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Dietary effect of varying linseed oil compositions on growth response, survival and polyunsaturated fatty acid levels in Tilapia (*Oreochromis niloticus*)

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The present study evaluated the impact of varying dietary linseed oil composition on growth, survival and tissue polyunsaturated fatty acids (PUFA) profiles in Nile tilapia (*Oreochromis niloticus)*. Five iso-nitrogenious diets with varying linseed and sunflower oil concentrations were formulated and fish fed twice daily to for 3 months. Commercial diet was used as a control in triplicate tanks set for each diet treatment. Growth parameters were measured from changes in body weight and length. A 75:25 ratio of sunflower oil to linseed oil gave a better survival and specific growth rate than 100% linseed oil or 100% sunflower oil. Tissue PUFA composition were determined using gas chromatography. High dietary linseed oil composition (100%) resulted into significantly high (P<0.05) total n3 fatty acids (9.9-25%) and DHA (1.8-7.9%) in muscles whereas liver n3 fatty acids and DHA composition ranged between 9.3-25.5 and 0.7-2.6%, respectively. Muscle and liver n3/n6 ratio ranged between 0.7-2.2 and 0.7-2.6 while tissue arachidonic acid (ARA 20.4 n6) content ranged between 2.6-3.5% in muscles and 3.4-4.5% in the liver. ARA 20.4 n6 values were low relative to the dietary precursor, linoleic acid, LA, 18.2 n6. Fatty acid deposition in the tissues increased with the feeding period with the third feeding month recording significantly higher DHA, total n3 and n3/n6 ratio. Based on the result, dietary linseed oil > 50% reduced growth and survival rate in tilapia, however, it increased tissue accumulation of essential fatty acids which also increased with the length of feeding period.

Key words: Growth, Tilapia, n3 fatty acids, linseed oil, polyunsaturated fatty acids (PUFA).

INTRODUCTION

Agricultural output originating from fisheries and aquaculture must increase by over 60% to feed the world in 2050 considering the perceived benefits of fish oil (Leaf and Weber, 1988; Bonaa et al., 1990) and the

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alarming number of people, mostly in developing countries, suffering from hunger and poverty (FAO, 2014). With the proven benefits of omega 3 highly unsaturated fatty acids (n3-HUFA) (Kris-Etherton et al., 2000; Gebauer et al., 2006; Kusunto et al., 2007), numerous governmental and non-governmental organizations across the world currently advise increased fish intake as a means of improving the health of their citizens (Munguti et al., 2014).

To meet the demand, quality and affordable feeds are integral component of sustainable intensive and semi-intensive aquaculture systems (Munguti et al., 2014). Recent studies have reported the existence of a correlation between the nutritional value of fish and the dietary composition of feeds (Župan et al., 2016; Ljubojević et al., 2015; Ljubojević et al., 2013). Fish oils (FO) are regarded as good lipid sources of aqua-feed formulations (National Research Council, NRC, 2011), however, the global fish oil production has reached a plateau and is not expected to raise much beyond the current level of production (Ng, 2002). Also, it is predicted that within a decade or so, fish oil production may not be sufficient to meet the demand of aquaculture (FAO, 2007). Therefore, the introduction of alternative lipid sources is necessary to enable sustainable aquaculture development (Jordal et al., 2007; Bouraoui et al., 2011).

Reducing the utilization of FO in aqua-feeds formulations while ensuring that appropriate n-3 long chain polyunsaturated fatty acids (n3 LCPUFA) proportion are available in the final product is a challenge (Turchini et al., 2009). However, vegetable oils which are rich in C_{18} polyunsaturated fatty acids have been considered the most sustainable alternatives to fish oil (Montero et al., 2003; Lee, 2001). In addition, studies have reported considerable success in partial or total replacement of FO with VO in many fish species (Ng, 2005; Turchini et al., 2009, 2010). Moreover, recent studies have also evaluated the effects of replacing high-quality FO with VO sources on growth performance, fatty acid profile or health parameters of fishes, such as gilthead seabream (*Sparus aurata*) (Menoyo et al., 2004) and European sea bass (*Dicentrarchus labrax L*.) (Mourente et al., 2007). However, the extent of the effect is determined by the vegetable oil source and level of dietary replacement. Linseed oil (LO) is distinguished by the highest content of a-linolenic acid (18:3n-3, ALA) compared to other vegetable oils (Popa et al., 2012). Alpha -linolenic acid is the metabolic precursor of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) such as eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) (Brenna, 2002).

Linseed oil has replaced dietary FO without affecting growth performance in various fish species (Menoyo et al., 2005; Francis et al., 2006). However, replacement of FO with LO has also led to decreased concentration of n-3 highly unsaturated fatty acids in fish flesh especially when higher dietary linseed oil were fed (Menoyo et al., 2005; Turchini et al., 2011). On the contrary, it has been shown that when substituted with sunflower oil, dietary linseed oil can increase the EPA/DHA content of Nile tilapia fillets (Justi et al., 2003; Visentainer et al., 2005). The effect of dietary linseed oil on immunity thus survival of various fish species has also been reported (Montero et al., 2010; Kiron et al., 2011).

In the recent past, fish production in Kenya has risen steadily from 1,012 metric tons produced in 2003 to the present production of 21,487 metric tons courtesy of Kenya government fish farming program (Charo-Karissa et al., 2010). However, the major challenges to Kenyan aquaculture sector are the unavailability of quality, efficient and inexpensive farm-made feeds (Munguti et al., 2014). The current research explored the possible use of linseed oil as a dietary replacement of FO in farm-made tilapia feeds and the impact on survival, growth parameters and fillet PUFA profile.

MATERIALS AND METHODS

Study site, experimental set up and sampling

Proximate composition analysis of experimental diets and fatty acid composition of tilapia muscles and liver were done in Food Biochemistry laboratory, Food Science department-Jomo Kenyatta University of Agriculture and Technology (JKUAT). Tilapias which were 4 weeks old were set up in a 1000-liters experimental tank for a period of 3 months. The fingerlings were obtained from Kenya Marine and Fisheries Research Institute, KEMFRI, Sagana fish hatcheries. Three tanks containing 40 tilapia were set for each experimental diet. A separate tank was used as a holding tank for two weeks prior to the feeding experiments for fish acclimatization. Feeding was done twice/day at 9.00 am and 4.00 pm.

