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THE EFFECTS OF COMBINED USE OF SOYBEAN EXTRACT AND MIXTURE OF SEVERAL PLANT OILS ON THE GROWTH PARAMETERS AND WHOLE BODY AND TISSUE AMINO ACIDS AND FATTY ACID COMPOSITIONS IN JUVENILE NILE TILAPIA (*OREOCHROMIS NILOTICUS* LINNAEUS, 1758)

Almila Uysal, Kenan Engin

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Department of Aquaculture, Faculty of Fisheries, Mersin University, Yenişehir Kampüsü C Blok Kat: 1, 33160 Mersin, Turkey

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Correspondence:
Kenan ENGIN
E-mail: kengin@mersin.edu.tr

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ABSTRACT

This study investigated the effects of combined dietary fish meal and oil replacement by soybean extract and increasing amount of plant oil mixture on growth performance, whole body and tissue amino acid and fatty acid compositions in Nile tilapia (*Oreochromis niloticus*). Diets in which 50% of the fish meal crude protein was replaced by soy bean extract along with 60, 80 and 100% of fish oil replacement by mixture of soy, canola and linseed oils (v/v, 1:1:1) were used in the study. Four iso-nitrogenous and iso-energetic experimental diets were prepared and fed to juvenile Nile tilapia (25 fish per tank with average wet weight of 16.24 ± 0.66 g) twice in equal portions during morning (09:00-10:00 h) and afternoon (17:00-18:00) at a fixed rate of 4% BW.d^{-1} for 84 days. At the end of 84 day grow out period growth parameters and whole body and muscle tissue amino acid and fatty acid compositions were assessed. Fish in each dietary treatment more than doubled its initial average weight and no statistical differences ($P>0.05$) were found among dietary treatments in terms of measured growth parameters. It was also demonstrated that diets supplemented with soy bean extract and increasing amount of plant oil mixture proportionally increased the crude protein contents and significantly decreased ($P<0.05$) the crude ash levels in fish whole body samples compared to that of fish fed fish meal and oil control diet. It appeared that except for one or two amino acids, fish fed diets supplemented with soy bean extract and increasing amount of plant oil mixture tended to increase the muscle tissue essential and non-essential amino acid levels and that L-lysine and DL-methionine used in these diets were effectively used for growth in juvenile Nile tilapia. Except for several fatty acid classes, fish whole body and tissue fatty acid compositions generally mimicked the feed fatty acids following the growth trial. It was also understood that DHA was probably deposited whereas EPA was used for energy production in fish fed diets supplemented with soybean extract and increasing amount of plant oil mixture. Furthermore, fish fed diets supplemented with soy bean extracts and plant oil mixtures had significantly higher ($P<0.05$) intermediate metabolites like 18:3n-6 and 20:3n-6 of the n-6 LC HUFA biosynthesis in whole body and tissue samples compared to that of fish fed the control diet implying Nile tilapia in this conditions might have activated the Δ5 and Δ6 elongation and desaturation enzymes. It was demonstrated that the partial replacement of dietary fish meal and 100% replacement of dietary

added fish oil by soy bean extract and plant oil mixture did not compromise growth rates or drastically change whole body and tissue amino acid and fatty acid compositions in juvenile Nile tilapia.

Keywords: Nile tilapia, Plant protein and oil sources, Nutrient utilization, Tissue amino acid, Fatty acid compositions

Introduction

The total production of aquatic organisms from wild and farming has almost reached 168 million tons in 2016 and nearly 60% of this production level comes from the farming activities only (FAO, 2016). Feed cost comprises of approximately 60% of total farm expenditures and sustainability of fish farming relies heavily on the availability of low cost good quality aqua feeds. Fishmeal and oil are finite and therefore expensive ingredients that are widely used in aqua feeds. Fishmeal is also considered an indispensable feed ingredient for farmed fish and crustacean species for several reasons such as its high protein content, balanced amino acid profile and palatability. However, the uncertainty in annual production levels and per unit price urges aquaculture scientist to find alternatives to fishmeal and oil that are cheaper and sustainable to produce in order to continuation of sustainability of aquaculture production (Tacon and Metian, 2008). Higher annual production rates and much lower per unit prices compared to that of fishmeal are the advantages of plant proteins to be considered as viable alternatives for fishmeal protein in aqua feeds (Teoh *et al.,* 2011). However, imbalanced amino acid compositions, several anti-nutritional factors and therefore the lower nutrient digestibility are the main disadvantages that may limit the effective use of plant protein sources in aqua feeds (Teoh *et al.,* 2011). Soya products have been used effectively in commercial diet formulations of many farmed fish species including Nile tilapia. But previous studies demonstrated that successful replacement of dietary fishmeal by soya products in Nile tilapia was only possible when dietary amino acid profile is balanced by AA supplementation (El-Saidy and Gaber 2002, Furuya *et al.,* 2004, Figueiredo-Silva *et al.,* 2015).

Dietary lipids provide fish not only with the energy they require for daily maintenance but also the essential fatty acids for healthy growth and development through the formation of many biologically active cellular compounds such as prostaglandins and eicosanoids, sterols, phospholipids and the bio activation of lipid soluble vitamins. Unlike fish oil, plant oils lack n-3 LC-PUFAs and contain high amounts of other fatty acid classes such as SFAs (Saturated Fatty Acids), MUFAs (Mono Unsaturated Fatty Acids) and n-3 / n-6 PUFAs (Short Chain Polly Unsaturated Fatty Acids) (Teoh and Ng, 2016). Perilla and linseed oils are examples of plant oils that are rich sources of n-3 PUFAs. However, soy, corn and sunflower oils are classified as plant oils that contain high amounts of n-6 PUFAs. Canola and palm oils are, on the other hand, categorized as MUFA and SFA rich plant oil resources respectively (Teoh and Ng, 2016). Previous studies conducted in many farmed warm freshwater fish species including tilapia demonstrated that partial and total replacement of dietary fish oil by vegetable oils did not compromised growth rates as long as dietary essential fatty acid requirements of fish has been provided (Steffens *et al.,* 1995; Alava, 1998; Li *et al.,* 2016). In addition, blend of different plant oil use in diets as a lipid source has been demonstrated to be more effective than a single plant oil source in meeting the fatty acid requirement of Nile tilapia thereby resulting in better growth and feed efficiency rates (Teoh *et al.,* 2011). However, major concern for dietary plant oil use in aqua feeds appear to be the lowering impact of these oils on the whole body and fillet n-3 LC-PUFA content of fish (Turchini *et al.,* 2011).

Studies investigating the effects of combined replacement of dietary fishmeal and oil on growth and whole body and tissue nutrient content and fatty acid composition of Nile tilapia are limited. Therefore, this study investigated the effects of dietary combined replacement of fishmeal and oil by soybean extract (50% of fishmeal crude protein) and blend of plant oil sources (60, 80 and 100% replacement of fish oil by soy, canola and linseed oil blend, v/v 1:1:1) on growth, nutrient utilization, whole body and tissue amino and fatty acid compositions of juvenile Nile tilapia using experimental diets with high crude lipid content.

