other hand, capture fisheries will not be able to meet the increasing demand of fish oil due to the fact that sustainable levels are threatened by over fishing, climate changes and increasing demand from other sectors (Sargent and Tacon, 1999). The most optimistic forecasts reveal that in few years, world fish oil production may not be able to meet the increasing demand for animal feed. On the other hand, global vegetable oil supply has increased volumes which are 100 times higher than that of fish oil in recent years (Piedecausa et al., 2007). It is a widely acknowledged fact that aquaculture industry cannot continue to depend on restricted stocks of marine fish for FO supply. Consequently; there is increasing interest in use of alternative oils in both marine and freshwater aquafeeds to partly substitute and decrease the dependence on FO. Fish cannot synthesize the essential fatty acid (EFA), such as linoleic (18:2 *n*-6) and linolenic acid (18:3 *n*-3) *de novo*. Therefore, these substances must be supplied through feed (Takeuchi et al. 1980). The requirements of fish for EFA for high growth rates

in farmed fish have been well examined and the demand for *n*-3 and *n*-6 FA seems to be species-specific. Generally, cold water fish require PUFA of *n*-3 series, while warm water species require PUFA from either the *n*-3 and *n*-6 series. In general, freshwater fish possess higher capacities for the conversion of C18 PUFA (polyunsaturated fatty acids) to the longer C20 and C22 homologues compared to marine species (Sargent et al., 2002). Tilapias have a higher requirement for *n*-6 than for *n*-3. They are known to have some capabilities to elongate and desaturate 18:3*n*-3 to20:5*n*-3 and 22:6*n*-3 (Olsen et al., 1990; Tocher et al., 2002). Substitution of fish oil by alternative lipid sources seems to possible if the EFA requirements are met (Sargent et al., 1999). Therefore, substitution of FO using different alternative oils would have a positive impact both on demand for FO and its price (Piedecausa et al., 2007).

The search for alternative FO is becoming more important because of the increasing prices due to the decrease in stocks of fish meal and oil. This trend, is of crucial importance aquaculture sector. However, the use of alternative oil sources for aquafeeds has not yet reached a sufficient level. The diets of protein, fat, carbohydrates, minerals and vitamins in the prepared feed mixes are influential on the growth needs of the individuals and fatty acid profile. Apart from aquaculture facilities, post-harvest quality of farmed fish is an important aspect that should be taken into considerations when evaluating the suitability of different source oils as dietary FO alternatives. However, it is well documented that fish consumption can have a positive impact on human health as well as risks related to cardiovascular disease (CVD) and coronary heart disease (CHD) (Mohebi-Nejad and Bikdeli, 2014). Therefore, the relations between fish as food and human health are strongly correlated with the fatty acid composition of the diet (Kris-Etherton et al., 2002). The fatty acid content of fish can be changed with diets containing different lipid sources (Naylor et al., 2009).

The effect of replacement of fish oil (FO) by Cod liver oil (CLO) in tilapia diets on physical quality of fillets and proximate composition of Nile tilapia are not well studied. Therefore, the purpose of this study was to understand the effect of complete replacement of dietary FO by CLO in tilapia diet on growth performance, feed utilization and fatty acid composition of Nile tilapia (*Oreochromis niloticus)*. Keeping in mind that the goal of sustainable aquaculture is to sustain aquatic products production without damaging the natural balance and producing high quality and healthy products.

2. Materials and Methods

2.1. Diet Preparation

Two isonitrogenous (38% crude protein), isocaloric (18.9 MJ GE/kg) diets containing two different oil sources, Fish oil (FO) and cod liver oil (CLO) were prepared **(**Table 1**)** to meet the nutritional requirements of tilapia (*Oreochromis niloticus*). The dietary fatty acid composition is shown in Table 2. Proximate analysis of the diets was performed according to AOAC (1990). After that, 2.0 mm diameter pellets were wet-extruded, air dried to about 12% moisture, and sealed in vacuum-packed bags and frozen (-20 ˚C) until feeding.

Table 1. Formulation and proximate analysis of the experimental diets (% dry matter)

	FO	CLO
Fish meal	6.00	6.00
Soybean meal	50.0	50.0
Wheat flour	15.0	15.0
Yellow corn meal	20.0	20.0
Vitamin premix[a]	1.00	1.00
Mineral premix[b]	1.00	1.00
Ethoxyquin	0.0125	0.0125
Lignobond (as binder)	1.9875	1.9875
Calcium phosphate	1.00	1.00
Fish oil[c] (FO)	4.00	-
Cod liver oil[d] (CLO)	-	4.00

	Proximate composition	
Dry matter	89.00	88.50
Crude protein	38.03	38.25
Crude lipid	8.52	8.25
Crude fiber	5.70	5.90
Ash	9.25	9.10
Ca	1.50	1.52
NFE[e]	38.50	38.50
Gross Energy[f] (Mj/kg)	18.95	18.89

[a] Vitamin premix (mg/kg diet): thiamin-H-Cl, 10.0; riboflavin, 12.0; niacin, 50; pyridoxine-HCl, 10.0; cyanocobalamine (1%), 4; pantothenic acid, 30; biotin (2%), 1,0; inositol, 400; folic acid, 3.0; choline chloride, 1500.0; L-ascorbyl-2-monophosphate-Ca, 300.0; vitamin A 500-P, 20.0; vitamin D_3, 4.0; vitamin E (all-rac-a-tocopheryl acetate, 50%) 150.0; vitamin K_3, 7.0; BHT, 10.0; α-cellulose, 7489.

