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RESEARCH PAPER

Fatty Acid Composition, Lipogenic Enzyme Activities and mRNA Expression of Genes Involved in The Lipid Metabolism of Nile Tilapia Fed with Palm Oil

Christian Larbi Ayisi [1], Jin-Liang Zhao[1,*]

[1] Shanghai Ocean University, Key Laboratory of Freshwater Fishery Germplasm Resources, Ministry of Agriculture, P. R. China.

* Corresponding Author: Tel.: +86 2161900435 ; Fax: +86 2161900405;
E-mail: jlzhao@shou.edu.cn

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Abstract

This study was aimed at elucidating the effects of replacing fish oil (FO) with palm oil (PO) on tissue fatty acid composition, lipogenic enzyme activities and mRNA expression of genes related to lipid metabolism in Nile tilapia, *Oreochromis niloticus* (6.72± 0.14g). An eight week feeding trial was conducted using five isonitrogenous and isolipidic diets containing 0% PO, 25% PO, 50% PO, 75% PO and 100% PO. PO supplementation led to a significant increase in total saturated fatty acid (SFA), total mono unsaturated fatty acids (MUFA) and 18: 2n-6, whiles DHA, EPA, total n-3 as well as 20: 2n-6 were reduced significantly in the liver ($P < 0.05$). With the exception of glycerol-3-phosphate acyltransferase (GPAT) enzyme activity, supplementing tilapia diet with PO significantly increased fatty acid synthesase (FAS), acetyl-CoA carboxylase (ACC), steroyl-CoA desaturase 1 (SCD1), ATP citrate lyase (ACYL), carnitine palmitoyltransferase Ia (CPTIa) and carnitine palmitoyltransferase Ib (CPT Ib) ($P < 0.05$). In addition, significant/positive correlations were observed among dietary PO and/or liver tissue FA with FAS, ACC, SCD1 and ACYL mRNA expression while a negative correlation was recorded for CPTI mRNA expression. Generally, inclusion of PO in tilapia diets resulted in lipid accumulation in the liver and altered the key gene expression of lipid metabolism.

Keywords: Stearoyl-CoA desaturate 1, Fatty acid synthesase, Carnitine palmitoyltransferase (CPT) I, Acetyl-CoA Carboxylase, ATP Citrate Lyase, Lipid metabolism, *Oreochromis niloticus*, Palm oil.

Introduction

Fish oils are considered as the main source of lipid in aquaculture feeds to promote growth and development of farmed species by providing essential polyunsaturated fatty acids (PUFAs), especially high unsaturated fatty acids (HUFAs) (Sargent *et al.*, 2002). However, as a result of increase in aquaculture activities as well as the limited availability of fish oil and fish meal, there are calls for the use of alternative lipid and protein sources to develop fish farming practices that are sustainable (Kazemi *et al.*, 2016).

Plant oils rich in C18 polyunsaturated fatty acids (PUFA) are potential and suitable candidates to replace fish oils in aquaculture feeds (Hafezieh *et al.*, 2010). Palm oil is currently the most abundant vegetable oil in the world (Ochang *et al.*, 2007a) with the global production of palm oil projected to increase by over 30% by 2020 due to the continuous supply by developing countries (Ayisi and Zhao, 2014).

At present, many preliminary studies have been conducted to determine the effects of replacing fish oil with palm oil in tilapia (Ochang *et al.*, 2007b; Ng and Wang, 2010), catfish (Ochang *et al.*, 2007a), large yellow croaker (Duan *et al.*, 2014) and Juvenile Chu's Croaker, *Nibea coibor* (Huang *et al.*, 2016).

Changes in dietary fatty acid composition can have effects on the regulation of fatty acid oxidation through a variety of genomic and non-genomic mechanisms (Morash *et al.*, 2009).

Recent studies indicated that replacing fish oil with palm oil could act as a modifier in lipid metabolism hence increasing lipid accumulation in fish tissue and whole body. For instance, replacing FO with PO led to an increase in liver lipid deposition in Juvenile Chu's Croaker, *Nibea coibor* (Huang *et al.*, 2016). Similarly, replacing FO with other VO resulted in increased lipid levels in liver in tilapia (Peng *et al.*, 2015) and rainbow trout (Guler and Yildiz, 2011).

Lipid accumulation results from the balance between synthesis of fatty acids (lipogenesis) and fat catabolism via β-oxidation (lipolysis), and many key enzymes and transcriptional factors are involved in these metabolic processes (Chen *et al.*, 2015). These enzymes include lipogenic enzymes

(such as fatty acid synthase (FAS), and acetyl-CoA carboxylase (ACC) , and lipolytic enzymes (such as carnitinepalmitoyltransferase I (CPT I), hormone-sensitive lipase (HSL) and adipose triacylglyceride lipase (ATGL) (Elliott and Elliott, 2009).

Acetyl-CoA carboxylase (ACC) is not only a key enzyme in fatty acid synthesis via the ACCa, but also plays an important role by regulating fatty acid oxidation via the ACCb. ACC is a biotin-dependent enzyme that catalyzes the irreversible carboxylation of acetyl-CoA to malonyl-CoA, the rate-limiting step in fatty acid biosynthetic pathway (Cheng *et al.,* 2011).

FAS is a key enzyme that regulates the de novo biosynthesis of long-chain fatty acids from acetyl-CoA and malonyl-CoA in the presence of NADPH (Dong, *et al*., 2014).

