

Full Length Research Paper

Effect of varying dietary proportions of linseed and olive oils on fatty acid composition of Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) under aquaponics culture system

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This study evaluated the effect of varying proportions of linseed and olive oils in fish diets on the fatty acid composition of tilapia and catfish liver and muscles under aquaponics culturing system after 150 days of feeding period. Experimental diets contained linseed, sunflower and olive oils at a varying composition of between 0 to 100% and commercial diet as a control feed. Gas chromatography was used to analyze fish tissue fatty acid profiles. Significantly high composition of n-3 fatty acids were observed in tilapia fed diet 1 ($p < 0.05$) with DHA (C22:6) being the dominant n-3 fatty acids at 12.2% and 10.8% in tilapia muscles and liver respectively. In catfish, muscle and liver had 10.4% and 9.7% DHA content respectively. The muscle accumulation of n-3 fatty acid was significantly higher than liver in both fish species and efficiency in their retention and relative resistance of DHA to β -oxidation in the fish muscles. In both tilapia and catfish, the tissue n-3 fatty acids decreased with the reduction in linseed oil proportion in the diet feed suggesting direct influence of dietary oil composition on tissue fatty acid composition.

Keywords: Catfish, tilapia, DHA, EPA, n-3 fatty acids, linseed oil.

INTRODUCTION

Fish is a major source of protein accounting for approximately 16% of animal protein consumed worldwide (Aguar *et al.*, 2011). In addition, fish contain omega-3 fatty acids especially DHA and EPA which are

an important component of human diet because of their health benefits. These fatty acids positively influence, blood pressure and hypotriglyceridemia (Kris-Etherton *et al.*, 2002). This reduction in coronary risk has been related to the capacity of EPA and DHA to lower serum triglyceride levels and decrease platelet aggregation (Leaf and Weber, 1988) and blood pressure (Bonaa, *et al.*, 1990).

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Dietary polyunsaturated fatty acids can specifically and rapidly effect changes in cellular metabolism, differentiation, and growth through alterations in gene expression patterns (Fonseca-Madrigal *et al.*, 2006). The heightened knowledge on health benefits has increased the utilization of fish as food, and with the increase in human population, there is decreased supply of wild fish. This has promoted fish farming across the world to meet the ever increasing market demand for fish. Because of limited land space, aquaculture systems, such as aquaponics have been employed in fish farming. However, there is a concern that the feed grade fisheries that provide fish oil and fish meal have reached their limit of sustainability (Barlow & Pike, 2001). As a result, research on the substitution of dietary fish oil has indicated that it may be possible to replace fish oil by plant seed oils (soybean oil, linseed oil, rapeseed oil, olive oil, palm oil and corn oil) (Mourente *et al.*, 2005). Several studies involving warm water freshwater species like catfish *Heterobranchus longifilis* (Ng *et al.*, 2003) and tilapia *Oreochromis* sp. (Bahurmiz, 2007) have demonstrated the possibility of inclusion of high levels of plant oils such as sunflower and canola oil (CO) (Bell *et al.*, 2003b) without compromising growth or feed conversion. In comparison with marine fish which generally require substantial amounts of HUFA-rich fish oil (FO) in their diet, freshwater fish possess a better ability to synthesize HUFA from linoleic acid and alpha linolenic acid. This is because of $\Delta 5$ and $\Delta 6$ fatty acyl desaturases and elongases which appears more active in freshwater than marine fish and are important in the HUFA biosynthetic pathway (Panserat *et al.*, 2003). Studies suggest that different vegetable oils may have different effects on fish metabolism and that different fish species respond in different ways to dietary oil (Regost *et al.*, 2003; Menoyo *et al.*, 2003). For instance, replacement of fish oil with linseed oil or sunflower oil in diets of marine fish could reduce the growth and survival, and modulate a number of physiological processes such as lipid metabolism, energy utilization, and immune response (Bell *et al.*, 1999; Tocher *et al.*, 2000; Ringø *et al.*, 2002; Mourent *et al.*, 2005; Martins *et al.*, 2009; Ganga *et al.*, 2011; Friesen *et al.*, 2013a). On the other hand, dietary vegetable oil inclusion does not result in reduced growth performance and feed conversion in Atlantic salmon (Torstensen *et al.*, 2000; Bell *et al.*, 2001, 2003a).