Continuous water circulation was maintained using a water pump with water conditions and quality checked and maintained regularly for optimum water quality. After the feeding period, 5 tilapia were randomly sampled after 24 h fasting period, anaesthetized and dissected for liver and muscles.

Diet ingredients

Experimental diets used in this study includes freshwater shrimps *(Caridina nilotica)* as the main protein source, rice bran, wheat flour, popcorn maize flour, vegetable oil blends and vitamin and mineral premixes. Fresh water shrimps were obtained from Wich lum beach along Lake Victoria in Siaya County, Kenya.

Vegetable oil blend comprised of linseed oil, olive oil and sunflower oil. Popcorn maize, wheat flour, olive oil and sunflower oil were obtained from local supermarkets vitamin and mineral premixes obtained from Tam feeds and linseed oil was extracted from linseeds using extruder machine at the Biomechanical engineering workshop, Jomo Kenyatta University of Agriculture and Technology (JKUAT).

Diet formulation

Powdered experimental ingredients were weighed and pre-mixed prior to the addition of vegetable oil blends. They were then dry mixed thoroughly for 2 min in a bench top food mixer before addition of distilled water and vegetable oil blends and mixing

Table 1. Macronutrient content of experimental diets.

Macronutrients (g/100 g)	Washout	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish shrimps	51.7	51.7	51.7	51.7	51.7	51.7
Rice bran	10.3	10.3	10.3	10.3	10.3	10.3
Wheat flour	10	10	10	10	10	10
Popcorn flour	10	10	10	10	10	10
Yeast	1	1	1	1	1	1
Vegetable oil	14	14	14	14	14	14
Oil proportions (% w/w in 14% oil)						
Sunflower[1]	0	0	25	50	75	100
Olive oil[1]	100	0	0	0	0	0
Linseed oil[2]	0	100	75	50	25	0
Premixes[3]	2	2	2	2	2	2

[1]Obtained from local supermarket; [2]Extracted from linseeds using oil extruder machine, BEED Department, JKUAT-Kenya; [3]Obtained from Tam feeds, Nairobi, Kenya.

continued for further 10 min. All the experimental diets were made on grade 12 meat mincer as an extruder fitted with a die plate with 2 mm diameter holes. The soft feed dough was then cold extruded into the 2 mm die-size strand, pelleted and dried at ambient temperature for 3 h. The feeds were then placed on a sieve and oven dried at 40°C for approximately 24 h until the moisture content was 10% (w/w). The dried feeds were then broken into 2 to 3 mm pellets, sealed in plastic bags and stored at -20°C until commencement of feeding trials.

All equipment used for making up feeds were washed and dried before the next diet was produced to avoid cross-contamination. Pearson's square method was used in feed formulation to determine the proper dietary proportions of high and low protein feed stuffs to add to a feed to meet the dietary requirements (Table 1).

Proximate analysis

Moisture content, crude protein, crude fat, crude fiber and ash for diet ingredients and experimental diets were determined according to AOAC methods specification 950.46 (AOAC, 1995) (Table 1). In brief, moisture content was determined by weighing 2 g of sample into a moisture dish and transferred to an oven previously heated to temperatures of 105°C and drying done for 1 h.

The final weight of the sample was taken after the drying period and cooling in a desiccator. The flour residue was then reported as total solids and loss in weight as moisture by following formula (AOAC, 1995, method 925. 10):

$$\text{Moisture (\%)} = \frac{W1-W2}{W1} x100$$

W_1 = Weight of sample before drying and W_2 = Weight of sample after drying.

Crude protein was determined by Semi-Micro Kjeldahl method where about 1 g of sample was weighed into a digestion flask together with a catalyst composed of 5 g of K_2SO_4 and 0.5 g of $CuSO_4$ and 15 ml of concentrated H_2SO_4. The mixture was heated in a fume hood till the digest color turned blue signifying the end of the digestion process. The digest was cooled, transferred to a 100 ml volumetric flask and topped up to the mark with distilled water. A blank digestion with the catalysts and acid was also made. Ten milliliter of diluted digest was transferred into a distilling flask and washed with about 2 ml distilled water. 15 ml of 40% NaOH was added and this was also washed with about 2 ml distilled water. Distillation was done to a volume of about 60 ml distillate.

The distillate was titrated using 0.02 N-HCL to an orange colour of the mixed indicator which signified the end point (AOAC, 1995; Method 20.87-32.1.22). Calculations were done using the following formula:

$$\text{Nitrogen\%} = (V_1 - V_2) \times N \times f \times 0.014 \times \frac{100}{V} \times \frac{100}{S}$$

V_1 = Titer for the sample (ml); V_2 = Titer for blank (ml); N = Normality of standard HCL solution (0.002); F = Factor of standard HCL solution; V = Volume of diluted digest taken for distillation (10ml); S = Weight of sample taken (g).

Crude protein % = Nitrogen × protein factor

Crude fat was determined through soxhlet extraction method which gives intermittent extraction of oil with excess of fresh organic solvent used. About 5 g of samples were weighed into extraction thimbles and the initial weights of the extraction flasks taken. Fat extraction was done using petroleum ether in soxhlet extraction apparatus for 8 h. The extraction solvents were evaporated and the extracted fat dried in an oven for about 15 min before the final weights of the flasks with extracted fat were taken (AOAC, 1995; Method 920.85-32.1.13). Calculations were done using the following formula:

$$\text{Crude fat (\%)} = \frac{W1-W2}{W1} x\ 100$$

W_1 = Weight of sample before extraction; W_2 = Weight of sample after extraction.

Crude fiber was determined by approximately weighing 2 g of the sample was weighed into a 500 ml conical flask. About 200 ml of boiling 1.255 H_2SO_4 was added and boiling done for 30 min under reflux condenser. Filtration was done under slight vacuum with Pyrex glass filter and the residue washed to completely remove the acid with boiling water. Approximately 200 ml of boiling 1.25% NaOH was added to the washed residue and boiling done under reflux for another 30 min. Filtration was done using the same glass filter previously used with the acid. The residue was rinsed with boiling water followed by 1% HCL and again washed with boiling

water to rinse the acid from the residue.