[b] Mineral premix (g/kg diet): Ca $(H_2PO_4)_2$- H_2O, 10.00;$MgSO_4$- H_2O,3.00;$NaHCO_3$, 2.00; $FeSO_4$-$7H_2O$, 0.60; $ZnSO_4$-$7H_2O$,0.35; $MnSO_4$-$7H_2O$, 0.18; KI, 0.01; Na_2SeO_3,0.01; CoCl-$6H_2O$(1%), 0.05; $CuSO_4$-$5H_2O$, 0.01; zeolite 13.79

[c] Fish oil (Anchovy) was supplied by Agromarin Fish Feed Co. Ltd-Turkey

[d] Cod liver oil (500ml) Produced by Henry Lamotte GmbH/Germany supplied by Akimtaş A.Ş. Istanbul/Turkey

[e] Nitrogen-free extract (NFE) (calculated by difference) = 100- (moisture + crude protein + crude fat + ash + crude fiber)

[f] Gross energy, calculated based on 0.17, 0.237, 0.398 MJ/g for carbohydrate, protein and lipid, respectively (Brett, 1973).

Table 2. The percentage fatty acid profile (% of total fatty acids) of feed used in the research

Fatty acids	**FO (%)**	**CLO (%)**
14:0	6.51±0.00	4.57±0.05
15:0	1.15±0.00	0.61±0.01
16:0	22.03±0.02	17.18±0.04
17:0	1.71±0.01	1.62±0.01
18:0	4.40±0.01	3.79±0.03
20:0	0.90±0.01	0.33±0.01
21:0	NA	NA
22:0	0.24±0.00	0.13±0.01
23:0	1.15±0.04	NA
24:0	0.33±0.01	0.38±0.01
∑SFA	38.42	28.61
14:1	0.17±0.00	0.15±0.01
16:1	5.43±0.02	6.90±0.02
17:1	0.62±0.01	0.55±0.01
18:9	19.73±0.05	25.29±0.05
20:9	1.16±0.01	8.96±0.02
22:9	0.51±0.01	6.62±0.03
24:9	1.72±0.01	1.68±0.06
∑MUFA	29.34	50.15
18:3*n*-3	2.45±0.02	1.01±0.02
20:3*n*-3	0.04±0.04	NA
20:5*n*-3	5.52±0.03	3.93±0.02
22:6*n*-3	9.99±0.05	5.04±0.01
∑*n*-3 PUFA	18.00	9.98

18:2*n*-6	13.31±0.04	10.34±0.02
18:3*n*-6	0.12±0.00	0.10±0.01
20:4*n*-6	NA	NA
22:2*n*-6	1.17±0.00	0.39±0.01
∑*n*-6 PUFA	14.6	10.83
∑PUFA	32.6	20.81
n-3/*n*-6	1.23	0.91
EPA/DHA	0.55	0.77
PUFA/MUFA	1.11	0.41

2.2. Experimental Systems and Animals

The experiment was carried out at the outdoor installations of the Çukurova University, Faculty of Fisheries Fresh Water Fish System Culture Unit in Adana, Turkey. The experimental systems consisted of 12 experimental cages (1×1×1.25 m). Every cage was located inside big concrete pond and cages joined with each other to one side of the cage. Wooden walkways connected the cages to the pond bank. Water depth in the pond was kept at 1 m throughout the experiment by adding water continuously. Fresh water was supplied from Seyhan Dam Drainage systems by pipes. All cages were cleaned every 2 weeks after fish sampling. Dissolved oxygen was measured every other day using YSI model 58 oxygen meters (Yellow Springs Instrument Company, Yellow Springs, OH). pH was monitored twice weekly using an electronic pH meter (pH pen. Fisher Scientific, Cincinnati, OH). Total NO_3, NO_2 and N-NH_4 were monitored once a week using a Merck-Spectroquant® Nova 60 A.

Nile tilapias, *O. niloticus*, with an average initial body weight of 18.18 ± 0.02 g were obtained from same culture unit. The fingerlings were stocked into three cement ponds at fresh water fish unit before the experiment started. After stocking into the cages fish were allowed to acclimatize for 2 days without feeding. Total of one hundred two fish were weighted individually and stocked in every cage (17 fish/ per cage-three replicate per group). Before the experiment started fish were fed a commercial diet at a level of 3% of body weight. (protein:32%; lipid:5.6%, Çamli-Yem-İzmir/Turkey). Fish were fed the calculated quantity of fish oil (FO; Diet A) and Cod liver oil-based (CLO; Diet B) diets two times a day (08:00, 16:00) until the end of the experiment. Feeding rates were 3% of body weight daily during the experiment. Fish from each cage were weighted biweekly. On the days of weighing no feed was offered as fish experienced stress.

2.3. Sample collection and analytical methods

At the end of 60-day feeding trial, fish in each cage were individually weighed and sampled for proximate and fatty acid composition. six fish from each cage were used for muscle proximate composition analysis and the livers and viscera of nine fish per cage were weighted for calculation of hepatosomatic index (HSI) and viscerosomatic index (VSI). Six fish from each cage were sampled for fatty acid composition. The fish muscle tissue samples were homogenized in a blender and sealed in plastic bags, and stored frozen (-20 ˚C) until analyzed. Prior to analysis samples were thawed at 4 ˚C for 24 h. Homogenized and thawed samples from each cage were pooled in equal amounts before proximate and fatty acid analyses were made. Moisture and crude ash contents were calculated according to standard methods (AOAC, 1990). Crude lipid content was determined according to Bligh and Dyer (1959). Crude protein content was analyzed using Macro Kjeldahl method (N x 6.25) (AOAC, 1990).