Linseed is distinguished to have high levels of alpha-linolenic acid (C18:3, n-3) which is a precursor for the synthesis of highly polyunsaturated omega-3 fatty acids such as DHA and EPA (Popa *et al.*, 2012). However, there is no adequate information on the effect of dietary linseed oil on the fatty acid profiles of catfish and tilapia which are the most commercially cultured fish species in Kenya today. In this study, vegetable oil blends were included in the diet at 14% with varying composition of

linseed oil and olive oil. The effect of diet-oil composition on catfish and tilapia fatty acid composition was analyzed after 150 days of feeding period.

MATERIAL AND METHODS

Study site, experimental set up and sampling

Proximate composition analysis of experimental diets and fatty acid composition in fish muscles and liver was done in Food Biochemistry laboratory, Food Science department-Jomo Kenyatta University of Agriculture and Technology. A polyculture of monosex fish, 8 weeks old *O. niloticus* and 4 weeks old *C. gariepinus* which were previously fed on commercial diets were set up in a 1000-liters experimental tanks for a period of 5 months under aquaponics. The fingerlings were obtained from Kenya Marine and Fisheries Research Institute, KEMFRI, Sagana fish hatcheries. Three tanks containing 40 tilapia and 40 catfish were set for each experimental diet. Separate tanks were used as holding tanks for two weeks prior to the feeding experiments for fish acclimatization. Feeding was done twice/day at 9.00am and 4.00pm daily. 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, 3 tilapia and 3 catfish were randomly sampled after 24 hours fasting period, anaesthetized and dissected for liver and muscles.

Diet ingredients

The 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 Wichlum beach along Lake Victoria in Siaya County, Kenya. The 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

The powdered experimental diets were carefully weighed and pre-mixed prior to the addition of vegetable oil blends. The powdered ingredients were dry mixed thoroughly for 2 minutes in a bench top food mixer before addition of distilled water and vegetable oil blend was

Table 1. Macronutrient content of experimental diets.

Macronutrients (g/100g)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Freshwater 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)						
Olive oil ¹	0	25	50	75	100	0
Sunflower oil ¹	0	0	0	0	0	100
Linseed oil ²	100	75	50	25	0	0
Vitamin/Mineral premixes ³	2	2	2	2	2	2

¹Obtained from local supermarket

²Extracted from linseeds using oil extruder machine, BEED department, JKUAT-Kenya

³Obtained from Tam feeds, Nairobi, Kenya

added and mixing continued for further 10 minutes. All the experimental diets were made on grade 12 meat mincer as an extruder fitted with a die plate with 2mm diameter holes. The soft feed dough was then cold extruded into the 2mm die-size strand, pelleted and dried at ambient temperature for 3 hours. The feeds were then placed on a sieve and over dried at 40°C for approximately 24 hours until the moisture content was 10% (w/w). The dried feeds were then broken into 2-3mm 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 2).

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, fish 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 20minutes. The mixture was then centrifuged at 3000rpm for 10minutes and the aqueous layer was then separated from organic layer using a micropipette. The bottom layer, chloroform, was then transferred to

100ml reflux flask, quick fit, and evaporated to dryness under vacuum evaporator. Fatty acid methyl esters (FAME) were then prepared from vegetable oils and extracted total lipid by acid-catalyzed trans-esterification by adding 5ml of 1% H₂SO₄ (v/v) in methanol at 70°C, for 3hrs.

FAME were then extracted into 750ml of distilled water and 10 ml of hexane, dehydrated using an hydrous sodium sulphate, Na₂SO₄ and concentrated to 0.5 ml under vacuum evaporator. The concentrated FAME were then transferred to GC vials for later GC analysis

GC-analysis

FAME were separated and quantified by gas-liquid with on-column injection, equipped with a fused silica capillary column (SUPELCO Column Omega waxtm530, 30m x 0.5mm x 0.5µm) with nitrogen as carrier gas and temperature programming from 170°C to 220°C for 18 min⁻¹ and final time of 47 minutes totaling to a run time of 75 minutes. Injection and detection temperatures were 240°C and 260°C respectively. The programmer rate for both GC and decoder were set at 5min⁻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.