The residue was washed twice with alcohol and thrice with ether. It was then dried in an oven at 105°C in a porcelain dish to a constant weight (W_1). Incineration was done in a muffle furnace at 550°C for 3 h, the dish was then cooled in a desiccator and the final weight (W_2) taken (AOAC, 1995, Method 920.86-32.1.15). Calculations were done as follows:

$$\text{Crude fiber (\%)} = = \frac{W1-W2}{W} \times 100$$

Where, W_1 = Weight of acid and alkali digested sample; W_2 = Weight of incinerated sample after acid and alkali digestion; W = Weight of sample.

Ash was determined from Sample weights of between 2 and 5 g were weighed in pre-conditioned crucibles. The samples were first charred by flame to eliminate smoking before being incinerated at 550°C in a muffle furnace to the point of white ash. The residues were cooled in desiccators and the weights taken (AOAC, 1995, Method 925.03-32.1.05).

Calculations were done as shown below:

$$\text{Crude ash (\%)} = \frac{\text{Weight of Ash(g)}}{\text{Weight of sample (g)}} \times 100$$

Extraction of total lipids and preparation of fatty acid methyl esters

Lipids extraction was done according to the procedure by Bligh and Dyer (1959). Lipids in experimental diets, tilapia liver and muscles were extracted by homogenization of finely ground 0.5 g of samples in chloroform-methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant and cold isotonic saline, 0.9% sodium chloride. This was mixed vigorously and allowed to stand for 20 min. The mixture was then centrifuged at 3000 rpm for 10 min and the aqueous layer was then separated from organic layer using a micropipette. The bottom layer, chloroform, was then transferred to 100 ml reflux flask, quick fit, and evaporated to dryness under vacuum evaporator. Fatty acid methyl esters (FAME) were prepared from vegetable oils and total lipid extracted by acid-catalyzed trans-esterification and addition of 5 ml of 1% H_2SO_4 (v/v) in methanol at 70°C, for 3 h. FAME were then extracted into 750 ml of distilled water and 10 ml of hexane, dehydrated using anhydrous sodium sulphate, Na_2SO_4 and concentrated to 0.5 ml under vacuum evaporator. The concentrated FAME were stored in GC vials for later GC analysis

GC-analysis

FAME were quantified using gas chromatography with on-column injection, equipped with a fused silica capillary column (SUPELCO Column Omegawax^{tm}530, 30 m × 0.5 mm × 0.5 µm) with nitrogen as carrier gas and temperature programming from 170 to 220°C for 18 min^{-1} and final time of 47 min totaling to a run time of 75 min. Injection and detection temperatures were 240 and 260°C respectively. The programmer rate for both GC and decoder were set at 5 min^{-1} with an attenuation of 3.

All the GC analyses were done under same conditions. Individual methyl esters in the sample were identified by comparison with known FAME standards obtained from Kobian chemicals.

Growth performance of tilapia

Growth performance was measure through the following ways:

Initial body weight (IBW) (g): Measured before transferring to experimental tanks

After washout body weight (g) (WBW): Measured at the 3-weeks washout period

Final Body weight (g) (FBW): Measured at the end of the experimental feeding period

Length (cm): Measured from the tip of the snout to the end of the tail

Weight gain (%): Calculated as [(FBW-WBW)/WBW] x 100

Specific growth rate (SGR, %): Calculated based on [(log of FBW-log of WBW)/ feeding days] ×100

Survival (%): Calculated as [(initial number of fish before feeding - dead fish number during feeding/initial number of fish before feeding) x 100]

Statistical analyses

Performed using GenStat version 41.0. Significance in polyunsaturated fatty acid compositions of liver and muscles of tilapia fed different experimental diets was determined by analysis of variance (One-way ANOVA). When significant differences were discerned, treatment means were compared using Duncan`s Multiple Range Test (DMRT) (Duncan DB. 1955). Values throughout the text are expressed as means ±standard error. In all the analysis, treatment significance was accepted at $P < 0.05$.

RESULTS

Growth and survival

Commercial diet (control) and diet 4 had significantly high ($P<0.05$) final body (147, 97) and final length (21.44, 17.4) respectively (Table 5). Weight gain (%) and specific growth rate were also significantly higher ($P<0.05$) in both diet 4 (1689%, 1.3) and commercial diet (2625%, 1.5) respectively (Table 5). Significantly high survival rate was observed in commercial diet (98.3%) with relatively low survival rate observed in diet 1 (Table 5). Survival and specific growth rate significantly increased with reduction of dietary linseed oil. Body weight and length before and after washout were 2.6/5.4 and 1.6/ 2.7 respectively (Table 4)

Proximate and fatty acid composition of diet

There was no significant difference ($P<0.05$) in the experimental dietary proximate composition of protein, crude fats, ash and fiber except for commercial diet which had significantly lower crude protein and fiber (Table 2). Fatty acid composition of diets varied significantly ($p<0.05$) among the diets with significantly high linolenic acid (C18:3), oleic acid (C18:1) and linoleic acid (C18:2) composition in diet1, washout diet and diet 5 respectively (Table 3). The dietary fatty acid composition variation was attributed to the vegetable oils supplemented at different proportions in the diets (Table 1) and difference in fatty acid composition of linseed oil, olive oil and sunflower oil (Figure 1). Polyunsaturated fatty acid compositions of tilapia muscle and liver following 3-

Table 2. Proximate composition of feed ingredients and diets.

Feed ingredients	Proximate compositions				
	Protein	Crude fat	Ash	Moisture	Fibre
Diet 1	45.9±0.21^{d}	23.2±0.09^{ab}	13.6±1.2^{ab}	9.1±0.16^{a}	3.3 ±0.23^{c}
Diet 2	45.4±0.9^{d}	25.0±6.46^{ab}	13.2±1.02^{ab}	8.9±0.16^{a}	2.9±0.03b^{c}
Diet 3	44.6±0.4^{d}	25.1±1.5^{ab}	11.7±0.36^{ab}	10.1±0.22^{a}	3.3±0.25^{c}
Diet 4	45.5±0.8^{d}	25.0 ±3.3^{ab}	12.7±0.73^{ab}	10.3±0.35^{a}	3.3±0.1^{c}
Diet 5	44.2±0.4^{d}	24.9±1.2^{ab}	12.8±1.05^{ab}	9.8±0.32^{a}	3.4±0.2^{c}
Washout	45.6±0.6^{d}	25.4±2.3^{ab}	14.9±0.33^{b}	9.4±0.16^{a}	3.3±0.2^{c}
Commercial[1]	36.0±3.97^{c}	16.3±0.4^{ab}	13.5±0.06^{ab}	7.7±0.12^{a}	2.2±0.13^{b}
Caridina[2]	61.1±0.14^{e}	18.2±2.67^{ab}	15.6±3.71^{b}	9.7±0.11^{a}	0.6±0.26^{a}
Popcorn flour[3]	10.8±0.21^{b}	7.5±0.45^{a}	6.4±4.2^{a}	9.3±0.95^{a}	3.3±0.18^{c}
Wheat flour[3]	10.9±0.31^{b}	7.9±0.98^{a}	8.7±2.03^{ab}	8.7±1.17^{a}	1.9±0.04^{b}
Rice Bran[4]	8.7±0.35^{a}	9.9±5.96^{a}	16.2±2.1^{b}	8.7±0.69^{a}	6.1±0.17^{d}