Carnitine palmitoyltransferase (CPT) I is considered as the main regulatory enzyme in mitochondrial fatty acid oxidation because it catalyses the conversion of fatty acyl-CoAs into fatty acyl-carnitines for entry into the mitochondrial matrix (Kerner and Hoppel, 2000). Various nutrients modulate the mechanisms involved in CPT I regulation and consequently mitochondrial β-oxidation of fatty acids. For example, CPT I expression was increased in the red muscle, adipose tissue and liver between three and seven fold in rainbow trout (*Oncorhynchus mykiss*) fed high PUFA diet compared to the mixed fatty acid control diet (Morash *et al.,* 2009). Also, PUFAs can also act on CPT I activity indirectly via changes in the mitochondrial membrane composition. The fatty acid composition of the outer mitochondrial membrane has been shown to be of particular importance to the regulation of CPT I because it can affect membrane properties and the binding affinity of the allosteric regulator M-CoA to the enzyme (Morash *et al.,* 2009). Fatty acid can serve as a substrate for β-oxidation to provide energy. β-oxidation is the major process by which fatty acids are oxidized, by sequential removal of two carbon units from the acyl chain. CPT I is frequently described as the 'rate-limiting enzyme' of β-oxidation flux in liver, heart and skeletal muscle (Eaton, 2002).

SCD are known for their roles in synthesizing unsaturated fatty acids (Ardiyanti *et al.,* 2012). Stearoyl-CoA desaturase (SCD) synthesizes oleate necessary for the biosynthesis of triglycerides and other lipids (Miyazaki *et al.,* 2004). Although studies have investigated the replacement of fish oil with palm oil on growth and lipid deposition in fish, the underlying molecular processes involved in fatty acid metabolism and the change of lipid deposition as a response to dietary fish oil replacement by palm oil are seldom known.

The aim of the present study was to investigate the underlying mechanisms of dietary fish oil replacement with palm oil on fatty acid metabolism, lipogenic enzyme activities as well as lipid metabolism related gene expression. Fatty acid composition of the liver and muscle were analyzed. Also enzyme activities of Fatty acid synthesase (FAS), Acetyl-CoA Carboxylase (ACC), Steroyl-CoA desaturase 1 (SCD1), ATP Citrate Lyase (ACYL), Glycerol-3-phosphate acyltransferase (GPAT), Carnitine Palmitoyltransferase I were examined in the liver. Finally, mRNA expression of SCD1, ACC, FAS, ACYL, CPTI a and CPTI b as well as GPAT were studied.

Materials and Methods

Feed and Feeding Trial

Five isonitrogenous (33% crude protein) and isolipidic diets (10%) were prepared for the eight weeks feeding trial. The variation was made to have effects on fish oil and palm oil as shown in Table 1. Fish meal, soybean meal and rapeseed meal were used as the protein sources, while palm oil and fish oil were used as the sources of lipid. All dry ingredients (defatted fish meal, soybean meal, rapeseed meal, wheat meal, mineral mix and vitamin mix) were mixed using the progressive enlargement method. The experimental diets were prepared by mixing the dry ingredients with palm oil, fish oil and distilled water in a Hobart mixer, and the resulting moist dough pelleted using a meat mincer through a 1-mm die. The 1-mm diameter pelleted diets were wet extruded, air dried, broken up and sieved into proper pellet size. All experimental diets were stored at -20 C until time of feeding. The fatty acid composition of the test diets are given in Table 2.

Experimental Procedures

Nile tilapia fingerlings (6.72±0.14, initial weight) were obtained from Tilapia Germplasm Station of Shanghai Ocean University, China. Prior to the start of the experiment, fish were transported to aquarium facilities at Shanghai Ocean University and acclimated for two weeks. Fish were fed commercial diets obtained from Shanghai Jin Yuan Trade containing 30% crude protein twice daily to apparent satiation. Fish were starved for 24 hours before the feeding trial, weighed, and randomly distributed into 15 rectangular fiber glass tanks (150× 60× 40 cm) at 40 fish per tank with water maintained at 210 litres. Dissolved oxygen (DO) concentration, pH and water temperature were monitored on daily basis using YSI 556 instrument (YSI, Yellow Springs, Ohio). Ammonia-N and Nitrite-N were analyzed spectrophotometrically on a weekly basis following standard methods (APHA, 1998). Each diet was randomly offered to a tank and its replicates to sum up to 15 experimental tanks. During experimental period of eight weeks, fish were offered the experimental diets to apparent satiation twice daily at

Table 1. Formulation and proximate composition of experimental diets (g/100 g in dry matter)

	Dietary PO replacement level (%)				
Ingredient	PO 0	PO 25	PO50	PO75	PO100
Fish meal*	6.00	6.00	6.00	6.00	6.00
Soybean meal*	30.00	30.00	30.00	30.00	30.00
Wheat meal*	22.50	22.50	22.50	22.50	25.50
Rapeseed meal*	30.00	30.00	30.00	30.00	30.00
Fish oil*	6.00	4.50	3.00	1.50	0.00
Palm oil*	0.00	1.50	3.00	4.50	6.00
Soybean phospholipid*	2.50	2.50	2.50	2.50	2.50
Mineral mix**	0.55	0.55	0.55	0.55	0.55
Vitamin mix***	0.40	0.40	0.40	0.40	0.40
$Ca(H_2PO_4)$	1.50	1.50	1.50	1.50	1.50
Choline chloride	0.50	0.50	0.50	0.50	0.50
Inositol	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100
Proximate composition (%)					
Moisture	10.30	10.53	10.82	10.70	10.50
Protein	33.18	33.15	33.09	33.23	33.17
Lipid	9.82	9.81	9.87	9.88	9.85
Ash	5.25	5.60	5.53	5.40	5.50

*Fish meal, Soybean meal, Wheat meal, Soybean phospholipase, Palm oil, Vitamin premix, Mineral mix and Ca (H_2PO_4) were supplied by Nonghao Feed Company (Shanghai, China).

**Mineral mix (mg kg^{-1} dry diet): Cu ($CuSO_4$), 2.0; Zn ($ZnSO_4$), 34.4; Mn ($MnSO_4$), 6.2; Fe ($FeSO_4$), 21.1; I (Ca ($IO_3)_2$), 1.63; Se (Na_2SeO_3), 0.18; Co ($CoCl_2$), 0.24; Mg ($MgSO_4.H_2O$), 52.7.