Materials and Methods

Fish and the maintenance

Juvenile Nile tilapia used in this experiment was obtained from the freshwater fish culture unit of Faculty of Fisheries, Çukurova University, Adana, Turkey. Following their arrival, fish were acclimatized to feeding and laboratory conditions for approximately 2 weeks using commercial fish pellets (Çamli Feed Company: 45% CP and 15% CL). After the acclimatization period, a total of 300 Juvenile Nile tilapia (16.2 ± 0.7 g AIBW) was randomly assigned to 12 (25 fish per tank) rectangular fiber glass culture tanks (120 cm L × 50 cm H × 32 cm W and 190 l rearing volume) designed as a semi-recirculating rearing unit. Before the experimental feeding began, fish were starved for 2 days. During the experimentation, fish were fed twice a day in the morning (09:00-10:00 h) and the afternoon (17:00-18:00 h) at a fixed feeding rate of 4% $BW.d^{-1}$. Uneaten feed and feces were siphoned out daily throughout the experimental period and the water taken out was replenished with the same temperature freshwater

kept in the reservoir tank. Utmost care was given to the fact that diet was totally consumed during the feeding periods and actual feeding consumption was measured only twice a week by collecting the uneaten pellets and drying them. Water quality parameters were maintained within the desired range for tilapia species. Water temperature and dissolved oxygen (DO) measurements were made daily during the experimental period whereas pH and nitrogenous compounds such as total ammonia and nitrite in rearing tank water were recorded twice weekly. Briefly; water temperature was kept around 25°C (24.7 ± 0.6°C); DO averaged 6.6 ± 0.4 mg.L^{-1}, pH was maintained at 6.8 ± 0.5; Total ammonia and nitrite levels were also measured to be 0.01 ± 0.005 and 0.02 ± 0.01 mg.L^{-1} throughout the experiment.

Diet formulation and preparation

Four iso-nitrogenous and iso-energetic diets containing 31% Crude Protein and 10% Crude Lipid on a dry matter basis were formulated. Fifty percent of the fishmeal crude protein and 60 (60 SCL), 80 (60 SCL) and 100 (100 SCL)% of dietary added fish oil in control diet (FO) was replaced by soybean extract and the equal mixtures of soy, canola and linseed oils respectively in other dietary treatments. All the feed ingredients were obtained locally. Canola and linseed oils were purchased from a grocery store. Dextrin was used as a carbohydrate source. Diets were also supplemented with L-lysine and DL-methionine in order to meet the recommended essential amino acid requirement of Nile tilapia in accordance with National Research Council (1993) (Table 1). Before being combined with the wet ingredients, all the dry ingredients were mixed thoroughly for at least 45 minutes. Following mixing, oils and water were incorporated individually into dietary mixtures and continued mixing for a further 30 minutes each until the pelleting consistency reached. Dietary mixtures were then screwed through a 2 mm die using a kitchen meat grinder and all the feed strands, after being dried at room temperature, were broken into mouth size pellets by hand. All the experimental diets were individually bagged and stored at -20°C until using.

Sampling and evaluation of growth rate: Before the beginning of the experimentation, 20 fish were sampled for initial whole body and tissue nutrient, amino and fatty acid composition analysis and kept at -80°C until analysis. Feeding lasted 84 days. Every 20 days, fish were individually weighed and amount of feed to be given to each tank was calculated accordingly using fixed feeding rate of 4% BW.d^{-1}. Fish were also starved one day before the weight gain measurements throughout the experimentation. To minimize the stress, fish were weighed quickly and put into a container filled with rearing water before returning to their respectful tanks. After the rearing period, 6 fish from each tank (18 fish per treatment) were sampled randomly and killed by an overdose of MS222 followed by a sudden blow to the back of the head. Thereafter fish were immediately dissected for muscle and liver tissue samples to be used for nutrient, amino acid and fatty acid composition analysis. Homogenized whole body (6 fish per treatment), skinned right muscle and liver tissue samples (12 fish per treatment) were stored individually in plastic jars at -80°C. Before using in chemical analysis, all the samples were freeze dried (LabConco, Missouri USA), homogenized, pooled and kept at -20°C.

At the end of the experimentation, Nile tilapia growth performances were evaluated according to following formulae: Specific Growth Rate as SGR (%.d^{-1}): [(ln final weight-ln initial weight)/84] × 100; Feed Efficiency Ratio as FER=[(Total weight gain (g))/(Total feed consumption (g DM))]; Protein Efficiency Ratio as PER (%)=[(Gain in weight (g))/(Protein intake (g))] ×100; Hepato Somatic Index as HSI=[(Liver weight/Fish body weight)] ×100.

Chemical analysis

Diets, dried whole body and muscle tissue homogenates were analyzed for crude protein and crude lipid using Kjeldahl technique (selenium catalyst; %N × 6.25) and chloroform/methanol extraction (2:1 v/v) (12) respectively. Dietary gross energy values were calculated based on the standard physiological fuel values of 19 kJ.g^{-1} for protein, 36 kJ.g^{-1} for lipid and 15 kJ.g^{-1} for carbohydrate (13). Dry matter (g.kg^{-1} DM) and ash in samples were analyzed using standard methods (AOAC 1995).

Fatty acid methyl esters (FAME) were prepared according to Metcalfe and Schmitz, 1961 and analyzed using slightly modified method described previously by Czesny and Dabrowski, 1998. Fame was then separated by gas chromatography (Agilent 7890 A series, Santa Clara, USA) equipped with a flame ionization detector and fitted with an HP-88 capillary column (100 m, 0.25 mm i.d. and 0.2 µm). The oven temperature was first set up at 140°C for 5 min. then increased 4°C per min. up to 240°C where it stayed for 20 min. The carrier gas was helium with fixed flow rate of 0.82 mL.min.$^{-1}$ and the split ratio was adjusted as 10:1. Fatty acids were then identified by comparing their retention times to that of a standard mix of fatty acids (Sigma Germany) and quantified by comparing their peak area with that of the internal standard.

Essential and non-essential amino acid composition analysis of diets, whole body and muscle tissue samples were commissioned to food laboratory of Marmara Research Centre of The Scientific and Technological Research Council of Turkey (TUBITAK) and were according to Dimova, 2003. Briefly, homogenized samples weighing between 0.1 to 1.0 g were taken into 50

Table 1. Formulation (g.kg^{-1}) and chemical composition of the experimental diets.

Ingredients	*FO*	*60 SCL*	*80 SCL*	*100 SCL*
Fish Meal	375	223	223	223
Corn Gluten Meal	106	39	39	39
Soybean Extract	0	283.7	283.7	283.7
Fish Oil	50	20	10	0
Soy Oil	0	10	13.3	16.7
Canola Oil	0	10	13.3	16.7
Linseed Oil	0	10	13.3	16.7
Dextrin	260	240	240	240
Vitamin Mix[a]	10	40	40	40
Mineral Mix[b]	10	30	30	30
DCP[c]	46	21.3	21.4	21.2
CMC	60	30	30	30
Bentonite	78.5	36	36	36
L-Lysine	3	4	4	4
DL-Methionine	1.5	3	3	3
Moisture	76.5	81.6	73.6	96.2
Crude Protein	307.6	311.4	291.6	294.7
Crude Lipid	116.4	105.8	97	89.5
NFE[d]	375.9	428	453.4	461.8
Crude Ash	200	154.5	157.8	153.9
Gross Energy[e] (MJ.kg^{-1})	14.5	13.9	13.9	13.9

[a,b]According to recommendations of NRC (1993) for Tilapia; [c] Di Calcium Phosphate; [d] Nitrogen Free Extract: 1000-(moisture+crude protein+crude lipid+crude ash); [e] Calculated based on the standard physiological fuel values: 19 kJ.g^{-1} for proteins, 36 kJ.g^{-1} for lipids and 15 kJ.g^{-1} for carbohydrates (Smith, 1989).

mL glass jars and added with 20 mL of 6 N HCl solution. Immediately after the addition of nitrogen gas, sample jars were tightly closed with the lids and hydrolyzed in an oven at 110°C for 24 h. Thereafter samples were cooled to room temperature and filtered using filter paper. 0.2 mL of filtrate was then pippeted into different experimental tubes and evaporated under the nitrogen gas at 50°C. The evaporation was further continued adding 0.5 mL acetonitrile to the tubes. Approximately 0.5 mL of acetonitrile: methanol: triethylamine mixture and 0.1 mL of derivatization solution were then added to the tubes and derivatized in the oven set at 40°C for 30 min. Following derivatization, samples were added 0.2 mL acetonitrile and further evaporated under the nitrogen gas. Before injecting into UFLC (Ultra-Fast Liquid Chromatography) samples were added with 5 mL 0.02 M ammonium acetate solution and filtered through 0.2 µm filter papers. UFLC conditions used in the analysis were as follows; Mobile Phase A: 1 l of Sodium dihydrogen phosphate dehydrates and disodium hydrogen phosphate dehydrate buffer solution was prepared and pH was adjusted to 6.8-6.9. Mobile Phase B: UV detector and acetonitrile were used and column temperature was set up at 40°C. Wavelength, injection volume and flow rate were adjusted as 254 nm, 10 µL and 1 mL.min^{-1} respectively.