[1]Skretting, Fontaine-Les-Vervins-France obtained from Jambo fish farm, Kiambu, Kenya; [2]Obtained from Wich lum beach of Lake Victoria, Kenya; [3] Obtained from local supermarket; [4]Obtained from Mwea rice mills.

Table 3. Fatty acid composition (%) of diet.

Acid	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Washout	Commercial
C10:0	1.4^{a}±0.21	1.8^{ab}±0.61	1.8^{ab}±0.17	2.1^{b}±0.19	3.2^{c}±0.23	3.5^{c}±0.5	1.5^{ab}±0.37
C12:0	1.9^{ab}±0.35	2.2^{b}±0.41	2.3^{b}±0.27	2.9^{c}±0.34	3.4^{c}±0.41	3.3^{c}±0.22	1.4^{a}±0.33
C14:0	2.4^{b}±0.31	3.2^{c}±0.4	3.7^{c}±0.24	3.5^{c}±0.16	2.6^{b}±0.2	2.2^{ab}±0.13	1.8^{a}±0.36
C16:0	11.7^{a}±0.19	12.4^{b}±0.17	13.7^{c}±0.11	14.5^{d}±0.31	15.5^{e}±0.45	15.3^{e}±0.34	12.8^{b}±0.45
C18:0	3.3^{a}±0.36	4.4^{b}±0.23	4.9^{bc}±0.26	5.1^{cd}±0.17	5.3^{cd}±0.18	5.5^{d}±0.15	3.4^{a}±0.12
C20:0	3.1^{c}±0.21	2.9^{c}±0.21	2.6^{bc}±0.42	2.3^{ab}±0.51	2.1a±0.37	1.8^{a}±0.17	2.6^{bc}±0.24
^{a}∑SFAs	23.3^{a}±0.41	26.4^{b}±0.51	28.5^{c}±0.23	29.7^{d}±0.19	31.6^{e}±0.14	31.1^{e}±0.1	23.1^{a}±0.13
C16:1	3.9^{ab}±0.16	4.2^{abc}±0.28	4.4^{bcd}±0.37	4.7^{cd}±0.26	4.9^{d}±0.19	4.9^{d}±0.2	3.7^{a}±0.18
C18:1	18.1^{a}±0.13	20.0^{b}±0.31	20.3^{b}±0.23	20.4^{b}±0.26	22.4^{d}±0.15	29.8^{e}±2.3	21.6^{c}±0.15
^{b}∑MUFAS	21.8^{a}±0.63	24.1^{b}±0.25	24.6^{bc}±0.13	25.1^{cd}±0.38	27.3^{e}±1.4	34.6^{f}±2.1	25.2^{d}±0.13
C18:2	16.3^{a}±01.7	19.5^{b}±0.81	22.3^{e}±0.63	24.7^{f}±0.34	28.5^{g}±1.5	20.5^{c}±0.22	21.4^{d}±0.41
C18:3	18.7^{f}±1.1	16.5^{e}±1.2	14.5^{d}±0.31	12.8^{c}±0.51	4.5^{b}±1.3	3.2^{a}±0.36	14.5^{d}±0.24
C20:5	1.2^{a}±0.18	1.3^{a}±0.16	1.1^{a}±0.12	1.1^{a}±0.14	1.1^{a}±0.03	1.2^{a}±0.31	1.5^{a}±0.18
C22:6	2.4^{a}±0.21	2.3^{a}±0.19	2.3^{a}±0.22	2.1^{a}±0.17	2.3^{a}±0.25	2.2^{a}±0.32	3.21^{b}±1.56
^{c}∑PUFAS	38.3^{c}±0.61	39.3^{d}±0.31	39.9^{e}±0.21	40.2^{e}±0.35	36.0^{b}±0.5	26.8^{a}±2.3	40.3^{e}±0.43
^{d}∑n3	22.2^{g}±0.38	19.9^{f}±0.7	17.6^{d}±0.8	15.6^{c}±0.83	7.6^{b}±0.73	6.4^{a}±0.51	19.1^{e}±0.26
∑n6	16.3^{a}±0.21	19.5^{b}±0.43	22.3^{e}±0.19	24.7^{f}±0.33	28.5^{g}±0.27	20.5^{c}±0.17	21.4^{d}±0.52
∑n3/n6	1.5^{c}±0.59	1.1^{bc}±0.22	0.9^{ab}±0.28	0.7^{ab}±0.18	0.4^{a}±0.05	0.4^{a}±0.05	1.1^{bc}±0.17

Values reported are means± standard error (n=*3*) as determined using Duncan's multiple range test. Means within the same row with different superscripts varied significantly (p<0.05). Fatty acids: C10:0 Capric Acid, C12:0 Lauric acid, C14:0 Myristic acid, C14:1 Myristoleic acid, C16:0 palmitic acid, C16:1 palmitoleic acid, C18:0 Stearic acid, C18:1 Oleic acid C18:2 Linoleic acid, C18:3 α-Linolenic acid, C20:0 Arachidic acid, C20:5 Eicosapentaenoic acid, C22:6 docosahexaenoic acid [a]∑SFAs: Total saturated fatty acids b∑MUFAs: Total monounsaturated fatty acids [c]∑PUFAs: Total Polyunsaturated fatty acids. [d]∑n3: Total omega-3 fatty acids ∑n6: Total omega-6 fatty acids.

months feeding period are presented in Tables 6 and 7.

In all the experimental diets, there was significant (P<0.05) changes in fatty acid composition in tilapia tissues with feeding period. The tilapia liver and muscles fatty acid composition trends were also related to the dietary fatty acid composition.