*** Vitamin premix (IU or mg kg^{-1} diet): vitamin A, 16000 IU; vitamin D, 8000 IU; vitamin K, 14.72; thiamin, 17.8; riboflavin, 48; pyridoxine, 29.52; cynocobalamine, 0.24, tocopherols acetate, 160; ascorbic acid (35%), 800; niacinamide, 79.2; calcium-D-pantothenate,73.6; folic acid, 6.4; biotin, 0.64; inositol, 320; choline chloride, 1500; L-carnitine, 100.

Table 2. Main fatty acid composition (% of total fatty acids) of experimental diets

FATTY ACID(S)	Dietary PO replacement level (%)				
	PO 0	PO 25	PO 50	PO 75	PO 100
12:0	0.15±0.00	0.14±0.01	0.12±0.00	0.12±0.01	0.11±0.00
14:0	5.54±0.21	4.52±0.04	3.20±0.05	2.25±0.09	1.27±0.1
16:0	23.88±0.49	25.90±0.08	27.00±0.39	28.06±0.37	29.19±0.27
18:0	5.31±0.04	5.76±0.06	5.43±0.12	5.30±0.13	5.15±0.05
Total SFA's	34.88±0.46	36.32±0.83	35.75±0.19	35.73±0.33	35.72±0.72
16:1(n-7)	6.02±0.34	5.23±0.59	3.15±0.04	2.06±0.15	0.89±0.01
18:1(n-9)	23.56±0.68	25.42±0.09	28.40±0.16	31.16±0.20	33.77±0.16
TOTAL MUFAs	29.58±0.85	30.65±0.47	31.55±0.77	33.22±0.43	34.66±0.32
18:2(n-6)	20.65±0.77	21.57±0.05	23.07±0.22	23.62±0.32	24.33±0.03
20:4(n-6)ARA	0.56±0.05	0.47±0.03	0.41±0.00	0.25±0.05	0.20±0.02
Total n-6	21.21±0.22	22.04±0.89	23.48±0.33	23.87±0.18	24.53±0.62
18:3(n-3)	5.52±0.23	3.96±0.15	3.98±0.04	3.79±0.09	3.63±0.03
18:4(n-3)	0.32±0.01	0.33±0.00	0.32±0.01	0.30±0.00	0.29±0.01
20:5(n-3)EPA	4.25±0.14	3.19±0.01	2.20±0.06	1.41±0.04	0.62±0.00
22:6(n-3)DHA	5.66±0.35	4.15±0.04	2.85±0.16	1.69±0.02	0.58±0.02
Total n-3	15.75±0.19	11.63±0.57	9.35±0.04	7.19±0.39	5.12±0.31
DHA/EPA	1.33±0.05	1.30±0.21	1.29±0.33	1.19±0.43	0.93±0.05
Total PUFAs	34.96±0.18	33.67±0.34	32.83±0.18	31.06±0.09	29.65±0.14
Total SFA/total PUFA	0.87	1.07	1.08	1.15	1.20
n-3:n-6	0.74	0.52	0.39	0.30	0.20

ARA= Arachidonic acid; EPA= Eicosapentanoic acid; DHA= Decosahexanoic acid; SFA= saturated fatty acids; MUFA= mono unsaturated fatty acid; PUFA= polyunsaturated fatty acid.

08:00 and 16:00.

Sample Collection

Fish were starved 24 hours prior to harvest after completion of the trial period. Seventy-five (five per tank) fish at the end of the trial were randomly sampled, euthanized with an overdose of tricaine methane sulfonate (MS-222 at 200mg/L in culture water), liver and muscle samples taken, pooled and

stored at -80°C for subsequent determination of fatty acid composition, lipogenic enzyme activity and mRNA expression.

Fatty Acid Composition of Feed, Liver and Muscle

Before the assay was performed, tissues (liver and muscle) and feed were grinded to powder individually. (1) Total lipid (TL) was extracted from freeze – dried samples with chloroform–methanol (2:1, V/V), according to the method of (Folch *et al*., 1957). (2) Fatty acid methyl esters (FAME) were prepared by transesterification with 0.4 M KOH-methanol, and then detected by gas chromatograph (GC-7890A, USA) using methyl heneicosanoate (C21:0) as the internal standard. (3) Fatty acid content was determined using the normalization method. All measurements were performed in triplicates and the fatty acids content expressed as % total FA.

Lipogenic Enzyme Activities

Enzyme activities of Fas, Acc, Acyl, CPTI, Gpat and Scd1 were measured using enzyme linked immunosorbent assay (ELISA). A total of 0.5-1.0 g of liver were homogenized using a ground glass homogenizer on ice. The homogenates were centrifuged (20,000 rpm, 50 mins at 4 C), and the clear phase between the top layer and the pellets used for the analysis. Samples were analyzed in a 96-well plates by ELISA (Shanghai MLBIO Biotechnology Co. Ltd, China). Optical Density (OD) was measured in an ELISA microplate reader (Bio Tek Synergy, USA) at 450nm. A standard curve was generated according to the manufacturer's instruction, and the standard diversity calculated with Excel 2003. Enzyme activity units (IU), defined as moles of substrate converted to product per minute at assay temperature, were expressed per mg of hepatic soluble protein specific activity or per gram of liver tissue wet weight.

RNA Extraction and Real-Time Quantitative Polymerase Chain Reaction (RT-Qpcr)

Expression analysis of FAS, ACC, SCD 1, ACYL, CPTIa and CPTIb was performed using real-time PCR with beta-actin as house-keeping gene (Oku *et al*. 2006). Total RNA was isolated from the samples using Trizol Reagent (Invitrogen). RNA concentration was determined by conventional agarose electrophoresis and through absorbance measurements (ratio 260/280 ≥2). The genomic DNA contaminating the RNA samples was digested by RNase-free DNase I (TaKaRa) incubation for 15 min at 37 C. Next, 2ug of RNA was transcribed into cDNA using M-MLV reverse transcriptase. All cDNA samples were stored at –20C until analysis.