Statistical analyses

Statistical analyses were performed using Genestat version 41.0. Significance in fatty acid compositions of liver and muscles of tilapia and catfish 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).

Table 2. Proximate composition of different diets and dietary 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
Diet 6	45.6±0.6 ^d	25.4±2.3 ^{ab}	13.9±0.33 ^{ab}	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

¹Skretting, Fontaine-Les-Vervins-France obtained from Jambo fish farm, Kiambu, Kenya

² Obtained from Wichlum beach of Lake Victoria, Kenya

³ Obtained from local supermarket

⁴ Obtained from Mwea rice mills

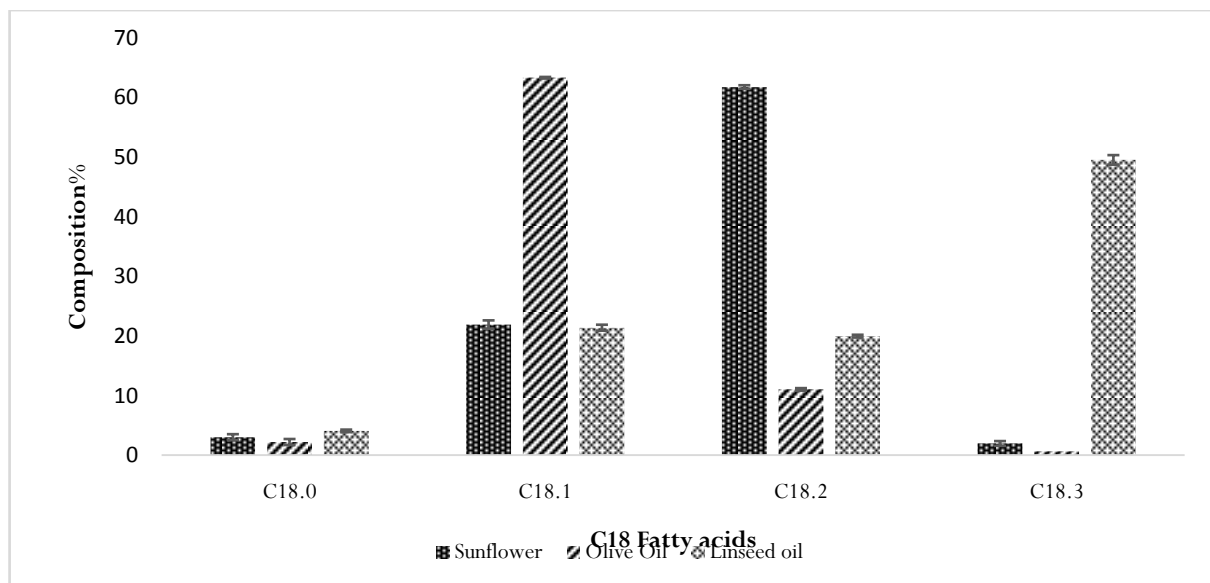


Figure1: Compositions (%) of C18 fatty acids in vegetable oils used for experimental diets formulation. *C18.0 Stearic acid, C18.1 Oleic acid C18.2 Linoleic acid, C18.3 α-Linolenic acid

Values throughout the text are expressed as means ±standard error. In all the analysis, treatment significance was accepted at P < 0.05.