Even though there was no particular trend in linoleic acid composition in the feeding period in all experimental diets, significant increase in the tissues composition of linolenic acid, arachidonic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), omega -3 and omega-3/omega-6 ratio was observed (Tables 6 and 7). Tissue composition of linolenic acid increased with increased inclusion of linseed oil in the diet with relatively

Table 4. Mean body weight and length before and after washout in tilapia.

Body weight (g)		Body length (cm)	
Before washout	**After washout**	**Before washout**	**After washout**
2.6±0.2	5.4±0.4	1.6±0.1	2.7±0.1

Table 5. Different growth parameters for tilapia.

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Washout	Commercial
FBW[1]	46.8±6.3^{a}	55±4.0^{ab}	64.4 ^{b} ±6.4	97.4±4.9^{c}	69.2±6.8^{b}	66.4±8.8^{b}	147.6±2.9^{d}
FBL[2]	13.5±1.2^{a}	14.9±0.7^{ab}	16.5±0.3^{bc}	17.4±0.3^{c}	16.5±0.4^{bc}	16.5±0.3^{bc}	21.44±0.4^{d}
Weight gain (%)	750^{a}	937^{ab}	1099^{ab}	1689^{c}	1216^{b}	1175^{ab}	2625^{d}
SGR[3] (%)	0.9±0.05^{a}	1.1±0.04^{ab}	1.16±0.01^{b}	1.3±0.02^{c}	1.2±0.06^{bc}	1.2±0.08^{b}	1.5±0.01^{d}
Survival (%)	94^{a}	95.67^{a}	96^{b}	97.67^{c}	97^{c}	97.33^{c}	98.33^{d}

[1]FBW, Final body weight (grams); [2]FBL-Final body length (cm); [3]SGR, Specific growth rate.

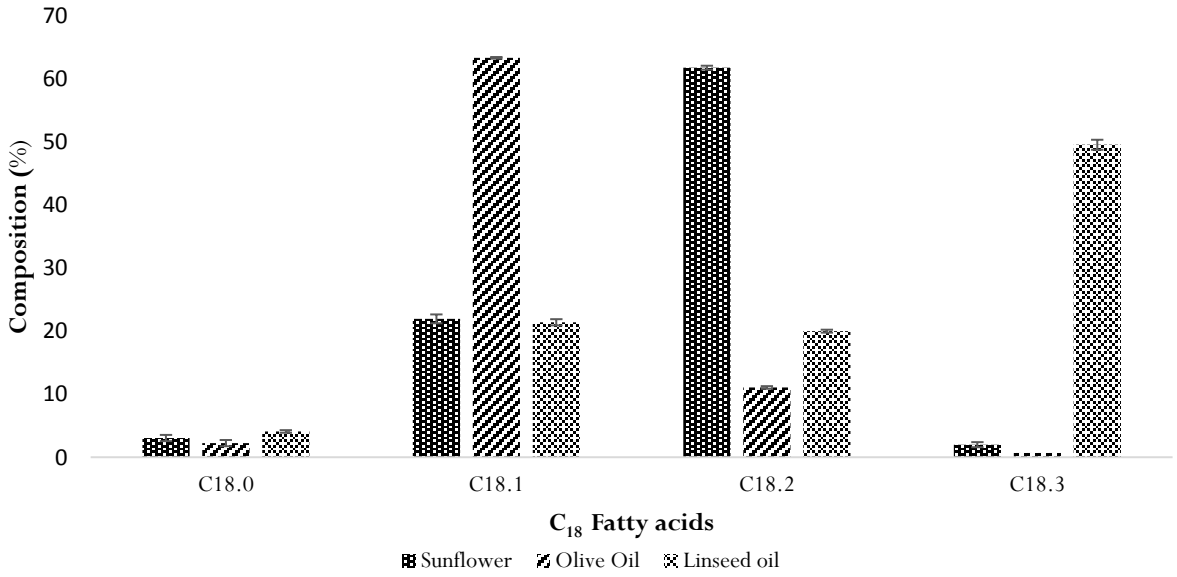

Figure 1. Compositions (%) of C18 fatty acids in vegetable oils used for experimental diets formulation. *C18:0; Stearic acid, C18:1; Oleic acid, C18:2; Linoleic acid, C18:3; α-Linolenic acid.

high tissue composition of linolenic acid observed in diet 1.In addition, the accumulation of linolenic acid increased with feeding period in both tilapia tissues with significantly high composition observed in month 3 (Tables 6 and 7). The compositions of DHA, EPA, arachidonic acid, ∑n3, and n3/n6 also increased with feeding period in both tilapia tissues with significantly high composition observed in diet 1 (Tables 6 and 7)

DISCUSSION

The possible use of VO as a replacement for dietary FO in fish diets has been a subject of study in the recent past. The data from these studies indicate that FO can partially or totally be replaced with VO, however, with varying outcomes on growth performance and fatty acid profile for different fish species. Results from this study show that > 50% dietary linseed oil lowered growth parameters and survival ability of tilapia (*Oreochromis niloticus*).

Present finding is consistent with earlier reports that high dietary linseed oil lowered the growth performance of tilapia (Li et al., 2016; Francis et al., 2006). Moreover, earlier reports indicate that high dietary n-3 polyunsaturated fatty acids (PUFAs) depressed growth in hybrid tilapia and tilapia zillii (Huang et al., 1998; Kanazwa et al., 1980). In contrast, replacing FO with

Table 6. Monthly changes of muscles polyunsaturated fatty acid composition in tilapia fed diets containing varying composition of linseed, sunflower and olive oil.