Real-time PCR was conducted on a Mini Option Real-time PCR machine (Bio-Rad). The 20-µl reaction contained 1-µl cDNA sample, 10 µl SYBR green I Master Mix (TaKaRa), 0.5µl of each primer and 8 µlH2O. PCR amplification was performed in triplicate wells using the following protocol: 3 min at 95C, 45 cycles consisting of 10s at 95 C, 15s at 63 C and 25 s at 72C. A melting curve analysis was performed to confirm that a single PCR product had been amplified. Approximately, there was an equal amplification efficiency for all genes ranging between 95.1% and 99.3%. β-actin gene was used as the reference to quantify the target genes relatively. In order to quantify the transcripts of lipid metabolism related genes, the normalized gene expression of the group fed the control diet (0% PO) was set to 1. The expression of the target genes were expressed relating them to the control group. At the end of the reaction, the fluorescent data were converted into Ct values. Each transcript level was normalized to b-actin using the 2 -DDCT Method (Livak and Schmittgen, 2001).

Statistical Analysis

All data were analyzed by one-way analysis of variance and Turkey's multiple test to compare treatment means. Differences were considered significant at 0.05 probability level for all data. All analysis was performed using the GraphPad Prism V.5.03 and results presented as mean ± standard error of the mean (SEM).

Results

Fatty Acid Compositions in Liver and Muscle

The fatty acid composition of liver and muscle following 56 days of feeding experimental diets are presented in tables 4 and 5, respectively. Replacing FO with PO had significant influence on the fatty acid composition of the liver and muscle. Whereas total SFA, total MUFA and 18:2n-6 (LA) increased significantly ($P<0.05$) along increasing dietary PO inclusion, EPA, DHA, total n-3, total PUFA, n-3: n-6 and 20:2n-6 decreased significantly ($P<0.05$). In addition, there were no significant differences ($P\geq0.05$) among groups in the liver with respect to 18:3n-3 (LNA) and 20:4n-6 (ARA).

The fatty acids reported in the muscle were similar to that of the liver as well as the feed. Dietary PO inclusion resulted in an increasing total SFA and total MUFA. Also, EPA, DHA, total n-3, total PUFA, n-3: n-6 and 20:2n-6 increased as PO levels increased. Contrary to the liver, 18:3n-3 (LNA) and 20:4n-6 (ARA) were significantly different ($P<0.05$) among treatments.

Lipogenic Enzyme Activities

The results of lipogenic enzyme activities in liver were presented in Table 6. The inclusion of

Table 3. Primer sequences, annealing temperatures (Tm) used for real-time quantitative PCR (qRT-PCR)

Targeted genes	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Tm (°C)	GenBank accession no
FAS	TTTGAGATGTGCTCACAGCTGCAGA	TCTGCAGCTGTGAGCACATCTCAAA	62	GU433188.1
ACC	ATTAAACACTAAAGAAGAAGAGCTT	AAGCTCTTCTTCTTTAGTGTTTAAT	59	XM_003442879.3
SCD1	GACATTTTTGCCTTTTTAGA	TCCTAATTAATAAGAGTATA	59	AJ55697
ACYL	GCACTGTTCAGATGGTTTATGTTTATGACCT	AGGTCATAAACCATCTGAACAGTGC	60	XM_005470605.2
CPTIa	GTACGTCTGTTCGCTCCTGCACCAA	TTGGTGCAGGAGCGAACAGACGTAC	62	DQ011056.1
CPTIb	AAGCCACTTCAAGGCATAGGAATC	GATTCCTATGCCCTTGAAGTGGCTT	62	NM_001171855.1
β-Actin	ATCGTGGGGCGCCCCAGGCATCAGG	CCTGATGCCTGGGGCCCACGAT	59	EU887951.1

FAS: Fatty acid synthesase
ACC: Acetyl-CoA Carboxylase
SCD1: Steroyl-CoA desaturase 1
ACYL: ATP Citrate Lyase
CPTIa: Carnitine Palmitoyltransferase Ia
CPT Ib: Carnitine Plmitoyltransferase Ib
GPAT: Glycerol-3-phosphate acyltransferase
β-Actin: Beta actin

Table 4. Fatty acid composition (% of total fatty acids) and lipids in the liver of *Oreochromis niloticus* juvenile fed diets with different levels of palm oil (PO) for 8 weeks