Extracted lipids were stored under liquid nitrogen at -20 ˚C for the determination of fatty acid profile in the whole body and muscle samples. The fatty acids in the total lipid were saponified into the free form by saponification with 0.5 N methanolic NaOF, followed by esterification with 14% BF_3 (w/v) in methanol (IUPAC, 1979). Esterified samples were analysed using a thermoquest trace

gas chromatograph equipped with a Supelco-SP-2330 fused-silica capillary column (30m×0.25 mm i.d., 0.20 µm film thickness of polyethylene glycol) (Supelco Inc., Bellefonte, PA, USA) and a flame-ionization detector (FID). Helium (30 ml min^{-1}) was used as a carrier gas. The samples were injected at 120 ˚C. After 2 min. temperature was raised 5 ˚C min^{-1} to 220 ˚C where it was kept for 8 extra minutes. The temperatures of the injector and the detector were set at 240 and 250 ˚C, respectively. Fatty acid methyl esters were identified by comparing their retention times with those of the commercial fatty acid methyl ester standards (FAME mix C4-C24; Supelco LB41302; Code Number:18919). The relative concentrations of fatty acids were expressed as percentages of their total.

2.4. Statistical analyses

Results are reported as mean ± SEM throughout the text. Differences between fish fed with FO and CLO group were analyzed using the Student`s *t* test. The significance was accepted at the probability value of 0.05 or less. The SPSS ver. 20.0 statistical programs were used to evaluate data.

3. Results

Fish appeared healthy during the experiment duration. Fish in both experimental groups performed their feeding activity. Different oil sources did not change water quality parameters. Water temperature was monitored every day by a mercury thermometer suspended at 30-cm water depth. During the 60 days feeding trial, the average water quality parameters were: water temperature, 27.5 ± 0.8 ˚C, dissolved oxygen, 5.3± 0.07 mg /l, pH, 8.3±0.15, NO_3, 0.4±0.1 mg/l, NO_2, 0.02±05 mg/l, NH_4, 0.06±0.5 mg/l.

Fish grown with FO contained diet had significantly higher average growth rate, live weight gain and daily growth rate than fish fed with CLO diet in the end of the experiment (P<0.05; Table 3). Specific growth rate (SGR) and feed conversion ratio (FCR), did not significantly affected by oil source in tilapia diet (Table 3).

Table 3. Growth, feed utilization and biometrical parameters of tilapia, *O. niloticus* fed with different oil sources

	FO	**CLO**	
Initial body weight (g)	18.16±0.18	18.11±0.19	*NS*
Final body weight (g)	74.96±1.46[a]	73.50±1.07[b]	*
Weight gain (g)	56.80±1,25	55.39±1.06	*
Live weight gain (%)	286.87±0.62[a]	276.55±0.89[b]	*
Daily growth rate (g)	0.87±0.01[a]	0.83±0.01[b]	*
SGR (%/d)	2.79±0.07	2.22±0.01	*NS*
FCR	2.09±0.04	2.11±0.01	*NS*
HSI	4.12±0.25	3.66±0.18	*NS*
VSI	13.58±0.45	13.74±0.18	*NS*

Values in the same row with the same superscript are not significantly different NS = (P>0.05), * (P<0.05). Values are expressed ±SEM of three replicates in each groups (n=3x17 except for HIS and VSI where n = 9). Weight gain (WG) = final body weight (g)-initial body weight (g); specific growth rate (SGR) = ln final body weight-ln initial body weight (g)/t (time); feed conversion ratio (FCR) = dry feed fed (g)/weight gain; Hepatosomatic index (HSI) = 100 × Liver weight (g)/ Body weight (g); Viscerosomatic index (VSI) =100 × Viscera weight (g)/ Body weight (g)

The fatty acid composition of the tilapia muscle is presented in Table 4. Dominant fatty acids in both fish fed with FO and CLO based diet were 14:0 (Myristic acid), 15:0 (Pentadeclic acid), 16:0 (Palmitic acid), 18:0 (Stearik acid), 20:0 (Arachidic acid), 21:0 (Henicosonoic acid), 17:1 (Heptadeconoic acid), 18:1*n*-9 (Oleic acid), 20:1*n*-9 (Eicosonoic acid), 18:2*n*-6 (Linoleic acid), 18:3*n*-6

(Gamma-linolenic acid), 18:3*n*-3 (Alpha-linolenic acid), 20:4*n*-6 (Arachidonic acid), 20:5*n*-3 (Eicosapentaenoic acid/EPA), 22:6*n*-3 (Docosahexaenoic acid/DHA). (Table 3). Fish fed with FO based diet contained significantly higher (P<0.01) proportions of 15:0, 20:0, 24:1*n*-9 (Nervonic acid), 22:6*n*-3, 18:2*n*-6, 20:4*n*-6 and lower proportions of 16:0, 16:1(Palmiteloic acid), 20:1*n*-9, 22:2*n*-6 in muscle tissues than that of fish fed with CLO added diet (Table 3). 14:0, 16:0 and 18:0 were also the major saturated fatty acids (SFA), contributing approximately 90% to the total SFA content of the lipids in both fish fed with FO and CLO added reared tilapia muscle tissue samples. Furthermore, the total SFA content of muscle tissue lipids were 36.53% and 38.10% in FO and CLO reared fish respectively.