Statistical analysis

Data are presented as mean ± SD throughout the text. Means were compared by one-way ANOVA. Prior to ANOVA, the assumption of normality and homogeneity of variance was assessed by Shapiro-Wilk (Zar 1996) and Levene's tests respectively. When a significant treatment effect was observed, a Tukey-HSD test was used to compare means. Significance was accepted at probabilities of 0.05 or less. All the data were analyzed using SPSS 22.0 Statistical Software Package (SPSS, Chicago, IL).

Results

Diets were prepared as iso-nitrogenous (varying from 295 to 311 $g.kg^{-1}$), iso-lipidic (90 to 116 $g.kg^{-1}$) and iso-energetic (13.9 to 14.5 $Mj.kg^{-1}$) (Table 1). Dietary essential and non-essential amino acid values of FO diet were slightly higher than that of other dietary treatments (Table 2). L-lysine was also found to be a little higher (6 to 12 g per kg of diets) in BY diet than that of other dietary treatments. Fatty acid composition of the diets mirrored the fatty acid composition of the oils used in the diets. Briefly, increased level of plant oil mixture in diets (60 SCL, 80 SCL and 100 SCL) significantly decreased (P<0.05) the level of EPA and DHA compared to that of FO diet (Table 3). EPA and DHA levels in 60SCL diet were also found to be significantly higher (P<0.05) than that of 80SCL and 100SCL diets. Linoleic acid (18:2n6) levels were significantly higher (P<0.05) in diets containing increasing amount of plant oil mixtures than that of FO diet and it was proportional to the fish oil replacement levels (Table 3). Linolenic acid (18:3n3) levels almost tripled in diets in which fish oil was replaced by increasing amount of plant oil mixture (60 SCL, 80 SCL and 100 SCL) compared to that of FO diet. In addition, oleic acid (18:1n9) levels were found to be similar in all the dietary treatments (Table 3).

There were no mortalities during the experimentation. Fish doubled its initial weight at the end of the experiment. In addition, no statistically significant differences in any of the growth parameters measured were found among the dietary treatments (Table 4). However, HSI was tended to increase with the increasing level of fish oil replacement by plant oil mixture in diets (Table 4). Regardless of soybean extract and increasing plant oil mixture inclusion in diets, the essential and non-essential amino acid values of muscle tissue of fish fed all the dietary treatments were higher than that of initial fish muscle tissue samples at the end of the grow-out period (Table 5).

Whole body and muscle tissue nutrient composition data are presented in Table 6. Whole body crude protein levels varied 63.6 to 67.6% (on a DM basis) among dietary treatments and were higher in fish fed diets containing soybean extract and vegetable oil blend than that of fish fed FO diet. The crude protein levels of fish fed the dietary treatments 60 SCL and 80 SCL were significantly higher (P<0.05) than that of fish fed FO diet (Table 6). However, whole body crude lipid contents of fish varied 18.9 to 22.4% (on a DM basis) among dietary treatments and tended to decrease in fish fed diets containing soybean extract and plant oil mixture. Whole body crude ashes contents of fish followed the same trend and decreased with the use of soybean extract and plant oil mixture in diets. Tilapia fed the 60SCL diet had significantly lower (P<0.05) whole body crude ash content (11.7% DM basis) compared to that of fish fed FO diet (Table 6). Muscle tissue crude protein and ash did not significantly vary with the dietary treatments (Table 6). However, crude lipid

Table 2. Essential and non-essential amino acid composition of the experimental diets ($g.kg^{-1}$ diet) (n=1).

Essential amino acids	*FO*	*60SCL*	*80SCL*	*100SCL*
Methionine (Met)	11.1	9.5	10.6	9.5
L-Phenilalanine (Phe)	15.4	16.5	17.6	13.3
L-Lysine (Lys)	37.8	31.2	31.7	25.3
L-Histidine (His)	7.3	7.7	9	8.3
L-Valine (Val)	21.1	21.7	21.5	20.3
L-Leusine (Leu)	32.2	30.2	31.8	26
L-Isoleusine (Ile)	19.2	20.2	21.6	17.7
L-Threonine (Thr)	15.5	13.4	15.1	13.7
L-Arginine (Arg)	9.4	8.7	8.4	9.2
Non-Essential amino acids				
L-Alanine (Ala)	19.6	17.2	18	17.1
L-Aspartic Acid (Asp)	15.5	12.8	12.1	14.9
Glycine (Gly)	16.5	14.7	14.6	14.8
L-Glutamic Acid (Glu)	17.7	16.7	17.6	20.3
L-Proline (Pro)	18.3	16.8	17.3	15.6
L-Serine (Ser)	8.7	9.7	11	10
L-Tyrosine (Tyr)	10.2	10	11.1	9.9

contents were significantly lowers (P<0.05) in fish fed the diets containing soybean extract and increasing amount of plant oil mixtures (Table 6).

Whole body, muscle tissue and liver fatty acid compositions of Nile tilapia following 84-day grow-out period are shown in Tables 7-9 respectively. Except DHA, whole body and tissue fatty acid compositions of Nile tilapia at the end of the study mirrored the dietary fatty acid compositions in all the dietary treatments. It was found that total Saturated fatty acids in whole body fatty acid composition of fish fed diets containing soybean extract and increasing levels of plant oil mixture were significantly higher (P<0.05) than that of fish fed FO diet (Table 7). In contrast to whole body total saturated fatty acid contents, liver tissue total saturates levels (specifically, meristic (14:0), palmitic (16:0) and stearic (18:0) acids) were found to be significantly lower (P < 0.05) compared to that of fish fed FO diet (Table 9). Total whole body and muscle tissue monoenes were found to be similar among all the dietary treatments although the levels were 8 to 9% higher and 3 to 6% lower than that of dietary levels respectively (Tables 7 and 8). However, liver total monoenes were found to be more than 10% higher in fish fed soybean extract and increasing plant oil mixture compared to dietary levels. No significant differences were measured in whole body and muscle tissue oleic acid (18:1n9) levels of fish fed all the experimental diets (Tables 7 and 8) whilst liver oleic acid levels were slightly but significantly higher (P<0.05) in fish fed diets 60SCL, 80SCL and 100SCL (Table 9) than that of fish fed FO diet. Total n-6 fatty acid content of whole body, muscle and liver tissue of Nile tilapia fed diets 60SCL, 80SCL and 100SCL was significantly higher (P<0.05) than that of fish fed FO diet (Tables 7-9). In contrast to total n-6 fatty acids, whole body and liver total n-3 fatty acid contents of fish fed diets 60SCL, 80SCL and 100SCL were significantly lower (P<0.05) than that of fish fed FO diet. However, no significant differences were measured in muscle tissue total n-3 fatty acid contents of fish among dietary treatments (Table 8). The total whole body and liver tissue n-3 HUFA contents ranged from 5.6 to 7.2 and 2.8 to 3.6% of total fatty acids respectively in fish fed diets 60 SCL, 80 SCL and 100 SCL and were significantly lower than that of fish fed FO diet (Tables 7 and 9). However, the muscle tissue total n-3 HUFA contents of fish ranged from 14.3 to 15.8% of total fatty acids and the levels found in fish fed diets 80 SCL and 100SCL were slightly but significantly lower (P<0.05) than that of fish fed diets FO and 60 SCL (Table 8). It was found that whole body and liver tissue EPA contents of fish fed diets 60 SCL, 80 SCL and 100 SCL were significantly lower (P<0.05) than that of fish fed FO diet (Tables 7 and 9). EPA contents of muscle tissue of fish fed diets containing soybean extract and increasing amount of plant oil mixture were measured to be slightly