FATTY ACID(S)	Dietary PO replacement level (%)				
	PO 0	PO 25	PO 50	PO 75	PO 100
Liver lipid	7.48±0.86	7.63±0.43	8.46±0.59	8.61±0.78	9.06±0.41
12:0	0.12±0.00^{b}	0.10±0.00^{ab}	0.09±0.00^{ab}	0.11±0.01^{ab}	0.08±0.00^{a}
14:0	6.25±0.02^{d}	3.86±0.15^{c}	2.60±0.07^{ab}	2.98±0.30^{b}	2.16±0.02^{a}
16:0	23.88±0.49^{a}	27.90±0.08^{b}	29.01±0.39^{c}	30.06±0.37^{d}	31.19±0.27^{e}
18:0	6.85±0.04	6.76±0.06	6.43±0.12	6.30±0.13	6.15±0.05
20:0	0.15±0.01	0.14±0.00	0.12±0.00	0.12±0.01	0.10±0.01
ΣSFA's	37.18±0.46^{a}	37.76±0.83^{b}	38.25±0.19^{c}	39.57±0.33^{d}	39.70±0.72^{e}
16:1(n-7)	7.03±0.03	6.49±0.15	6.28±0.01	6.89±1.71	6.39±0.05
16:1(n-9)	0.8±0.00	0.57±0.01	0.66±0.01	0.95±0.31	0.85±0.00
18:1(n-7)	6.75±0.0	6.06±0.15	6.09±0.01	6.15±1.65	6.17±0.05
18:1(n-9)	24.41±0.16^{a}	26.68±0.31^{b}	29.08±0.27^{cd}	28.41±0.60^{c}	30.12±0.04^{d}
Σ MUFAs	38.98±0.85^{a}	39.79±0.47^{b}	42.14±0.77^{c}	42.42±0.43^{d}	43.55±0.32^{e}
16:3(n-3)	0.87±0.00^{d}	0.65±0.01^{c}	0.47±0.00^{b}	0.50±0.03^{b}	0.33±0.00^{a}
18:3(n-3) *LNA*	0.91±0.02	0.98±0.01	0.96±0.15	1.00±±0.49	1.15±0.00
20:4(n-3)	0.96±0.01^{b}	0.77±0.15^{b}	0.91±0.01^{b}	0.38±0.00^{a}	0.29±0.01^{a}
20:5(n-3) *EPA*	1.06±0.02^{b}	0.58±0.15^{ab}	0.20±0.03^{a}	0.27±0.03^{a}	0.13±0.00^{a}
22:6(n-3) *DHA*	6.35±0.18^{c}	5.85±0.31^{c}	4.33±0.09^{b}	1.47±0.19^{a}	0.85±0.02^{a}
Σ n-3	10.16±0.22^{e}	8.81±0.89^{d}	6.88±0.33^{c}	3.65±0.18^{b}	2.77±0.62^{a}
18:2(n-6) *LA*	9.64±0.00^{a}	10.60±0.14^{b}	12.04±0.39^{b}	14.13±0.17^{c}	14.56±0.67^{c}
20:2(n-6)	1.49±0.01^{c}	1.36±0.01^{bc}	1.21±0.01^{b}	0.38±0.00^{a}	0.29±0.01^{a}
20:4(n-6)ARA	0.98±0.02	0.68±0.03	0.72±0.01	1.00±0.21	0.87±0.01
Σ n-6	13.10±0.02^{ab}	12.66±0.21^{a}	13.98±0.36^{abc}	15.52±0.13^{bc}	15.73±0.68^{c}
Total PUFAs	23.27±0.20^{c}	21.48±0.46^{bc}	20.86±0.43^{b}	19.17±0.43^{ab}	18.50±0.70^{a}
ΣSFA/Σ PUFA	1.59±0.41^{a}	1.76±0.08^{b}	1.83±0.04^{c}	2.06±0.31^{d}	2.15±0.05^{e}
n-3:n-6	0.77±0.04^{c}	0.69±0.02^{c}	0.49±0.01^{b}	0.24±0.01^{a}	0.18±0.00^{a}

dietary PO increased activities of SCD 1, FAS, ACYL and ACC. Fish fed PO 100 diet recorded the highest SCD 1 and ACYL activities and were significantly different from those fed PO 0 diet. Also fed diets PO 100 recorded the highest FAS and was significantly higher than those fed diets PO 0 and PO 25. GPAT activity was however not affected significantly by the inclusion of dietary PO but increased along increasing dietary PO inclusion levels.

Gene Expression Profiles

The mRNA expression levels of *cptIa, cptIb, acyl, acc, scd1, fas and gpat* are shown in Figure 1 (A-G). CPT1a and b were down regulated by the addition of PO to the experimental diets. Fish fed diets with 0% PO recorded the highest mRNA expression in both CPT1a and b and were significantly higher than all other groups ($P < 0.05$). On the other hand, Acyl, ACC, SCD1 and FAS were up regulated by the addition of PO. Fish subjected to diets with 75% and 100% PO recorded the highest FAS mRNA expression and were significantly higher than all other groups. SCD1 mRNA expression of fish fed 100% PO were significantly higher than those fed 0%, 25%, 50% and 75% PO. Also there was statistical

Table 5. Fatty acid composition (% of total fatty acids) in the muscle of *Oreochromis niloticus* juvenile fed diets with different levels of palm oil (PO) for 8 weeks

FATTY ACID(S)	Dietary PO replacement level (%)				
	PO 0	PO 25	PO 50	PO 75	PO 100
Liver lipid	9.48±0.12	9.82±0.38	9.54±0.05	9.89±0.74	9.15±0.08
12:0	0.14±0.01^{b}	0.11±0.00^{ab}	0.10±0.00^{ab}	0.10±0.00^{ab}	0.09±0.00^{a}
14:0	4.80±0.07^{d}	4.63±0.04^{cd}	4.08±0.22^{bc}	3.95±0.09^{b}	2.53±0.05^{a}
16:0	23.42±0.09^{a}	25.06±0.27^{b}	29.66±0.18^{c}	30.52±0.27^{c}	32.30±0.19^{d}
18:0	6.60±0.16	5.56±0.32	5.99±0.52	5.90±0.05	5.71±0.03
20:0	0.26±0.01^{b}	0.23±0.01^{b}	0.20±0.01^{b}	0.19±0.02^{b}	0.10±0.00^{a}
ΣSFA's	*35.23±0.13^{a}*	*35.60±0.34^{a}*	*40.06±0.34^{b}*	*40.68±0.35^{b}*	*40.75±0.19^{b}*
16:1(n-7)	7.95±0.07^{b}	7.88±0.12^{b}	6.13±0.75^{ab}	6.23±0.32^{a}	4.52±0.02^{a}
16:1(n-9)	0.50±0.00^{a}	0.47±0.02^{a}	0.66±0.07^{ab}	0.92±0.07^{c}	0.80±0.00^{bc}
18:1(n-7)	6.45±0.12	6.23±0.03	6.19±0.07	6.18±0.07	6.06±0.17
18:1(n-9)	28.92±0.25^{a}	28.69±0.08^{ab}	30.63±0.24^{bc}	31.68±0.60^{cd}	32.80±0.32^{d}
Σ MUFAs	*43.84±0.12*	*43.29±0.05*	*43.62±0.89*	*45.02±0.25*	*44.18±0.40*
16:3(n-3)	077±0.01^{c}	0.65±0.07^{bc}	0.55±0.01^{b}	0.52±0.03^{b}	0.32±0.03^{a}
18:3(n-3) LNA	*0.50±0.04^{a}*	*0.81±0.00^{ab}*	*1.06±0.11^{bc}*	*1.22±±0.03^{c}*	*1.16±0.09^{c}*
20:4(n-3)	0.54±0.01^{ab}	0.52±0.03^{a}	0.58±0.02^{ab}	0.58±0.02^{ab}	0.65±0.02^{b}
20:5(n-3) EPA	*0.97±0.03^{c}*	*0.84±0.07^{bc}*	*0.77±0.10^{abc}*	*0.56±0.05^{ab}*	*0.47±0.02^{a}*
22:6(n-3) DHA	*4.80±0.07^{c}*	*4.32±0.03^{c}*	*4.09±0.26^{c}*	*2.46±0.18^{b}*	*1.57±0.06^{a}*
Σ n-3	*7.59±0.03^{c}*	*7.17±0.13^{c}*	*7.06±0.36^{c}*	*5.37±0.08^{b}*	*4.18±0.07^{a}*
18:2(n-6) LA	*10.59±0.43^{ab}*	*12.48±0.61^{b}*	*10.36±0.19^{a}*	*10.30±0.21^{a}*	*10.99±0.43^{ab}*
20:2(n-6)	1.62±0.13^{d}	1.2±0.07^{c}	0.68±0.01^{b}	0.50±0.04^{ab}	0.26±0.00^{a}
20:4(n-6)ARA	0.84±0.02^{c}	0.71±0.06^{b}	0.57±0.10^{ab}	0.44±0.01^{a}	0.35±0.01^{a}
Σ n-6	13.04±0.38^{b}	14.46±0.70^{c}	11.62±0.15^{ab}	11.25±0.24^{ab}	11.61±0.43^{a}
Total PUFAs	20.63±0.38^{cd}	21.63±0.58^{d}	18.69±0.43bc	16.62±0.50^{ab}	15.80±0.50^{a}
ΣSFA/Σ PUFA	1.70±0.03^{a}	1.64±0.05^{a}	2.14±0.04^{b}	2.45±0.05^{c}	2.58±0.08^{c}
n-3:n-6	0.58±0.01^{cd}	0.49±0.03^{bc}	0.60±0.02^{d}	0.47±0.01^{b}	0.36±0.00^{a}