18:1*n*-9 was identified as a primary monounsaturated fatty acid (MUFA) in both groups fed with FO and CLO added diet in muscle tissue. But even though oleic acid composition was totally higher amount in CLO diet, muscle composition did not show any significant difference in tilapia muscle tissue (P>0.05). However, fish fed with FO based diet, 16:1*n*-9 and 20:*1*n-9 had significantly (P<0.01) lower amounts than that of fish fed with CLO based diet in muscle tissue samples (Table 3). Among *n*-6 series of fatty acids, 18:2*n*-6 was the one of the predominant polyunsaturated fatty (PUFA) in both FO and CLO based diet in muscle tissue samples of tilapia. Fish fed with FO based diet had also significantly (P<0.01) higher amount of 18:2*n*-6 than that of fish fed with CLO added diet in muscle tissue.

Table 4. The muscle tissue fatty acid profile of tilapia fed with FO and CLO diet for 60 days

Fatty Acids	**FO**	**CLO**	***F***
14:0	3.89±0.10[a]	3.93±0.11[a]	*NS*
15:0	0.76±0.02[a]	0.53±0.02[b]	**
16:0	22.90±0.42[a]	24.91±0.64[b]	*
17:0	1.61±0.02[a]	1.75±0.22[a]	*NS*
18:0	6.33±0.17[a]	6.16±0.19[a]	*NS*
20:0	0.30±0.01[a]	0.19±0.01[b]	**
21:0	0.61±0.11[a]	0.63±0.02[a]	*NS*
22:0	0.13±0.01	NA	
∑SFA	36.53	38.10	
14:1	0.17±0.01[a]	0.16±0.01[a]	*NS*
16:1	6.57±0.22[a]	8.68±0.14[b]	**
17:1	0.53±0.02[a]	0.48±0.03[a]	*NS*
18:9	24.49±0.19[a]	25.35±0.40[a]	*NS*
20:9	1.62±0.05[a]	3.21±0.13[b]	**
22:9	0.68±0.03[a]	0.74±0.11[a]	*NS*
24:9	3.76±0.27[a]	2.48±0.12[b]	**
∑MUFA	37.65	41.14	
18:3*n*-3	3.91±0.07[a]	4.75±0.39[a]	*NS*
20:3*n*-3	0.52±0.03[a]	0.45±0.01[a]	*NS*
20:5*n*-3	1.53±0.13[a]	1.37±0.36[a]	*NS*
22:6*n*-3	7.03±0.32[a]	3.57±0.19[b]	**
∑*n*-3 PUFA	12.99	10.14	
18:2*n*-6a	10.21±0.03[a]	8.81±0.11[b]	**
18:3*n*-6	0.49±0.01[a]	0.65±0.07[a]	*NS*
20:4*n*-6	0.50±0.02[a]	0.33±0.01[b]	**
22:2n-6	0.96±0.03[a]	1.78±0.29[b]	**
∑*n*-6 PUFA	12.16	11.57	

∑PUFA	25.15	21.77
n-3/*n*-6	1.07	0.88
EPA/DHA	0.22	0.38
PUFA/SFA	0.69	0.57

Values are mean ± SEM *(n* = 6) and expressed as percentages of total fatty acids.
Means in the same row with the same superscript are not significantly different NS = (*P*>0.05), * (*P*<0.05), ** (*P*<0.001)

It appeared that the substituting FO with CLO in diet caused an increase of the percentages of 16:1, 18:3*n*-3, 20:1*n*-9, 22:2*n* -6, and a decrease in the percentages of 15:0, 20:0, 24:9, 18:2n-6, 20:4n-6, 22:6*n*-3 in muscle tissues of the tilapia (Figure 1). However, the percentage of EPA in muscle tissue samples did not show any significant difference in fish fed with both oil sources diet (*P*>0.05). DHA in muscle tissue sample of fish fed with FO based diet were found to be significantly higher (*P*<0.01) than that of fish fed with CLO diet. Furthermore, it appeared that changing oil source in diet with CLO had a decreasing effect on the proportion of *n*-3 HUFA (especially DHA) in muscle tissues of tilapia (Table 4). Muscle tissue proximate composition values are given in Table 5. The fish fed with CLO diet group contained less lipid in their muscle than that of fish fed with FO contained diet group (*P*<0.05). In addition, crude protein, moisture and crude ash levels of the tilapia muscle tissues were not significantly affected by different oil source in diets (Table 5). Liver fat and viscera fat composition showed significantly lower than fish fed with FO added diet (*P*<0.001) (Figure 2).