Acid	Month	Treatment						
		Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Washout	Commercial
C18:2	1	12.0^{ab}±0.14	13.7^{fgh}±0.9	14.4^{gh}±0.83	15.7^{i}± 0.62	18.8^{j}±0.63	15.7^{i}±0.47	16.5^{i}±0.82
	2	10.2^{a}±0.82	11.6^{abc}±0.2	14.6^{h}±0.48	18.3^{j}±0.5	19.4^{k}±0.5	18.7^{j}±0.4	16.2^{i}±0.12
	3	11.9^{bcde}±0.38	12.9^{def}±0.15	13.2^{efg}±0.45	14.3^{gh}±0.5	16.3^{i}±0.36	14.3^{gh}±0.31	12.5^{ede}±0.14
C18:3	1	7.8^{i}±0.27	4.8^{cde}±0.73	4.3^{bcd}±0.19	3.7^{bc}±0.26	3.3^{b}±0.15	1.3^{a}±0.31	5.3^{ef}±0.41
	2	10.8^{kl}±0.23	9.3^{ij}±0.45	8.9^{i}±0.61	7.4^{gh}±0.21	5.5^{ef}±0.31	4.8^{cde}±0.13	8.5^{hi}±0.63
	3	11.9^{kl}±0.1	10.3^{l}±0.63	10.2^{jk}±0.42	8.4^{hi}±0.71	6.2^{fg}±0.37	5.3^{ef}±0.34	11.7^{l}±0.06
C20:4	1	1.6^{a}±0.11	1.9^{abc}±0.37	2.3^{bcde}±0.24	2.5^{cdef}±0.34	2.7^{efgh}±0.31	2.8^{efgh}±0.21	1.8^{ab}±0.13
	2	2.0^{abcd}±0.23	2.5^{cdef}±0.42	2.7^{efgh}±0.31	2.9^{efghi}±0.27	3.2^{ghi}±0.21	3.3^{hi}±0.43	2.4^{bcde}±0.27
	3	2.6^{defg}±0.31	2.9^{efghi}±0.33	3.1^{fghi}±0.23	3.3^{hi}±0.14	3.5^{i}±0.41	3.5^{i}±0.22	2.8^{efgh}±0.25
C20:5	1	3.5^{hi}±0.68	1.2^{bc}±0.73	0.8b±0.41	0.3^{a}±0.21	0.3^{a}±0.16	1.6^{c}±0.48	2.7^{efg}±0.93
	2	3.6^{hij}±0.36	2.9^{fg}±0.51	2.7^{efg}±0.21	2.2^{d}±0.23	3.2^{gh}±0.14	1.1^{b}±0.47	2.7^{efg}±0.74
	3	5.9^{kl}±0.73	5.3^{k}±0.33	4.1^{j}±0.38	3.7^{ij}±0.62	2.4^{de}±0.13	1.3^{bc}±0.41	2.4^{def}±0.41
C22:6	1	4.3^{g}±0.5	2.2^{de}±0.57	1.8^{cd}±0.19	1^{abc}±0.23	0.8^{ab}±0.14	0.4^{a}±0.11	3.1^{ef}±0.51
	2	6.5^{hi}±0.37	4.8^{g}±0.5	3.2^{f}±0.91	2.3^{def}±0.71	2.3^{def}±0.17	1.5^{bcd}±0.72	5.7^{gh}±0.17
	3	7.9^{ij}±0.52	7.4^{j}±0.21	7.3^{ij}±0.13	6.6^{hi}±0.5	2.5^{def}±0.13	1.8^{cd}±0.29	5.9^{h}±0.18
∑n3	1	17.2^{g}±0.34	8.2^{bc}±0.11	6.8^{b}±0.23	4.8^{a}±0.31	4.3^{a}±061	3.2^{a}±0.41	10.9^{de}±0.56
	2	20.6^{hij}±0.9	16.8^{g}±0.2	14.6^{f}±0.31	11.6^{e}±0.71	10.7^{de}±0.71	8.2^{bc}±0.72	16.6^{g}±0.93
	3	25.3^{k}±0.37	22.6^{j}±0.17	21.2^{ij}±0.36	18.2^{gh}±0.92	10.6^{de}±0.76	9.0^{cd}±0.43	19.5^{hi}±0.68
∑PUFA	1	28.4^{gh}±0.31	21.7^{cd}±0.18	20.9^{bc}±0.71	20.2^{b}±0.76	22.9^{de}±0.47	18.7^{a}±0.64	27.2^{fg}±0.88
	2	30.6^{jk}±0.43	28.1^{gh}±0.37	29.0^{hi}±0.27	29.8^{ij}±0.16	32.9^{l}±0.18	26.6^{f}±0.94	32.5^{l}±0.93
	3	37.0^{no}±0.25	35.3^{o}±1.2	34.2^{mn}±0.39	32.3^{m}±0.92	26.7^{kl}±0.62	23.1e±0.23	31.8^{kl}±0.31
∑n6	1	12.0^{ab}±0.41	13.7^{fgh}±0.93	14.3^{gh}±0.93	15.7^{i}±0.26	18.8^{j}±0.63	15.7^{i}±0.74	16.5^{i}±0.82
	2	10.2^{a}±0.82	11.6^{abc}±045	14.6^{h}±0.48	18.3^{j}±0.9	22.4^{k}±0.5	18.7^{j}±0.54	16.2^{i}±0.23
	3	11.9^{bcde}±0.38	12.9^{def}±0.51	13.2^{efg}±0.54	14.3^{gh}±0.23	16.3^{i}±0.36	14.3^{gh}±0.24	12.5^{cde}±0.14
n3/n6	1	1.5^{def}±0.64	0.8^{bc}±0.2	0.7^{abc}±0.25	0.5^{ab}±0.14	0.4^{a}±0.12	0.4^{a}±0.12	0.9^{c}±0.15
	2	2.2^{g}±0.4	1.7^{f}±0.9	1.2^{d}±0.14	0.8^{bc}±0.18	0.7^{abc}±0.1	0.6^{abc}±0.1	1.2^{d}±0.4
	3	2.2^{g}±0.46	1.8^{f}±0.61	1.7^{f}±0.14	1.3^{de}±0.12	0.7^{abc}±0.14	0.7^{abc}±0.25	1.6^{ef}±0.15

*Values reported are means± standard error (n=*3*) as determined using Duncan's multiple range test. Means within the same row with different superscripts varied significantly ($p<0.05$).C18.2: Linoleic acid, C18.3: α-Linolenic acid, C20.5: Eicosapentaenoic acid, EPA, C22.6: Docosahexaenoic acid, DHA, ∑ω3: Total omega-3 fatty acids, ∑PUFA: Total Polyunsaturated fatty acids, ∑ω6: Total omega 6 fatty acids.

linseed oil at higher levels had no effect in the growth of Atlantic salmon and Murray cod (Menoyo et al., 2005; Turchini et al., 2011). In addition, there was no reduction in growth rates of European sea bass, *Dicentrarchus labrax* when FO was replaced with rapeseed oil, linseed oil and olive oil at 60%. Furthermore, growth performance in Atlantic salmon, *Salmo salar*, was also not affected when FO was replaced with VO at 50% (Storebakken, 2002).