Table 6. Hepatic metabolic enzyme activities of *Oreochromis niloticus* juvenile fed diets with different levels of palm oil (PO) for 8 weeks

Lipogenic enzyme(U/L)	Dietary palm oil inclusion level (%)					
	PO 0	*PO 25*	*PO 50*	*PO 75*	*PO 100*	*P-value*
SCD 1	39.62±3.61^{a}	57.57±8.41^{ab}	74.52±17.69^{ab}	91.13±10.57^{bc}	105.9±11.90^{bc}	0.0031
FAS	219.7±24.08^{a}	214±28.39^{a}	227.9±34.47^{ab}	249.5±29.92^{ab}	356.8±31.36^{b}	0.0131
ACYL	72.09±4.06^{a}	97.46±19.78^{ab}	113.40±10.55^{ab}	123.30±3.91^{b}	143.10±12.81^{b}	0.0036
ACC	37.59±1.51^{a}	55.47±9.65^{ab}	72.31±10.45^{b}	73.51±8.50^{b}	61.60±7.40^{ab}	0.0285
GPAT	127.3±27.63	137.3±25.50	147.4±21.12	180.8±19.55	198.4±18.88	0.1680
CPTI	228.60±12.17^{a}	243.50±7.62^{ab}	234.0.1±21.68^{a}	264.81±22.88^{ab}	298.20± 7.62^{b}	0.0174

Scd 1: Stearoyl-CoA desaturate 1 (delta-9 desaturate)
Fas: Fatty acid synthesase
Acyl: ATP citrate lyase
Acc: Acetyl-CoA carboxylase
Gpat: Glycerol-3-phosphate acyltransferase

difference in Acyl mRNA expression among groups with fish fed higher levels of PO (50%, 75% and 100% PO) being significantly higher than those fed 0% and 25% PO. Finally, fish fed 100% PO had ACC mRNA levels significantly higher than those fed diets with 0% and 25% PO. Gpat mRNA levels were not significantly influenced by dietary PO inclusion although fish fed 75% PO had the highest expression level.

Discussion

The results of this study revealed that the liver and muscle FA profile of *O. niloticus* generally reflected the dietary FA composition and is in agreement with other studies (Li *et al.,* 2015a; Li *et al.,* 2016).

This study recorded a significant ($P<0.05$) decrease in n-3/n-6 ratio in both liver and muscle. The n-3/n-6 ratio reduced from 0.77 and 0.58 in the liver and muscle in fish fed 100% FO to 0.18 and 0.36, respectively. This shows that omega 3 fatty acid had been spared by mono saturated fatty acid and to a lesser extent by saturated fatty acids (González-Félix *et al.,* 2016). This could be attributed to the increase in 18:2*n*-6 content (Bransden *et al.,* 2003) coupled