Table 5. Muscle proximate composition, liver and viscera fat of *Oreochromis niloticus* fed different oil sources

	FO	**CLO**	***F***
Moisture (%)	76,97±0.54[a]	78.09±0.40[a]	*NS*
Crude protein (%)	23.95±0.19[a]	22.18±0.34[b]	*
Crude lipid (%)	1.70±0.02[a]	2.35±0.05[b]	*
Crude ash (%)	1.82±0.02[a]	1.77±0.07[a]	*
Liver lipid (%)	13.88±0.22[a]	20.20±0.22[b]	**
Viscera lipid (%)	10.00±0.69[a]	13.60±0.58[b]	**

Values are mean ± SEM *(n* = 6). Means in the same row with the same superscript are not significantly different NS = (*P*>0.05), * (*P*<0.05), ** (*P*<0.001)

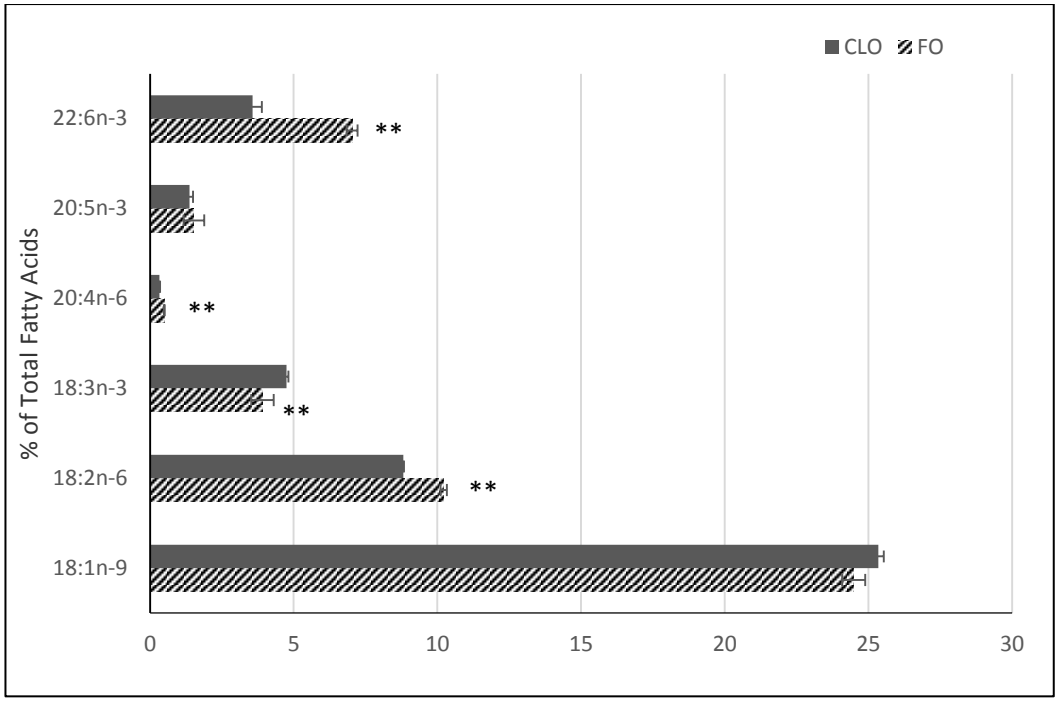

Fig. 1. Percentages (% total fatty acids) of 18:1*n* -9, 18:2*n* -6, 18:3*n* – 3, 20:4*n*-6, 20:5*n*-3, 22:6*n*-3 in muscle tissue of fish fed with FO and CLO diets. * Indicates significant difference compared with the FO and CLO groups (Student's t-test, $P<0.05$), ** $P<0.01$

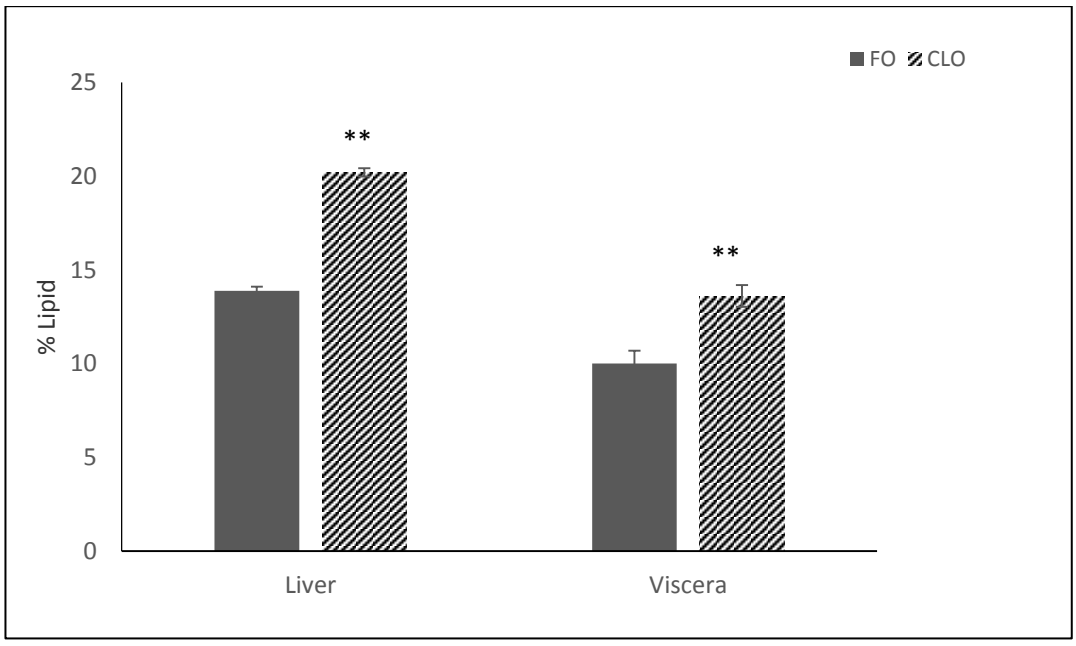

Fig. 2. Percentages (% total fatty acids) of tilapia liver and viscera of fish fed with FO and CLO diets. * Indicates significant difference compared with the FO and CLO groups (Student's t-test, $P<0.05$), ** $P<0.01$

4. Discussion

In the present study, tilapia in both experimental groups were healthy. No difference in mortality was found and this may indicate that the tested FO or CLO added diets did not have any adverse effect on the health of the fish. Both FO and CLO can meet the energy requirements of the fish by providing positively reacted EFA and, generate fillet fatty acid composition that are beneficial to the consumer by maximizing retention of required EFA such as DHA and EPA.