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

	Diets			
Fatty Acid Classes	***FO***	***60 SCL***	***80 SCL***	***100 SCL***
Σ Saturates	25.2 ± 0.2^{c}	21.2 ± 0.1^{b}	20.6 ± 0.4^{ab}	20.1 ± 0.4^{a}
Σ Monoenes	41.0 ± 0.1^{b}	35.1 ± 0.2^{a}	32.6 ± 0.9^{a}	31.8 ± 0.1^{a}
Σ n-9	34.8 ± 0.1^{b}	31.4 ± 0.2^{a}	29.2 ± 0.5^{a}	28.9 ± 0.1^{a}
18:1n9	30.0 ± 0.1	30.6 ± 0.5	28.5 ± 1.0	28.4 ± 0.2
Σ n-6	12.4 ± 0.2^{a}	24.0 ± 0.2^{b}	27.4 ± 0.5^{c}	30.0 ± 0.4^{d}
18:2n6	10.2 ± 0.0^{a}	23.1 ± 0.2^{b}	26.5 ± 0.5^{c}	29.5 ± 0.4^{d}
20:4n6	0.6 ± 0.0^{a}	0.3 ± 0.0^{b}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}
Σ n-3	19.9 ± 0.1^{c}	19.0 ± 0.1^{a}	18.6 ± 0.7^{b}	17.7 ± 0.1^{b}
18:3n3	3.5 ± 0.0^{a}	9.6 ± 0.1^{b}	10.5 ± 0.1^{c}	10.1 ± 0.2^{bc}
Σ n-3 HUFA	16.5 ± 0.1^{c}	9.4 ± 0.1^{b}	8.4 ± 0.2^{ab}	7.6 ± 0.1^{a}
20:5n3	8.7 ± 0.1^{c}	5.2 ± 0.1^{b}	4.9 ± 0.1^{a}	4.8 ± 0.0^{a}
22:6n3	5.2 ± 0.1^{c}	2.8 ± 0.1^{b}	2.5 ± 0.1^{a}	2.2 ± 0.1^{a}
n-3/n-6	1.6 ± 0.0^{d}	0.8 ± 0.0^{c}	0.7 ± 0.0^{b}	0.6 ± 0.0^{a}
ARA/EPA	0.07	0.06	0.04	0.04

Values are mean ± SD (n=3). Different superscripts in the same row denote significant differences (P<0.05)

lower than that of fish fed FO diet and the levels were only found significantly lower ($P<0.05$) in fish fed diets 80S CL and 100 SCL (Table 8). No significant differences were found in muscle tissue DHA contents of fish fed all the experimental diets and it ranged from 11.4 to 12.5% of total fatty acids among dietary treatments (Table 8). The DHA contents of fish whole body samples were also found to be similar to each other among dietary treatments but fish fed 100SCL diet had significantly lower DHA levels than that of fish fed 80 SCL and FO diets (Table 7). However, liver DHA levels were measured to be significantly lower in fish fed 60SCL, 80SCL and 100 SCL diets than that of fish fed FO diet (Table 9). It was also found that n-3/n-6 ratios in muscle tissue of fish fed soybean extract and plant oil mixture were closest to the ratio obtained from fish fed FO diet (Table 8).

Discussion

Fish doubled its initial weight at the end of the growing

Table 4. Growth performance of Nile tilapia fed experimental diets for a 84-day feeding period.

	Diets			
Growth Parameters	***FO***	***60 SCL***	***80 SCL***	***100 SCL***
Initial Weight (g.fish^{-1})	16.4 ± 0.8	16.4 ± 0.3	16.5 ± 0.9	15.7 ± 0.6
Final Weight (g.fish^{-1})	34.3 ± 1.2	33.8 ± 2.7	31.4 ± 1.7	32.5 ± 1.5
SGR (%.d^{-1})	0.7 ± 0.0	0.7 ± 0.1	0.6 ± 0.0	0.7 ± 0.1
FER	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
PER (%)	89.5 ± 3.5	84.7 ± 11.7	78.6 ± 4.4	87.4 ± 9.1
HSI (%) (n=9)	1.8 ± 0.3	1.7 ± 0.2	2.2 ± 0.2	2.1 ± 0.3
Survival (%)	100	100	100	100

Values mean ± SD (n=3)

Table 5. Whole body and muscle tissue essential and non-essential amino acid compositions of Nile tilapia following a 84-day feeding period with diets containing soybean extract and increasing amount of plant oil mixture (g.kg^{-1} diet) (n=1). M=Muscle Tissue, WB=Whole Body.

		Diets							
	Initial	***FO***		***60 SCL***		***80 SCL***		***100 SCL***	
Essential Amino Acids M	M	M	WB	M	WB	M	WB	M	WB
L-Methionine (Met)	13.7	14.5	10.3	15.4	11.9	18.4	12.3	15.9	11.7
L-Phenilalanine (Phe)	20.6	21.5	17.7	23.8	19.4	26.7	18.9	23	20
L-Lysine (Lys)	89.3	96.9	76.9	107.6	78.1	73.8	73.6	100.2	83.5
L-Histidine (His)	19.6	20.3	12.2	21.9	16.7	23.8	16.5	22.1	17
L-Valine (Val)	25.6	30.7	28.7	32.6	27.9	34.9	27.5	33.3	27.6
L-Leusine (Leu)	40.2	44.6	36.5	47.9	38.9	53.1	37.6	47.8	38.8
L-Isoleucine (Ile)	30.7	34.1	25.3	35.4	27.6	40.1	27.2	65.2	27.9
L-Threonine (Thr)	45.8	44.6	30.6	45.2	33.3	55.7	39.1	46.3	34.9
L-Arginine (Arg)	47	50.2	44.2	48.8	37.5	31.8	35.9	52.2	40.5
Non-Essential Amino Acids									
L-Alanine (Ala)	24.9	30.6	19.8	28.3	23.8	33.4	18.9	26	21.9
L-Aspartic Acid (Asp)	179	182	111	194.6	125.6	190	120.3	206.8	128.3
Glycine (Gly)	21.9	28.2	27.9	29.8	28.7	33.4	27.2	29.6	25.8
L-Glutamic Acid (Glu)	117.9	135.5	85.6	135.1	101.1	139.5	95.9	140.1	101.7
L-Proline (Pro)	22.1	22.7	23.8	26.7	24.5	28.3	27.5	28.9	24.7
L-Serine (Ser)	28.2	29.1	18.2	30.8	24.5	34.7	25.7	29.9	24.1
L-Tyrosine (Tyr)	16.4	17.7	13.5	18.2	13.8	23.1	15.1	19.9	15

Table 6. Whole body and muscle tissue nutrient composition of Nile tilapia fed the experimental diets containing soybean extract and increasing plant oil mixture for a 84-day feeding period.