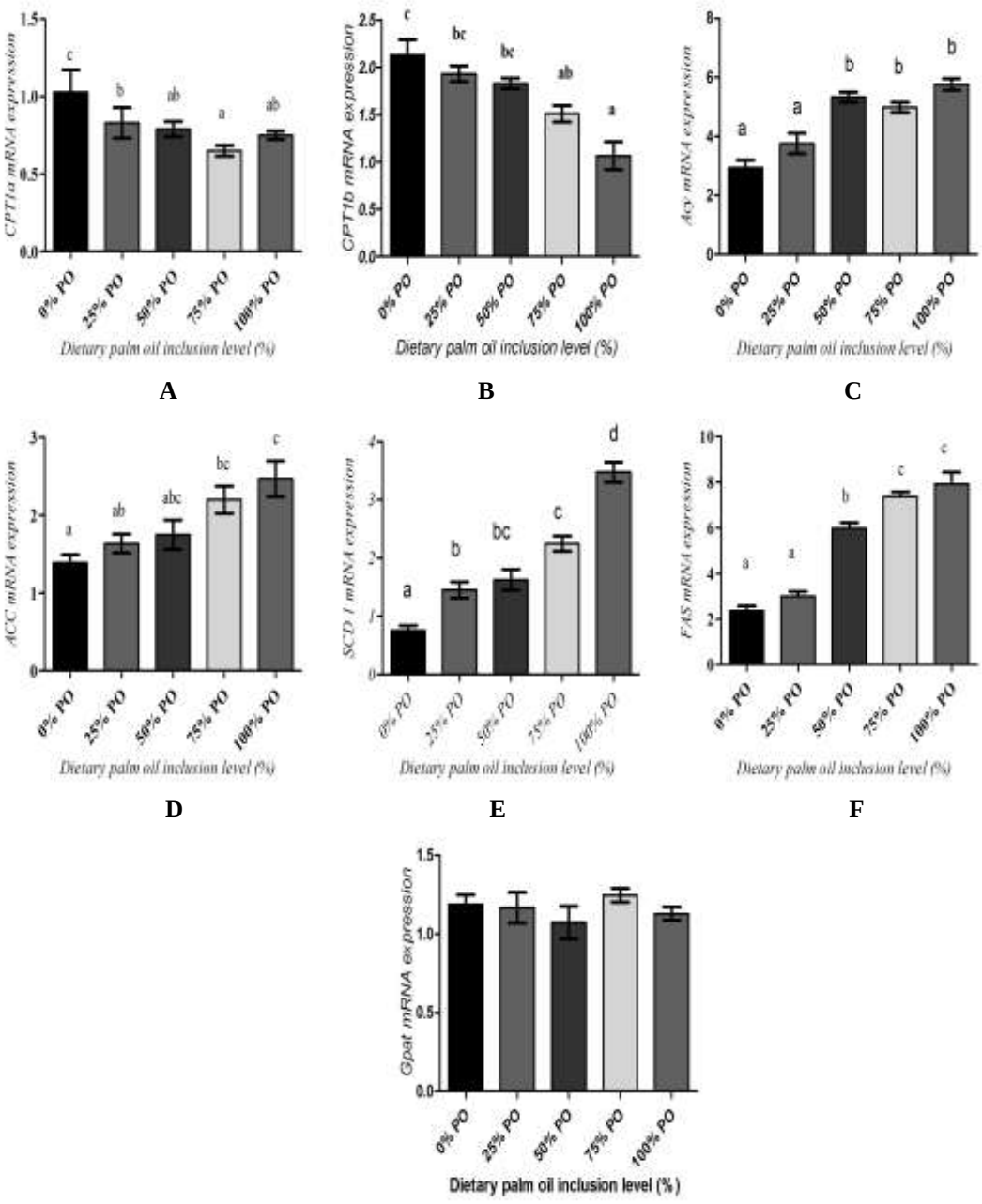

Figure 1. Real time PCR gene expression profiles of A) carnitine palmitoyltransferase Ia (CPT Ia), B) carnitine palmitoyltransferase Ib (CPT Ib), C) ATP citrate lyase (Acyl), D) acetyl-CoA carboxylase (ACC), E) Steroyl-CoA desaturase 1 (SCD1), F) fatty acid synthesase (FAS) and G) Glycerol-3-phosphate acyltransferase in liver f *O. niloticus* juvenile fed diets with different levels of palm oil (PO) for 8 weeks. Values are expressed relative to β-actin and are presented as means ± S.E.M.

with decreased levels of long-chain n-3 PUFA in vegetable oils (Mateos *et al.*, 2012). Other studies had previously documented this phenomenon in Atlantic salmon (Grisdale-Helland *et al.*, 2002), murray cord (Turchini *et al.*, 2011) and short fin corvina (González-Félix *et al.*, 2016). The concentration of SFA in both liver and muscle was generally higher than the concentration in the feed of all groups; this is an indication that these fatty acids were not mainly catabolized for energy purposes.

The n-3LC-PUFAs such as EPA and DHA of fish fed vegetable oils are usually lower compared to those fed fish diets with fish oil (Taşbozan *et al.*, 2015). EPA, DHA as well as ARA reduced as palm oil levels increased in diets in both muscle and liver. This is in agreement to previous studies in African catfish (Ng *et al*., 2003) and hybrid tilapia (Han *et al.*, 2013). DHA in the liver (6.35-0.85) were relatively

higher than that of the feed (5.66-0.58) indicating there was a selective retention of the DHA (Mozanzadeh *et al.,* 2016). This also indicates that when *O. niloticus* are fed diets with increasing PO levels, their ability to synthesize EPA and DHA from short-chain n-3 fatty acids such as ALA is reduced (Han *et al.,* 2013). Comparatively, LA (18:2n-6) values in the liver and muscle were smaller compared that of the feed indicating that LA was used to the meet energy demands of the fish (Li *et al.,* 2016).

Inclusion of dietary PO resulted in a significant decrease of n-3 PUFA in both liver and muscle (from 10.16 to 2.77 and 7.59 to 4.18 respectively), and is in agreement with other studies (Mourente and Bell, 2006; Gao *et al.,* 2012). Contrary to this study, Visentainer *et al*. (2005) observed a significant increase in n-3 fatty acids in adult Nile tilapia fed diets with increasing levels of flaxseed oil. Also, Kanari *et al.* (2010) recorded a higher n-3 PUFA in fish fed FO diets than in those fed on blends of vegetable diets. These differences in results could be attributed to factors such as differences in species and size, nutritional status of the fish at stocking as well as feeding duration (Li *et al.,* 2013).

Oleic acid (C18:1n-9) in both liver and muscle showed a trend of increasing values with increasing PO inclusion levels. This suggests that feeding *O. niloticus* with diets containing PO could increase active biosynthesis (liponeogenesis) (Eroldoğan *et al.,* 2012).

The activities and gene expressions of lipid metabolism-related enzymes are known to be influenced by dietary lipids (Yilmaz *et al.,* 2004; Geay *et al.,* 2011).