In this research, there was a decrease in body lipid composition, but an increase in crude protein content of muscle composition of fish fed with FO diet group. This could probably be due to quantity of oil used which was not in excess of the fish requirement resulting in better utilization of protein for growth and evident in the protein efficiency in this study. The use of some animal and vegetable oils reduce the *n*-3 series fatty acid (Bell et al. 2002; Piedecausa et al., 2007). The fish muscle n-3/n-6 lipid ratio is not strongly influenced by the diets n-3/n-6 ratio. Feeding on vegetable oils lowered the muscle content of EPA, DHA and ARA and this kind of effect has been documented earlier in trout (Caballero et al. 2002) and African catfish (Ng et al., 2003). Fish fed with CLO containing diet showed a significantly higher liver fat than those fed FO but HSI or VSI were not affect negatively in either diet groups. This could be attributed to the digestibility of fish fed in FO added diet, leading to lipid deposition in the liver enterocytes (Babalola et al., 2011). If diet is unbalanced in terms of FA, this can cause fat metamorphosis and steatosis, characterized by the abnormal accumulation of trigliserids (TGs) in hepatocytes. An excessive (or unbalanced) dietary intake of lipids saturate the physiological capacity of the liver to handle, and leads to lipid TGs accumulation. Synthesis and degradation of FA occurs mainly in the liver, and many enzymes involved in regulating these pathways show varying affinities for the different fatty acids in the organ (Kiessling, and Kiessling, 1993) Liver steatosis has been frequently seen in association with nutritional imbalances in farmed fish (Tacon, 1996). This could be happening as result of an EFA deficiency (Montero et al., 2001), the use of artificial diets (Spichni et al., 1998) and the presence of vegetable oils (Alexis, 1997). Some studies have showed similar results where, steatotic livers were observed in fish fed on diets characterized by low PUFA/MUFA ratio. In freshwater fish, the degradation of excess oleic acid predominantly occurs in peroxisomes (Shimada et al., 2014). In this study fish fed CLO contained diet had more oleic acid content and this why steatotic liver might have developed. Moreover; our findings also showed that fish fed with CLO diet had lower ratio of (0.41) PUFA/MUFA than fish fed with FO (1.11) contained diet (Table 2). The specific fatty acid composition of fish diets, and certainly human diets, is also of critical importance. Fish have a species-specific and varying requirement for n-3 or ω3 long-chain polyunsaturated fatty acids (PUFA), EPA; 20:5n-3, and DHA; 22:6n-3, sometimes referred to as n-3 HUFA (highly unsaturated fatty acids; (Sargent et al., 1989). Humans have a limited capacity to synthesize n-3 HUFA from shorter chain precursors, and fish have become vitally important as the only significant source of n-3 HUFA. Fish oil, rich in n-3 HUFA and resulting from industrial fisheries, e.g., capelin, herring, sand eel, mackerel, anchovy, and sardine, has been the standard ingredient of substance feeds for intensively farmed fish. Until recently, the availability and relatively low cost of FO has resulted in its widespread use in aquafeeds. However, global capture fisheries production has reached its sustainable limits, and the yield of FO from industrial fisheries, circa 1.4 million tonnes in 1996 (Sargent and Tacon, 1999), is unlikely to be significantly exceeded in future (Tacon, 2004). It is clear that increasing demand from aquaculture for FO will soon exceed supply and threaten the viability of fish farming activities and sustainability of wild fish stocks. Therefore; identifying suitable alternatives to FO in aquafeeds or improving efficiency of FO used are gaining more and more importance as critical issues. A variety of oils, especially those from plant oil-seeds (vegetable oils, VO) are available. However, any dietary alternative to FO must meet the issues of balancing lipid storage with lipid burning for growth and supplying essential fatty acids, while maintaining the health of the fish (Leaver et al., 2008).

Medicinally important fatty acids like PUFA and ω-3 are abundant in the marine fish of equatorial waters. These acids when given in high doses (20-25 g/day) have been proven to reduce blood triglycerides, platelet aggregation and blood pressure and thus effectively prevent cardiovascular diseases (Ahmad, 1991). Diets enriched with seafood or fish would be helpful in avoiding preventing heart problems. A minimum value of PUFA/SFA ratio recommend is 0.45 for humans in their diets (HMSO, 1994). Therefore, the PUFA/SFA ratio calculated for fish fed with FO

contained diet (0.69); and fed with CLO contained diet (0.57) in this study were actually higher than the recommended for both group for tilapia rearing in terms of human consumption. Further research should be focusing on different fat sources in fish diets in order to draw a clear picture about the lipid deposition in different tissue of farmed tilapia.