It has been established that Nile tilapia and hybrid tilapia require both n-3 and n-6 polyunsaturated fatty acids as essential fatty acids for optimal growth (Chen et al., 2013; FAO, 2014). In addition, dietary n-3 and n-6 fatty acids requirements in tilapia are in the form of α-linolenic and linoleic acids (Izquierdo et al., 2003; Tocher, 2003), therefore, dietary VO should not alter growth in fish because the needs of essential fatty acids (EFAs) are covered within the VO (Corraze and Kaushik, 2009). Dietary linoleic (C18:2) and α-linolenic (C18:3) acids were provided in the present study through sunflower and linseed oil respectively.

High dietary linseed oil (>50%) in the diets 1 to 3 (Table 1) might have resulted into n3/n6 imbalance creating oxidative stress as n-3 fatty acids physiological tolerance for tilapia might have been exceeded thus possible compromised immune system in the study subjects (Li et al., 2016) reduced survival rate in this study. In addition, it has been established that n-6 fatty acids have superior

Table 7. Monthly changes of liver polyunsaturated fatty acid composition in tilapia fed diets containing varying composition of linseed and sunflower.

Acid	Month	Treatments						
		Diet1	Diet 2	Diet 3	Diet 4	Diet 5	Washout	Commercial
C18:2	1	11.9^{bc}±0.52	13.5^{ef}±0.4	14.1^{fg}±0.64	15.7^{h}±0.97	18.7^{j}±0.92	15.6^{h}±0.34	16.8^{i}±0.41
	2	10.2^{a}±0.82	11.6^{b}±0.24	14.6^{g}±0.4	18.3^{j}±0.91	22.4^{k}±0.51	18.7^{j}±0.53	16.1^{hi}±0.45
	3	10.3^{a}±0.72	12.5^{cd}±0.61	13.1^{de}±0.31	13.9^{fg}±0.82	16.2^{hi}±0.64	14.4^{g}±013	11.7^{b}±0.24
C18:3	1	7.32^{h}±0.23	4.9^{cd}±0.52	4.5^{c}±0.46	3.8^{b}±0.41	3.5^{b}±0.29	1.6^{a}±0.21	6.4^{fg}±0.23
	2	10.8^{k}l±0.32	9.3^{j}±0.54	8.9^{ij}±0.69	7.4^{h}±0.53	5.5^{de}±0.51	1.8^{a}±0.13	8.5^{i}±0.7
	3	12.2^{m}±0.86	11.3^{l}±0.55	10.3^{k}±0.42	9.4^{j}±0.6	6.5^{g}±0.51	2.5^{ab}±0.13	10.9^{kl}±0.94
C20:4	1	2.1^{a}±0.51	2.6^{abc}±41	2.9^{bcd}±0.12	3.1^{cdef}±0.22	3.3^{cdefg}±0.27	3.8^{fghij}±0.13	2.3^{ab}±0.16
	2	3.0^{cde}±0.81	3.3^{cdefg}±0.24	3.5^{defgh}±0.34	3.7^{efghi}±0.35	4.0^{ghij}±0.43	4.2^{hij}±0.41	3.1^{cdef}±0.24
	3	3.4^{defg}±0.23	3.6^{defghi}±0.33	3.8^{fghij}±0.16	4.0^{ghij}±0.32	4.3^{ij}±0.31	4.5^{j}±0.23	3.5^{defgh}±0.21
C20:5	1	3.8^{i}±0.12	1.8^{e}±0.68	1.0^{c}±0.24	0.2^{a}±0.01	0.5^{b}±0.17	2.0^{ef}±0.27	2.3^{f}±0.86
	2	3.6^{i}±0.36	2.9^{gh}±0.51	2.7^{g}±0.21	2.2^{f}±0.31	2.2^{ef}±0.14	1.1^{c}±0.47	2.7^{g}± 0.74
	3	6.3^{k}±0.52	5.2^{j}±0.59	5.1^{j}±0.22	3.7^{i}±0.24	2.1^{ef}±0.27	1.5^{d}±0.29	3.8^{i}±0.17
C22:6	1	5.4^{h}±0.43	3.0^{f}±0.12	1.9^{cd}±0.31	1.4^{b}±0.12	1.2^{b}±0.19	0.6^{a}±0.23	3.1^{f}±0.61
	2	6.5^{i}±0.14	4.8^{g}±0.45	2.4^{de}±0.71	2.3^{de}±0.21	1.5^{bc}±073	1.2^{b}±0.24	3.3^{f}±0.91
	3	7.4^{l}±0.16	7.4^{j}±0.91	7.3^{j}±0.17	7.0^{m}±0.51	2.8^{ef}±0.31	1.6^{bc}±0.21	7.8^{kl}±0.95
∑n3	1	16.3^{i}±0.31	9.6^{e}±0.43	7.3^{c}±0.21	5.2^{b}±17	5.0^{b}±0.62	4.1^{a}±0.72	11.6^{g}±0.18
	2	20.6^{k}±0.15	16.8^{i}±0.43	14.6^{h}±0.32	11.6^{g}±071	10.7^{f}±0.24	8.2^{d}±0.92	16.6^{i}±091
	3	25.5^{n}±0.51	23.8^{m}±0.51	22.4^{l}±0.34	19.8^{j}±0.62	11.1^{f}±0.24	9.3^{e}±0.23	22.3^{l}±0.61
∑PUFA	1	28.0^{f}±0.31	22.9^{c}±0.26	21.1^{b}±016	20.7^{b}±0.52	23.5^{d}±0.82	19.4^{a}±0.53	28.2^{f}±0.92
	2	30.6^{i}±0.41	28.1^{f}±0.71	29.0^{g}±0.36	29.8^{h}±0.23	32.9^{j}±0.41	26.6^{e}±0.91	32.5^{j}±0.9
	3	36.6^{n}±0.83	36.1^{m}±0.71	35.3^{l}±0.51	33.5^{k}±0.94	27.1^{e}±0.17	23.4^{d}±0.62	33.8^{k}±0.34
∑n6	1	11.7^{b}±0.52	13.3^{de}±0.92	13.9^{ef}±0.32	15.6^{hi}±0.43	18.6^{k}±0.41	15.4^{h}±0.85	16.6^{j}±0.94
	2	10.2^{a}±0.81	11.6^{b}±0.21	14.6^{g}±0.52	18.3^{k}±0.91	22.4^{l}±0.51	18.7^{k}±0.53	16.1^{ij}±0.56
	3	10.3^{a}±0.72	12.5^{c}±0.62	13.1^{cd}±0.31	13.9^{ef}±0.81	16.2^{ij}±0.61	14.4^{fg}±0.15	11.7^{b}±0.25
n3/n6	1	1.4d^{ef}±0.96	0.8^{abc}±0.17	0.6^{a}±0.22	0.4^{a}±0.26	0.3^{a}±0.17	0.3^{a}±0.18	0.7^{abc}±0.14
	2	2.3g^{h}±0.94	1.7^{def}±0.19	1.2^{bcd}±0.59	0.8^{abc}±0.12	0.7^{ab}±0.12	0.6^{a}±0.17	1.3^{cde}±0.94
	3	2.6^{h}±0.81	2.0^{g}±0.17	1.8^{efg}±0.22	1.5^{def}±0.29	0.7^{abc}±0.18	0.7^{ab}±0.4	2.0^{fg}±0.27