	Diets			
Parameters	***FO***	***60 SCLb***	***80 SCL***	***100 SCL***
Whole Body				
Crude Protein (% DM)	63.6 ± 1.3^{a}	67.6 ± 0.6^{c}	65.5 ± 0.1^{b}	64.9 ± 0.3^{ab}
Crude Lipid (% DM)	22.4 ± 0.0	18.9 ± 2.1	19.3 ± 1.2	21.4 ± 0.8
Ash (% DM)	14.9 ± 0.0^{b}	11.7 ± 0.0^{a}	12.2 ± 0.0^{ab}	12.8 ± 0.0^{ab}
Muscle Tissue				
Crude Protein (% DM)	82.6 ± 0.3	83.4 ± 1.0	83.8 ± 1.6	84.3 ± 0.4
Crude Lipid (% DM)	7.2 ± 0.0^{b}	5.1 ± 0.0^{a}	4.9 ± 0.2^{a}	5.7 ± 0.3^{a}
Ash (% DM)	8.5 ± 0.0	7.6 ± 0.5	7.5 ± 0.6	8.1 ± 0.4

Values are ± SD (n=3). Means with different superscripts in the same row are significantly different (P<0.05)

Table 7. Fatty acid composition of the whole body samples of Nile tilapia fed the experimental diets containing soybean extract and increasing plant oil mixture for a 84-day feeding period (% of total fatty acids).

		Diets			
Fatty Acid Classes	***Initial***	***FO***	***60SCL***	***80SCL***	***100SCL***
Σ Saturates	31.2 ± 0.4	16.9 ± 0.5^{a}	26.8 ± 0.4^{b}	28.2 ± 0.5^{b}	26.1 ± 0.4^{b}
Σ Monoenes	39.8 ± 0.1	50.4 ± 6.4	40.7 ± 1.2	39.0 ± 0.1	39.9 ± 0.4
Σ n-9	34.9 ± 0.1	46.9 ± 2.5^{d}	36.4 ± 0.2^{c}	33.8 ± 0.0^{a}	34.9 ± 0.4^{ab}
18:1n9	30.8 ± 0.2	39.9 ± 5.5	33.0 ± 0.3	30.4 ± 0.1	32.1 ± 0.4
Σ n-6	16.2 ± 0.3	15.3 ± 1.7^{a}	19.7 ± 0.4^{b}	19.9 ± 0.3^{b}	22.4 ± 0.5^{b}
18:2n6	14.5 ± 0.3	13.5 ± 1.5^{a}	17.8 ± 0.4^{b}	18.3 ± 0.6^{bc}	20.2 ± 0.4^{c}
20:4n6	0.5 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.0
Σ n-3	10.5 ± 0.1	15.2 ± 0.9^{c}	8.5 ± 0.2^{a}	10.3 ± 0.3^{b}	9.2 ± 0.2^{ab}
18:3n3	2.5 ± 0.1	5.8 ± 0.0^{b}	3.0 ± 0.1^{a}	3.1 ± 0.2^{a}	3.5 ± 0.2^{a}
Σ n-3 HUFA	8.0 ± 0.0	9.5 ± 0.4^{c}	6.5 ± 0.1^{b}	7.2 ± 0.0^{b}	5.6 ± 0.0^{a}
20:5n3	1.2 ± 0.0	2.7 ± 0.0^{c}	1.0 ± 0.1^{b}	1.0 ± 0.0^{b}	0.8 ± 0.0^{a}
22:6n3	5.8 ± 0.1	5.8 ± 0.8^{bcd}	4.7 ± 0.1^{ab}	5.4 ± 0.1^{c}	4.0 ± 0.0^{a}
n-3/n-6	0.7 ± 0.0	1.0 ± 0.1^{c}	0.4 ± 0.0^{a}	0.5 ± 0.0^{b}	0.4 ± 0.0^{a}
ARA/EPA	0.4 ± 0.0	0.1 ± 0.0^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.4 ± 0.0^{c}

Values are ± SD (n=3). Means with different superscripts in the same row are significantly different (P<0.05).

Table 8. Muscle tissue fatty acid composition of Nile tilapia fed the experimental diets containing soybean extract and increasing plant oil mixture for a 84-day feeding period (% of total fatty acids).

		Diets			
Fatty Acid Classes	***Initial***	***FO***	***60 SCL***	***80 SCL***	***100 SCL***
Σ Saturates	33.7 ± 0.7	35.8 ± 1.0^{b}	34.7 ± 0.7^{b}	33.9 ± 0.5^{ab}	31.7 ± 0.7^{a}
Σ Monoenes	35.4 ± 0.1	30.0 ± 1.3	28.5 ± 1.6	28.5 ± 0.6	28.8 ± 0.8
Σ n-9	31.8 ± 0.2	26.9 ± 0.5	26.6 ± 0.4	26.4 ± 0.2	26.8 ± 0.4
18:1n9	28.0 ± 0.3	22.1 ± 1.1	22.5 ± 0.7	22.4 ± 0.6	23.0 ± 0.8
Σ n-6	13.8 ± 0.6	12.0 ± 0.2^{a}	15.7 ± 0.8^{b}	17.3 ± 0.5^{c}	18.9 ± 0.0^{d}
18:2n6	11.9 ± 0.5	9.9 ± 0.2^{a}	13.5 ± 0.5^{b}	15.3 ± 0.5^{c}	17.0 ± 0.1^{d}
20:4n6	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.1
Σ n-3	13.8 ± 0.5	17.2 ± 1.1	18.0 ± 1.7	16.6 ± 0.5	16.9 ± 1.2
18:3n3	2.1 ± 0.1	1.4 ± 0.1^{a}	1.7 ± 0.1^{b}	2.2 ± 0.1^{c}	2.6 ± 0.0^{d}
Σ n-3 HUFA	11.7 ± 0.2	15.8 ± 0.5^{b}	15.5 ± 0.3^{b}	14.4 ± 0.2^{a}	14.3 ± 0.6^{a}
20:5n3	1.5 ± 0.1	2.6 ± 0.2^{b}	2.2 ± 0.2^{ab}	2.1 ± 0.1^{a}	2.1 ± 0.1^{a}
22:6n3	9.4 ± 0.4	12.5 ± 0.9	12.6 ± 0.9	11.5 ± 0.5	11.4 ± 1.1
n-3/n-6	1.0 ± 0.0	1.4 ± 0.1^{c}	1.2 ± 0.2^{bc}	1.0 ± 0.1^{ab}	0.9 ± 0.1^{a}
ARA/EPA	0.20 ± 0.0	0.12 ± 0.0^{ab}	0.14 ± 0.0^{b}	0.14 ± 0.0^{b}	0.10 ± 0.0^{a}

Values are ± SD (n=3). Means with different superscripts in the same row are significantly different (P<0.05).

Table 9. Liver fatty acid composition of Nile tilapia fed the experimental diets containing soybean extract and increasing plant oil mixture for a 84-day feeding period (% of total fatty acids).