5. Conclusion

Aquaculture is accepted as the only way to meet the increasing demands for aquatic foods. According to FAO (Food and Agriculture Organization) currently aquaculture is responsible for nearly 50% of the global fishery production, and this figure is expected to rise as the demand for aquatic products increases. Fishmeal and fish oil are traditionally the chosen source in production of aquaculture feed because of its higher nutritional value. Moreover, the aquaculture feed industry competes in the marketplace with other sectors of the animal feed industry for fishmeal and fish oil use (FAO, 2016). However, the continue decline in the fishmeal and fish oil production relative to increasing demand is forcing the market price of fishmeal and oil upwards, thus threatening the profitability and economic sustainability of the aquaculture industry. The way to sustain the aquaculture industry into the future is to limit or replace the use of fish oil in commercial aquafeed. Complete or partial substitution of fish oil in fish feeds with alternative oils has been demonstrated in past studies without affecting the growth performance of several species of fish (Tacon, 2004; Kaushik et al., 2004). Dietary substitution of fish oil with alternative oil ingredient sources is evidently more feasible for omnivorous species than carnivorous species because of their FA requirements and ability to chain elongate and desaturate n-6 and n-3 PUFAs to n-6 and n-3 PUFAs, cultured tilapia are commonly fed diets that are supplemented with plant oils (Millikin, 1982). Numerous studies, however, have shown that the FA composition of the diet is the main factor affecting FA composition of fat in fish tissues. Thus, fillets of tilapia that are grown on diets supplemented with plant oils or cod liver oil will contain high levels of total MUFA low levels of PUFAs (especially 22:6*n*-3) relative to fish that receive diets supplemented with marine fish oil. Both 20:5*n*-3 and 22:6*n*-3 is known to play vital roles in human nutrition, disease prevention, and health promotion. They provide a protective effect in minimizing the development of several chronic degenerative diseases and have a therapeutic effect in certain cases. Therefore, to improve marketability and ensure continued growth of this industry, the quality of tilapia farming can be used fish oil or cod liver oil without have any problems.

References

Ahmad, F., Ali, S. S., Usmanghani, K., Ali, M. (1991) Pak. J. Pharm. Sci., 4, 91 (1991).

Alexis, M. N. (1997). Fish meal and fish oil replacers in Mediterranean marine fish diets, In: A. Tacon and B. Basurco, Eds., *Feeding Tomorrow's Fish*, *Proceedings of the Workshop of the CIHEAM Network on Technology of Aquaculture in the Mediterranean* (*TECAM*) *Cahiers Options Méditerranéennes*, Vol. 22:183-204.

AOAC (Association of Official Analytical Chemist) (1990) Official methods of analysis, 15th edn. Association of Official Analytical Chemist, Arlington.

Babalola, T. O., Apata, D. F., Omotosho, J. S., Adebayo, M. A. (2011). Differential effects of dietary lipids on growth performance, digestibility, fatty acid composition and histology of African catfish (*Heterobranchus longifilis*) fingerlings, Food and Nutrition Sciences, 2, 11-21.

Barlow, S. (2000). Fishmeal and fish oil; sustainable feed ingredients for aquafeeds. Glob. Aquacult. Advocate, 4, 85-88.

Bell, J.G., Henderson, R. J., Tocher, D.R., McGhee, F., Dick, J. R., Porter, A., Smullen, R.P., Sargent, J. R. (2002). Substituting fish oil with crude palm oil in the diet of Atlantic Salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism. J Nutr 132:222–230.

Bligh EC, Dyer WJ (1959). A rapid method of total lipid extraction and purification. Can J Biochem Physiol, 37:913–917.

Brett, J. R. (1979). Environmental factors and growth. in Fish Physiology, vol. 8. Bioenergetics and Growth, Pp. 599-675 W. S. Hoar, D. J. Randall, and J. R. Brett, eds. New York: Academic Press

Caballero, M.J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M., Izquierdo, M.S. (2002). Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout (*Oncorrynchus mykiss*). Aquaculture, 214(1-4): 253-271.

FAO, (2015). Fisheries Report (http://www.fao.org/fi/statist/FİSOFT/FİSHPLUS.asp, Accessed on March, 2018.

FAO, (2016). The State of World Fisheries and Aquaculture Contributing to Food Security and Nutrıtion for All, Rome, Italy

Grant, A. A. M., Baker, D., Higgs, D. A., Colin J. B., Richards, J. G., Balfry S. K., Schulte. P. M. (2008). Effects of dietary canola oil level on growth, fatty acid composition and osmoregulatory ability of juvenile fall chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture, 277: 303–312.

HMSO UK (1994) Nutritional aspects of cardiovascular disease (report on health and social subjects no. 46), London

Ioannis, T.K., Bell, M. V., Little, D. C., Yakupitiyage, A. (2007). Replacement of dietary fish oils by alpha-linolenic acid-rich oils lowers omega- 3 content in tilapia flesh. Lipids, 42:547–559.

IUPAC, (1979). Standards methods for the analysis of oils, fats and derivatives, 6th edn. Pergaman Press, London.

Kaushik, S.J., Coves, D., Dutto, G., Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. Aquaculture 230, 391-404.

Kiessling, K. H., Kiessling,(1993). Selective utilization of fatty acids in rainbow trout (*Oncorhynchus mykiss* Walbaum) red muscle mitochondria, Canadian Journal of Zoology, 71, 248-251.

Kumar, G., Engle, C. R. (2016). Technological advances that led to growth of shrimp, salmon, and tilapia farming, Reviews in Fisheries Science & Aquaculture, 24:2, 136-152.