This study recorded significant increase in SCD 1 enzymes as dietary PO increased in diets. This is in agreement with previous study when diets with elevated proportion of SFAs resulted in increased SCD activity in carp (Polley *et al.,* 2003). This might have caused the increase in hepatic liver lipids as dietary PO increased in diets. The upregulation of SCD 1 in fish fed higher dietary PO does not inhibit lipid synthesis in the liver of *O. niloticus* when PO is used in place of FO.

There was an increase in liver FAS activity (Table 6) as dietary PUFA increased. This is because dietary fatty acids, especially PUFAs (polyunsaturated fatty acids), inhibit the activity of FAS (Leng *et al.,* 2012). This is in agreement with previous study which reported that PUFA strongly inhibited FAS enzyme activity in rats (Kim *et al.,* 2004).

CPT1 enzyme activity correlated negatively with dietary C_{18} PUFA (specifically 18:3n-3). This is an indication that reducing dietary content of C_{18} as a result of increasing dietary PO levels could lead the storage of LC-PUFA rather than β-oxidation. There was also a negative correlation between liver 20:4n-3 and Cpt1 enzyme activity also indicating accumulation of 20:4n-3, an intermediate product rather than β-oxidation.

GPAT is involved in the first steps of glycerol-3-phosphate pathways (Castro *et al*. 2016). It is also known to be a competitor with CPT1 for acyl-CoAs at the outer mitochondrial membrane of tissues. There was no significant difference among groups in both the enzyme activity and mRNA expression. This non significant difference in the expression levels coupled with increase in CPT1 as palm oil levels increase in diets indicates that CPT1 plays a vital role in fatty acid synthesis compared to GPAT. We therefore assume that GPAT did not play any significant role in lipid synthesis in the liver. Similar to this study, there was no significant difference in GPAT mRNA expression in the liver of gilthead sea bream fed diets fish oil or a blend of vegetable by Castro *et al.* (2016).

The mRNA expression of ACC and FAS are regulated coordinately (Toussant *et al.,* 1981). The regulation of fatty acid synthesis is maintained by (ACCa) whiles its counterpart (ACCb) regulates fatty acid oxidation (Lopaschuk *et al.,* 1994). This mechanism was confirmed by the upregulation of both FAS and ACC mRNA expression in the liver. This is however contrary to the study of Dong *et al.* (2014) in grass carp where the mRNA expression of FAS and ACC was not regulated coordinately in the liver and muscle. Fish fed higher levels of PO had higher levels of FAS mRNA expression and was consistent with the lipid levels in the liver. This could suggest that the expression of FAS may have been regulated by the nutritional levels of the fish. The higher expression of mRNA of FAS coincided with the higher hepatic lipid deposition in 100% PO and could be attributed in the lower fatty acid oxidation in higher PO levels. This is in agreement to previous studies by Morais *et al.* (2011), Morais *et al.* (2012) in Atlantic salmon and Peng *et al.* (2014) in juvenile turbot.

There was a reduction in the CPT1a and CPT1b mRNA expression as PO inclusion levels increased in the diet. This could be as a result of the reduction in the n-3 LC-PUFA as PO increased. PUFA are known to act on the CPT 1 activity through the changes in the mitochondrial membrane composition (Jackson *et al.,* 2000). This change in the composition of the mitochondrial membrane composition may have caused the same changes in the CPT1a and CPT1b sensitivity in the liver. This study is in agreement to an earlier report by Morash *et al.* (2009) which documented higher mRNA expression of CPT1 in liver, adipose tissue as well as red muscle when rainbow trout were fed diets containing higher levels of PUFA as compared to the control diet. Also, this study agrees to that of Lu *et al*. (2014) which reported a significant alteration in the hepatic mitochondrial membrane FA composition and CPT1 kinetics and the down regulation of hepatic CPT1 when blunt snout were fed high levels of n-3LC-PUFA. Peng *et al.* (2014) reported that lower expression of CPT1 has the ability to lower non-esterified FA delivery leading to

the down-regulation of b-oxidation and finally resulting in higher hepatic lipid deposition. This study supports this assertion owing to the down-regulation of CPT1a and CPT1 b coupled with the increasing hepatic lipid deposition as PO levels increased. This could also be because dietary n-3 LC-PUFA can increase mitochondrial fatty acid oxidation hence stimulating CPT1 activity (Madsen *et al.,* 1999).

The activities of SCD primarily regulate the fatty acid composition in membrane lipids (Murata and Wada, 1995). Also, different individual fatty acids influence the regulation of SCD gene expression in tissues. There was an increase in mRNA expression of SCD1 in the liver when PO levels were elevated in the diets. This same observation was made by Hsieh *et al.* (2007) when tilapia were fed diets with different dietary lipid sources. This is probably due to the higher SFAs coupled with lower PUFAs which have been reported to upregulate SCD expression in tilapia (Hsieh *et al.,* 2007).

Conclusion

We investigated the liver and muscle tissue FA composition, liver lipogenic enzyme activities and hepatic gene expression response of Nile tilapia fed diets with varying levels of dietary palm oil. To our knowledge, this is the first report on lipogenic enzyme activities as well as mRNA expression of lipid metabolism related genes in Nile tilapia fed diets with different levels of PO. Fatty acids recorded in both liver and muscle to a larger extent mirrored the dietary fatty acids. Replacing FO with PO significantly influenced the enzymatic activities (SCD1, FAS, ACYL, ACC and CPTI). PO induced these enzymatic activities with fish fed 75% PO and 100% PO recording higher enzymatic activities in all cases. The results suggest that *fas, scd1, acc, acyl* and *cpt1* could play vital roles in the metabolism of fatty acids in Nile tilapia when FO in diets are replaced or supplemented with PO. Replacing FO with PO up regulated the expression of *fas, scd1, acc* and *acyl* mRNA expression whiles the expression of *cpt1a* and *cpt1b* were down regulated. Further studies are however required to fully understand the molecular mechanism as well as clarifying the roles these enzymes play in the metabolism and synthesis of fatty acids/lipids.

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