		Diets			
Fatty Acid Classes	***Initial***	***FO***	***60 SCL***	***80 SCL***	***100 SCL***
Σ Saturates	37.3 ± 1.0	32.2 ± 0.3^{c}	25.5 ± 0.4^{b}	25.1 ± 0.3^{ab}	23.9 ± 0.3^{a}
Σ Monoenes	39.7 ± 0.2	45.5 ± 0.1^{a}	46.9 ± 0.4^{b}	47.2 ± 0.2^{b}	48.2 ± 0.0^{c}
Σ n-9	34.3 ± 0.1	39.5 ± 0.0^{a}	40.7 ± 0.1^{a}	41.4 ± 0.0^{a}	43.0 ± 0.1^{b}
18:1n9	31.6 ± 0.2	36.9 ± 0.1^{a}	38.0 ± 0.3^{b}	37.6 ± 0.1^{b}	39.0 ± 0.2^{c}
Σ n-6	12.6 ± 0.2	10.7 ± 0.1^{a}	19.8 ± 0.2^{b}	22.1 ± 0.0^{c}	22.8 ± 0.1^{d}
18:2n6	ND	9.6 ± 0.1^{a}	17.9 ± 0.2^{b}	20.2 ± 0.0^{c}	20.7 ± 0.2^{c}
20:4n6	0.2 ± 0.1	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.3 ± 0.1^{b}
Σ n-3	6.3 ± 0.9	9.9 ± 0.2^{c}	6.5 ± 0.0^{b}	5.0 ± 0.1^{a}	4.6 ± 0.1^{a}
18:3n3	2.7 ± 0.0	2.9 ± 0.0^{c}	2.9 ± 0.0^{c}	1.8 ± 0.0^{b}	1.7 ± 0.0^{a}
Σ n-3 HUFA	6.5 ± 0.2	7.1 ± 0.0^{d}	3.6 ± 0.0^{c}	3.2 ± 0.0^{b}	2.8 ± 0.1^{a}
20:5n3	0.5 ± 0.0	0.7 ± 0.0^{c}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}	0.3 ± 0.0^{a}
22:6n3	5.2 ± 0.6	5.7 ± 0.2^{d}	2.5 ± 0.1^{c}	2.0 ± 0.1^{ab}	1.7 ± 0.1^{a}
n-3/n-6	0.5 ± 0.1	0.9 ± 0.0^{c}	0.3 ± 0.0^{b}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}
ARA/EPA	0.40 ± 0.0	0.29 ± 0.0^{a}	0.75 ± 0.0^{b}	0.75 ± 0.0^{b}	1.0 ± 0.0^{c}

Values are ± SD (n=3). Means with different superscripts in the same row are significantly different (P<0.05); ND: Not Detected

period and no significant differences were found in final weight of fish among dietary treatments. Today fishmeal and oil comprise almost 20 and 5% of commercial Tilapia feeds respectively (Webster *et al.,* 2016). However, soybean products have been widely used in various amounts in aqua feeds including Tilapia feeds due mainly to its higher protein content and relatively balanced amino acid profiles compared to that of other plant protein sources (El-Saidy and Gaber, 2002, Webster *et al.,* 2016). Previous studies conducted in Nile tilapia reported that several alternative plant and animal by-product meals have been used to replace dietary fish meal in certain amounts either solely or in combination in diet formulations without compromising overall growth and feed utilization parameters (El-Saidy and Gaber, 2002; Ng and Wang, 2011; Koch *et al.,* 2016; Webster *et al.,* 2016). Although studies investigating the effects of combined use of alternative protein and oil sources on growth and nutritional physiology of important farmed fish species are on the increase recently (Torstensen *et al.,* 2008; Pratoomyot *et al.,* 2010), the knowledge available in literature for Tilapia is scarce. In this study, diets were formulated to include minimum 200 g of fishmeal per kg of diet in order to fish demonstrate better growth performance as this amount was suggested for Nile tilapia juveniles in the literature (Thompson *et al.,* 2012). No significant differences in SGR, FER and PER ratios among the dietary treatments implied that diets provided the nutritional requirements of fish in farming conditions used in this study. However, HSI was tended to increase with the increasing amount of plant oil mixture use in diets. Furthermore, it was evident that dietary L-lysine and DL-Methionine added into diets containing soybean extract and plant oil mixture based on the essential amino acid requirements of juvenile Nile tilapia (NRC, 1993) were effectively utilized by fish since similar growth rates and increment in muscular essential and non-essential amino acid levels were obtained compared to that of fish fed FO diet. Besides having imbalanced amino acid profiles, all the plant protein sources contain Anti-Nutritional Factors (ANFs) such as protease inhibitors, lectins, phytic acid, saponins, anti-vitamin compounds and high amounts of non-starch polysaccharides (Webster *et al.,* 2016). The fact that fish fed 60SCL, 80SCL and 100SCL diets attained similar growth rates to that of fish fed FO diet may also indicate that ANFs of soybean extract used in this study were in acceptable levels even though they were not specified as part of this study.

Soy products have previously been shown to replace dietary fishmeal in diets for tilapia and other important farmed species without negatively affecting the growth rates providing the diets are added with synthetic amino acids such as L-lysine, DL-methionine and taurine in order to make dietary amino acid profile balanced (El-Saidy and Gaber, 2002; Furuya *et al.,* 2004; Pratoomyot *et al.,* 2010; Figueiredo-Silva *et al.,* 2015; Koch *et al.,* 2016; Webster *et al.,* 2016). Furuya *et al.,* (2004) investigated the effects of diets in which 85% of the dietary protein supplied by soybean extract and supplemented with L-lysine, DL-methionine and L-threonine according to ideal protein concept on growth performance in Nile tilapia juveniles (5.3 g average initial weight). The diets were also supplemented with or without di-calcium phosphate. Only the fish fed diets supplemented with essential amino acids and di calcium phosphate attained a similar growth performance and feed utilization parameters to fish fed fishmeal control diet. The authors also concluded that dietary fishmeal in Nile tilapia diets could totally be replaced by plant proteins only if the ANFs are reduced by various mechanical, chemical and microbiological treatments and dietary essential amino acid profile is balanced (Furuya *et al.,* 2004). Recently Figueiredo-Silva *et al.,* (2016) studied the effects of dietary formulations in which 93% of fishmeal replaced by soybean meal and supplemented with increasing amount of DL-methionine (1.2 to 6.0 $g.kg^{-1}$ diet) but constant levels of L-lysine (3.8 $g.kg^{-1}$ diet) and L-threonine (0.6 $g.kg^{-1}$ diet) on growth and nutrient utilization efficiency in hybrid tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*). The results showed that SGR (Specific Growth Rate), FCR (Food Conversion Ratio) and protein utilization (gain and retention) efficiencies of fish improved significantly with increasing dietary DL-methionine supplementation (Figueiredo-Silva *et al.,* 2016). The authors also concluded that a dietary Met+Cys level of 15.7 and 12.5 $g.kg^{-1}$ diet (as fed) was required to reach 95% of maximum weight and protein gain respectively in hybrid tilapia fed soybean meal based diets with graded levels of DL-methionine indicating methionine might be the first limiting essential amino acid (Figueiredo-Silva *et al.,* 2016). Furthermore, the most recent study by Koch *et al.,* 2016 confirmed that fishmeal free diet formulation for Nile tilapia is possible if 30 and 20% of the dietary ingredients are composed of poultry by-product meal and soybean meal respectively and are supplemented with lysine, methionine and taurine. In addition, the authors reached a conclusion that the total deviations from an assumed ideal protein profile is more important in diet formulations than the combination of diet ingredients used to meet that profile (Koch *et al.,* 2016). However, similar studies conducted in different farmed species, specifically in salmonids, also demonstrated that dietary supplemental amino acids were not utilized effectively in protein synthesis due mainly to a delay in the digestion of dietary plant proteins (such as SBM and malt protein meals) (Larsen *et al.,* 2012). It also appeared that the effective utilization might be closely correlated

with the fish species and water temperature (Larsen *et al.,* 2012).