Kris-Etherton PM, Harris WS, Appel LJ. (2002). American Heart Association. Nutrition Committee. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation, 106 (21): 2747- 57.

Leaver, M. J.; Bautista, J. M.; Björnsson, B. T.; Jönsson, E., Krey , G.; Douglas, R. Tocher, B.;Torstensen, B. E. (2008). Towards Fish Lipid Nutrigenomics: Current State and Prospects for Fin-Fish Aquaculture, Reviews in Fisheries Science, 16:1, 73-94.

Millikin, M. R. (1982). Qualitative and Quantitative Nutrient Requirements of Fishes: A Review. Fishery Bulletin, 80:4, 655-686.

Mohebi-Nejad, A and Bikdeli, B. (2014). Omega-3 Supplements and Cardiovascular Diseases, Tanaffos, 13(1): 6-14.

Montero, D., Robaina, L. E., Socorro, J., Panseri, S., Bellagama, F., Moretti, V. M. (2001). Alteration of Liver and Muscle Fatty Acid Composition in Gilthead Seabream (*Sparus aurata*) Juveniles Held at High Stocking Density and Fed an Essential Fatty Acid Deficient Diet," *Fish Physiology and Biochemistry*, Vol. 24, No. ,2001, pp. 63-72.

Montero, D., Robaine, L., Caballero, M.J., Ginės, R., Izquierdo, M.S. (2005). Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: A time-course study on the effect of a re-feeding period with a 100% fish oil diet.

Naylor, R. L., Hardy,R. W., Dominique P. Chiu, B. A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D. M., Rebecca, J., Hua,G. K., Nichols, P. D. (2009). Feeding aquaculture in an era of finite resources, PNAS,106 (36) 15103-15110.

Ng, W.K., Lim, P.K., Boey, P.L. (2003). Dietary palm oil source affects growth, fatty acid composition and muscle α-tocopherol concentration of African catfish (*Clarias gariepinus*). Aquaculture, 215(1-4): 229-243.

Olsen, R. E., Henderson, R. J., McAndrew, B. J. (1990). The conversion of linoleic acid and linolenic acid to longer chain polyunsaturated fatty acids by Tilapia (*Oreochromis nilotica*) in vivo. Fish Physiol Biochem 8:261–270.

Piedecausa, M.A., Mazon, M.J., Garcia-Garcia, B. And Hernandez, M.D. (2007). Effects of total replacement of fish oil by vegetable oil in the diets of sharpsnout sea bream (*Diplodus pintazzo*). Aquaculture, 263(1-4)**:**211-219.

Sargent, J. R., Henderson, R. J. & Tocher, D. R. (1989) The lipids. In: Fish Nutrition (Halver, J. E., ed.), pp. 154–218. Academic Press, New York, NY.

Sargent, J.R., Tacon, A., (1999). Development of farmed fish: a nutritionally necessary alternative to meat. Proc. Nutr. Soc., 58, 377-383.

Sargent J. R.; Bell, J. G., McEvoy, L., Tocher, D. R., Estevez, A., (1999). Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177, 191-199.

Sargent J.R., Tocher, D.R., Bell, J. G. (2002). The lipids. In: Halver JE, Hardy RW (eds) Fish nutrition, 3rd ed. Academic, San Diego, pp. 181–257

Shepherd, J and Bachis, E. (2014). Changing supply and demand for fish oil, Aquaculture Economics & Management, 18:4, 395-416, DOI: 10.1080/13657305.2014.959212

Hepatic Steatosis in Cage-ReardYoung Cobia, RachycentronCanadum (Linnaeus, 1766), inBrazilShimada MT1, Claudiano GS2, Engracia Filho JR2,4, Yunis J1,Moraes FR2, Moreira RG3 and Moraes JRE1,2*

Shimada, M.T., Claudiano, G.S., Engracia Filho, J. R., Yunis, J., Moraes, F.R., Moreira, R.G., Moraes, J.R.E. (2014). Hepatic Steatosis in Cage-Reared Young Cobia, *Rachycentron canadum* (Linnaeus, 1766), in Brazil | J Vet Sci Med Diagn 2014, 3:2

Spisni, E., Tugnoli, Ponticelli, A., Mordenti, T., Tomasi, V. (1998). Hepatic steatosis in artificially fed marine teleosts, Journal of Fish Diseases, 21(3): 177-184.

Tacon,A. G. J. (1996). "Lipid Nutritional Pathology in Farmed Fish," *Archives in Animal Nutrition*, 1996, Vol. 49, No. 1, pp. 33-39.

Tacon, A.G.J. (2004). Use of fish meal and fish oil in aquaculture: a global perspective. Aquatic Resources, Culture & Development, 1(1):3-14.

Takeuchi, T., Arai, S., Watanabe, T., Shimma, Y. (1980). Requirement of eel A*nguilla japonica* for essential fatty acids, Bulletin of the Japanese Society of Scientific Fisheries, 46 (3), 345-353.

Tocher, D. R., Agaba, M., Hastings, N., Bell, J. G., Dick, J. R., Teale, A. J. (2002). Nutritional regulation of hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition in zebrafish (*Danio rerio*) and tilapia (*Oreochromis niloticus*). Fish Physiol Biochem., 24:309–320.

United Nations (UN, 2010) MDG Report 2010 En 20100604 r14 Final.indd Sec2:6.