*Values reported are means± standard error (n=3) as determined using Duncan's multiple range test. Means within the same row with different superscripts varied significantly ($p<0.05$). C18.2: Linoleic acid, C18.3: α-Linolenic acid, C20.5: Eicosapentaenoic acid, EPA, C22.6: Docosahexaenoic acid, DHA, ∑ω3: Total omega-3 fatty acids, ∑PUFA: Total Polyunsaturated fatty acids, ∑ω6: Total omega 6 fatty acids.

growth-promoting effects than n-3 fatty acids in red belly tilapia (Lim et al., 2009) confirming a relatively better growth rates at high (>50%) sunflower oil content in the present study.

Studies have shown that fish species, environmental factors, fish size and age as well as diets affect fatty acid composition of fish tissues (Saito et al., 1999; Kiessling et al., 2001). In this study, tilapia were raised under same exogenous conditions therefore culturing period and diet were the possible factors that could affect fatty acid profile of fish under study. From our findings, DHA, total n3 and n3/n6 ratio increased significantly ($P<0.05$) from month 1 to month 3 in both tissues (Table 6 and 7), an observation which relates with previous finding that fatty acid profile in fish is dependent on fish age (Parlov et al., 2009, Nemova et al., 2015a; Nemova et al., 2015b; Denis and Nina, 2016). A study on Atlantic salmon indicated that in two year feeding period, the levels of n3 fatty acids increased from 24 to 29.5%, DHA increased from 5.4 to 8.6% and n3/n6 ratio increased from 2.5 to 3 (Svetlana et al., 2003). In the finding, DHA, n3 fatty acids and n3/n6 ratios increased as follows DHA (4.3 to7.1%, diet 1 muscles; 5.4 to 7.4% diet 1 liver), n3 fatty acids (17 to 25% in diet 1 muscle; 5.4 to 7.4% in diet 1 liver) and n3/n6 ratios (1.5 to 2.2 in diet 1 muscles; 1.4 to 2.6 in diet 1 liver) (Tables 6 and 7).

The increase in these values correlated with dietary increase in linseed oil proportions suggests active

metabolism of fatty acid from dietary lipid source in tilapia.

Provision of dietary fatty acid in excess promotes intensive beta-oxidation for energy production (*Stubhaug et al., 2006, 2007*). The present findings is consistent with a study by Stubhaug et al. (2007), that there is preference in fatty acids beta-oxidation in which some fatty acids, specifically omega 3 long chain polyunsaturated fatty acids, LC-PUFAS such as DHA, are spared from catabolism. This explains the tissue accumulation of omega 3 fatty acid particularly, DHA, in our study. This accumulation correlates with the levels of linseed oil inclusion in the diet. Specifically, diet 1 and 2 with > 50% linseed oil inclusion had significantly high liver DHA and omega 3 fatty acids (7.4, 7.4 and 25.5,23.8%) respectively (Table 7) and muscle DHA and omega 3 fatty acids (7.9, 7.4 and 25.3, 22.6) (Table 6).

Tissue polyunsaturated fatty acid values in our study increased with increased dietary proportion of linseed oil, finding which is consistent with previous study indicating significant increase in total n3 fatty acids and DHA content as dietary linseed oil increases (Li et al., 2015). Tonial et al. (2009) observed that Nile tilapia fed dietary flaxseed oil had significant increase in muscle total n3 fatty acids and DHA content. The finding supports suggestion that tilapia is capable of converting dietary alpha- linolenic acid to LC-PUFA (Sargent et al., 2002) and that they can elongate and desaturate dietary alpha-linolenic acids to tissue DHA. However, the level of conversion of C18 PUFA to highly polyunsaturated fatty acids (HUFA) varies among species (Sargent et al., 2002). This variation reflects different rates of assimilation and catabolism of dietary fatty acids once consumed (Yones et al., 2013).The extent of interactions between dietary fatty acids may also determine final tissue lipids composition (Fonseca-Madrigal et al., 2005; Matsushita et al., 2006).

Arachidonic acid (C20:4) levels reported in the present study ranged between 3.4 and 4.5% in liver and 2.6 and 3.5% in tilapia muscles. These values were lower than values observed by Feirrera et al. (2011), 5.8 to 8.12% and Ribeiro et al (2008), 5.8 to 9.2% in tilapia. In addition, values reported in our study were low relative to the dietary linoleic acid (C18:2) (Table 3). These low values might have been due to varied roles played by arachidonic acid which includes being a source for eicosanoids formation and roles it play in resisting stressors prevalent under intensive culture system (Bell and Sargent, 2003; Li et al., 2016). In addition, previous studies indicate that excessive dietary supply of C18 polyunsaturated fatty acids may create selective competition disrupting bioconversion thus low levels of arachidonic acid (Ruyter et al., 2006). This is because alpha linolenic acid and linoleic acids use almost same enzymes for their metabolism (Visentainer, 2007).

The activities and expression of these enzymes are also affected by dietary lipids (Visentainer, 2007). In conclusion, dietary fish oil can successfully be replaced with linseed oil for adequate essential fatty acids in tilapia muscles, however, high dietary linseed oil (>50%) lowers survival and growth rate of tilapia. From our data, a ratio of 25:75 linseed to sunflower oil gave a relatively better survival and growth levels, however, studies are needed to establish the optimal dietary linseed oil that promote growth, survival and tissue deposition of essential fatty acids.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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