Crude protein content of whole body and muscle tissue of Nile tilapia fed soybean extract and increasing amount of plant oil mixture (60, 80 and 100SCL diets) was tended to increase whereas crude lipid contents were found to be significantly lower compared to that of fish fed FO diet. These results along with whole body and muscle essential amino acid compositions primarily indicated that Nile tilapia fed diets 60, 80 and 100 SCL supplemented with L-lysine and DL-methionine sufficiently obtained the balanced essential amino acids required for an optimal growth and development. It also appeared that fish were able to utilize dietary lipid sources and specifically the α-linolenic (18:3n3) and linoleic (18:2n6) fatty acids (predominant fatty acid classes in linseed and soy oils respectively) effectively to provide energy through β oxidation resulting in the dietary crude protein spared for growth (Torstensen *et al.,* 2008; Eroldogan *et al.,* 2013).

Whole body, muscle and liver fatty acid compositions of Nile tilapia following a 84-day grow-out period, except a few fatty acid classes, were found to be similar to that of feed fatty acid compositions in all the dietary treatments. Previous investigations conducted in Nile tilapia and other important farmed species targeting dietary fish oil replacement by plant oils also reported that feed fatty acid compositions were reflected specifically in the muscle tissue fatty acid compositions of fish (Montero *et al.,* 2005; Karapanagiotidis *et al.,* 2007; Ng and Wang, 2011, Li *et al.,* 2016). Although soy oil is used widely in commercial feed formulations in Nile tilapia, information regarding the effects of dietary use of other linoleic, α-linolenic and oleic acid rich plant oil sources (sunflower seed, corn, canola and linseed oils) and their combinations on growth and tissue fatty acid compositions and metabolism is limited. Tilapia species were demonstrated to utilize 18:2n6 rich plant oils (soy, corn and sunflower seed oils) more effectively for growth and development and these are considered as better quality oil sources than 18:3n3 rich plant oils (Takeuchi *et al.,* 1983; Yıldırım-Aksoy *et al.,* 2007). In this study whole body and tissue fatty acid compositions of fish fed diets 60, 80 and 100SCL demonstrated that 18:3n6 and 20:3n6 fatty acids were significantly higher than that of fish fed FO diet indicating the bio activation of Δ-5 elongation and Δ-6 desaturation mechanism of the n-6 PUFA. However, no bio activation of elongation and desaturation enzymes on n-3 PUFA was evident in fish fed those diets. All freshwater fish species including tilapia has the ability of bio converting the n-3 and n-6 PUFAs into their corresponding LC-HUFAs through the series of enzymatic desaturation and elongation pathways and that is much more effective in these fish compared to that of the marine counterparts (Eroldoğan et al. 2013; Herath *et al.,* 2016; Kabeya *et al.,* 2016). Previously the effectiveness of these metabolic pathways was demonstrated in Nile tilapia and other tilapia species (*Oreochromis aureus* and *Tilapia zillii*) via the dietary supplementation of linoleic and α-linolenic acid or the injection of fish with ^{14}C labelled fatty acids (Tocher *et al.,* 2002; Teoh *et al.,* 2011). Tocher *et al.,* (2002) reported that hepatic desaturation activity was twice as much in Nile tilapia fed plant oil based diets than that of fish fed FO diet. The authors also concluded that the enzymes responsible for fatty acid desaturation are always existent in Nile tilapia and their activities could be inhibited by LC-PUFAs (Tocher *et al.,* 2002). It appeared that Nile tilapia in this study failed to activate the elongation and desaturation enzymes on 18:3n3 since intermediate metabolites of the bioconversion pathway in fatty acid compositions of whole body and tissue samples of fish were almost absent or similar in all the dietary treatments. This has probably indicated that residual fishmeal oil in diets containing plant oil mixture was sufficient to meet the n-3 LC-PUFA requirement of juvenile Nile tilapia in this study. Furthermore, Teoh *et al.,* (2011) found that genetically improved Nile tilapia and red hybrid tilapia fed purified diets in which dietary fish oil was totally replaced by blend of vegetable oils (olive, sunflower, linseed and refined, bleached and deodorized palm) had significantly higher amounts of both n-6 and n-3 LC-PUFA in the fatty acid compositions of whole body samples. The authors also concluded that tilapia fed diets containing blend of vegetable oil sources as a lipid source exhibited efficient bioconversion of 18:2n6 to n-6 LC-PUFA indicating that the fatty acid metabolism of tilapia is actually able to fully compensate for the lack of dietary n-6 LC-PUFA in vegetable oil based diets (Teoh *et al.,* 2011). Although the diets in this study were formulated to contain low fat fishmeal (around 8%) and 10% crude lipid, upper limit levels of dietary fish oil replacement probably ensured Nile tilapia to demonstrate its capacity to bio convert 18:2n6 to n-6 LC-PUFAs but not as efficiently as it was demonstrated in Teoh *et al.,* (2011).

As it was shown in previous studies conducted in both tilapia and other important farmed species, the results of this study reiterated that the use of plant oil mixture in diets resulted in the decrement of 20:5n3 (EPA) while it caused 22:6n3 (DHA) to be increasingly deposited in whole body and tissue samples of Nile tilapia compared to their dietary levels (Teoh *et al.,* 2011; Eroldoğan *et al.,* 2013; Li *et al.,* 2016). Similarly a study conducted by Li *et al.,* 2016 reported that dorsal muscle tissue levels of EPA in genetically improved farmed tilapia were measured to be significantly lower in fish fed diets containing increasing amount of linseed oil compared to that of fish fed FO diet.

It is understood from previous research in Nile tilapia that EPA is more selectively and frequently used as a substrate for energy provision through β-oxidation compared to DHA (Karapanagiotidis *et al.,* 2007). EPA was also shown to be bio converted to DHA in Nile tilapia by Karapanagiotidis *et al.,* 2007 depending on the oxidative status of fish. Nile tilapia in this study had higher levels of SFA (Saturated Fatty Acids) and MUFA (Mono Unsaturated Fatty Acids) in whole body and muscle tissue samples compared to dietary levels of those fatty acid classes following grow-out period. This probably indicated that fish synthesized SFA and MUFAs through an active ex novo production (liponeogenesis) even though whole body fatty acid balance method was not utilized as part of this study to measure it (Teoh *et al.,* 2011). It is generally stated that feed utilization efficiency in fish could be substantially increased using diets containing plant oils that are rich sources of SFA and MUFAs since these fatty acid classes are considered as preferred substrate fatty acids for energy production through β-oxidation in many farmed fish species (Eroldogan *et al.,* 2013).

Conclusions

This study demonstrated that feeding Nile tilapia with diets containing soybean extract and increasing amount of plant oil mixture (soy, canola and linseed oils v/v 1:1:1) did not compromise the growth rate and n-3/n-6 PUFA ratios specifically in fish muscle tissue. Investigations targeting fatty acid metabolism using fatty acid balance method in Nile tilapia fed diets in which dietary fish meal and oil totally replaced by soybean extract and blend of soy, canola and linseed oils could further improve the chance of realizing commercial marine ingredient free diet formulations.

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REFERENCES

Alava, V.R. (1998). Effect of salinity, dietary lipid source and level on growth of milkfish (*Chanos chanos*) fry. *Aquaculture* 167, 229-236.

AOAC. (1995). Official Methods of Analyses of the Association of Official Analytical Chemists. Association of Official Analytical Chemists, Arlington, VA, USA.

Czesny, S., & Dabrowski, K. (1998). The effects of egg fatty acid concentrations on embryo viability in wild and domesticated walleye (*Stizostedion vitreum*). *Aquatic Living Resources* 11, 371-378.

Dimova, N. (2003). RH-HPLC analysis of amino acids with UV-detection. Bulgarian Academy of Science, Tome 56, No 12.

El-Saidy, D.M.S. & Gaber, M.M.A. (2002). Complete replacement of fishmeal by soybean meal with L-lysine supplement for Nile tilapia, *Oreochromis niloticus* fingerling. *Journal of the World Aquaculture Society* 33, 297-306.

Eroldogan, O.T., Yılmaz, H.A., Turchini, G.M., Arslan, M., Sirkecioglu, N.A., Engin, K., Ozsahinoglu, I. & Mumogullarında, P. (2013). Fatty acid metabolism in European sea bass (*Dicentrarchus labrax*): Effects of n-6 PUFA and MUFA in fish oil replaced diets. *Fish Physiology and Biochemistry* 39, 941-955.

FAO. (2016). The State of World Fisheries and Aquaculture. Rome, Italy.

Figueiredo-Silva, C., Lemme, A., Sangsue, D. & Kiriratnikom S. (2015). Effects of DL-methionine supplementation on the success of almost total replacement of fishmeal with soybean meal in diets for hybrid tilapia (*Oreochromis niloticus* X *Oreochromis mossambicus*). *Aquaculture Nutrition* 21, 234-241.

Folch, J., Lees, M. & Sloane-Stanley, G. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226, 497-509.

Furuya W.M., Pezzato L.E., Barros M.M., Pezzato A.C., Furuya V.R.B. & Miranda E.C. (2004). Use of ideal protein concept for precision

formulation of amino acid levels in fishmeal free for juvenile Nile tilapia (Oreochromis niloticus L.). Aquaculture Research 35, 1110-1116.

Herath, S.S., Haga Y. & Satoh S. (2016). Effects of long term feeding of corn co-product based diet on growth, fillet color and fatty acid and amino acid composition of Nile tilapia *Oreochromis niloticus*. *Aquaculture* 464, 205-212.

Kabeya, N., Takeuchi Y., Yazawa R., Haga Y., Satoh S., & Yoshizaki G. (2016). Transgenic modification of the n-3 HUFA biosynthetic pathway in nibe croaker larvae: improved DPA (docosapentaenoic acid; 22:5n3) production. *Aquaculture Nutrition* 22, 472-478.

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

Koch, J.F., Rawless, S.D., Webster, C.D., Cummins, V., Kobayashi, Y., Thompson, K.R., Gannam, A.C., Twibel, R.G. & Hyde N.M. (2016). Optimizing fishmeal free commercial diets for Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 452, 357-366.

Larsen, B.K., Dalsgaard, J., & Pedersen, P.B. (2012). Effects of plant proteins on postprandial free plasma amino acid concentrations in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 326, 90-98.

Li, F.J., Lin, X., Lin, S.M., Chen, W.Y., & Guan Y. (2016). Effects of dietary fish oil substitution with linseed oil on growth, muscle fatty acid and metabolism of tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition* 22, 499-508.

Metcalfe, L. & Schmitz, A., (1961). The rapid preparation of fatty acid esters for gas chromatographic analysis. *Analytical Chemistry* 33, 363-364.

Montero, D., Robaina, L., Caballero, M.J., Gines, R. & Izquierdo, M.S. (2005). Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oil: A time course study on the effect of a re-feeding period with a 100 % fish oil diet. *Aquaculture* 248, 121-134.

National Research Council (NRC) (1993). Nutritional requirements of Fish. National Academic Press, Washington, USA.

Ng Wang, Y. (2011). Inclusion of crude palm oil in the broodstock diets of female Nile tilapia, *Oreochromis niloticus*, resulted in enhanced reproductive performance compared to brood fish fed diets with added fish oil or linseed oil. *Aquaculture* 314, 122-131.

Pratoomyot, J., Bendiksen, E.A., Bell, J.G., & Tocher, D.R. (2010). Effect of increasing replacement of dietary fishmeal with plant protein sources on growth performance and body lipid composition of Atlantic salmon (*Salmo salar*). *Aquaculture* 305, 124-132.

Smith, L.S. (1989). Digestive functions in teleost fishes. In: J.E., Halver (editor) Fish Nutrition. 2nd edn. Academic Press, New York, USA, pp 331-421.

Steffens, W., Wirth, M. & Rennert, B. (1995). Effects of adding various oils to the diet on growth, feed conversion and chemical composition of carp (*Cyprinus carpio*). *Archive of Animal Nutrition* 47, 381-389.

Tacon, A.G.J. & Metian, M. (2008). Global overview on the use of fishmeal and fish oil in industrially compounded aqua feeds: trends and future prospects. *Aquaculture* 285, 146-158.

Takeuchi, T., Satoh, S., & Watanabe, W. (1983).

Dietary lipids suitable for practical feed of *Tilapia nilotica*. *Bulletin of Japanese Society of Scientific Fisheries* 49, 1361-1365.

Teoh, C.Y. & Ng, W.K. (2016). The implications of substituting dietary fish oil with vegetable oils on the growth performance, fillet fatty acid profile and modulation of the fatty acid elongase, desaturase and oxidation activities of red hybrid tilapia, *Oreochromis* sp. *Aquaculture* 465, 311-322.

Teoh, C.Y., Turchini, G.W. & Ng, W.K. (2011). Genetically improved farmed Nile tilapia and red hybrid tilapia showed differences in fatty acid metabolism when fed diets with added fish oil or a vegetable oil blend. *Aquaculture* 312, 126-136.

Thompson, K.R., Velasquez, A., Patterson, J.T., Metts, L.S., Webster, C.D., Brady, Y.J., Gannam, A.L., Twibel, R.G. & Ostrand, S.L. (2012). Evaluation of plant and animal protein sources as partial or total replacement of fishmeal in diets for Nile tilapia fry and juvenile stages. *North American Journal of Aquaculture* 74, 365-375.

Tocher, D.R., Angaba, M., Hastings, N., Bell, J.G., Dick, J.R. & Teale, A.J. (2002). Nutritional regulation of hepatic fatty acids desaturation and polyunsaturated fatty acid composition in zebra fish (*Danio rerio*) and tilapia (*Oreochromis niloticus*). *Fish Physiology and Biochemistry* 24, 309-320.

Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbo, R., Hemre G.I., Fontanillas R., Nordgarden, U., Hevrøy, E.M., Olsvik, P. & Berrntssen, M.H.G. (2008). Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fishmeal and fish oil with plant meal and vegetable oil blends. *Aquaculture* 285, 193-200.

Turchini G.M., Ng, W.G. & Tocher, D.R. (2011). *Fish oil replacement and alternative lipid sources in aquaculture feeds*. 1st ed., CRC Press, Taylor & Francis Group, Boca Raton, Florida, USA.

Yıldırım-Aksoy, M., Lim, C., Davis A.D., Shelby, R. & Klesius, P.H. (2007). Influence of dietary lipid sources on the growth performance, immune response and resistance of Nile tilapia, *Oreochromis niloticus*, to *Streptococcus iniae* challenge. *Journal of Applied Aquaculture* 19(2), 29-49.

Webster, C.D., Rawles, S.D., Koch, J.F., Thompson, K.R., Kobayashi, Y., Gannam, A.L., Twibel, R.G. & Hyde, N.M. (2016). Bio-Ag reutilization of distiller's dried grains with solubles (DDGS) as a substrate for black soldier fly larvae, *Hermettia illucens*, along with poultry by-product meal and soybean meal as total replacement of fishmeal in diets for Nile tilapia, *Oreochromis niloticus*. *Aquaculture Nutrition* 22: 976-988.

Zar, J.H. (1996). *Bio statistical Analysis*. Prentice Hall Inc., Upper Saddle River, New Jersey, USA.