RESULTS

There was no significant differences (P<0.05) in the proximate composition of experimental diets 1-6, however, the diets had higher protein and fat than the commercial diet (Table 2). The dietary variation in fatty acid composition was attributed by vegetable oils

supplemented at different proportions in the diet (Table 1) and difference in fatty acid composition of linseed oil, olive oil and sunflower oil (Figure 1). Fatty acid compositions of liver and muscles of catfish and tilapia fed on different diets are presented in Tables 4, 5, 6 and 7. The saturated fatty acids (SFAs) composition did not vary significantly between the experimental diets. Palmitic acid (C16:0) was the predominant SFA in all cases followed by stearic acid (C18:0) (Tables 4, 5, 6, 7). There was non-significant increase in tissue SFAs with the reduction of linseed oil (LO) content in the diets (Tables 4, 5, 6, and 7). Olive oil (C18:1) was the dominant

Table 3. Fatty acid composition (%) of different diets.

	Dietary treatments						
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Commercial
C10:0	1.2 ^a ±0.02	1.4 ^a ±0.05	1.5 ^a ±0.9	1.3 ^a ±0.81	3.8 ^b ±0.6	3.4 ^b ±0.21	1.5 ^a ±0.31
C12:0	1.3 ^b ±0.04	1.5 ^b ±0.08	2.5 ^c ±0.6	2.5 ^c ±0.02	3.2 ^d ±0.09	3.5 ^d ±0.18	0.4 ^a ±0.06
C14:0	1.2 ^a ±0.01	1.2 ^a ±0.04	3.9 ^d ±1.5	3.3 ^c ±0.08	2.2 ^b ±0.05	2.6 ^b ±0.08	1.05 ^a ±0.2
C16:0	10.5 ^a ±0.8	12.2 ^b ±0.12	14.7 ^c ±0.27	15.8 ^d ±0.7	16.3 ^d ±0.51	15.1 ^c ±0.7	12.2 ^b ±0.23
C18:0	2.2 ^a ±0.3	3.1 ^b ±0.8	4.2 ^c ±0.13	4.3 ^c ±0.14	5.1 ^d ±0.12	5.3 ^d ±0.16	3.4 ^b ±0.18
C20:0	2.8 ^b ±0.01	2.6 ^b ±0.09	2.3 ^b ±0.01	1.3 ^a ±0.4	1.4 ^a ±0.08	1.5 ^a ±0.03	2.4 ^b ±0.04
^a ∑SFAs	19.53 ^a	22.39 ^a	29.42 ^{bc}	28.73 ^b	32.2 ^c	31.7 ^{bc}	21.22 ^a
C16:1	3.3 ^a ±0.03	3.5 ^a ±0.6	3.7 ^{ab} ±0.14	4.2 ^{bc} ±0.18	4.6 ^c ±0.21	4.7 ^c ±0.7	3.6 ^a ±0.9
C18:1	22.3 ^a ±0.61	23.8 ^b ±0.41	24.6 ^c ±0.9	25.2 ^d ±0.27	29.8 ^e ±0.22	25.4 ^d ±0.17	22.6 ^a ±0.15
^b ∑MUFAs	25.7 ^a	27.4 ^b	28.4 ^b	29.5 ^c	34.5 ^d	30.2 ^c	26.3 ^a
C18:2	21.6 ^b ±0.32	22.1 ^{bc} ±0.6	22.5 ^{cd} ±0.41	22.9 ^d ±0.3	20.3 ^a ±0.63	28.4 ^a ±0.8	20.6 ^a ±0.6
C18:3	16.4 ^e ±0.32	14.8 ^d ±0.7	13.4 ^c ±0.5	12.8 ^b ±0.4	2.5 ^a ±0.9	2.7 ^a ±0.9	12.2 ^b ±0.6
C20:5	1.08 ^a ±0.81	1.2 ^{ab} ±0.3	1.04 ^a ±0.13	1.1 ^a ±0.23	1.02 ^a ±0.51	1.36 ^{ab} ±0.73	1.75 ^b ±0.25
C22:6	2.2 ^a ±0.31	2.2 ^a ±0.51	2.1 ^a ±0.93	2.1 ^a ±0.84	2.3 ^a ±0.23	2.2 ^a ±0.5	3.45 ^b ±0.71
^c ∑PUFAs	41.43 ^c	40.42 ^d	39.15 ^c	38.9 ^c	26.2 ^a	34.81 ^b	40.1 ^d
^d ∑n3	19.7 ^d	18.3 ^c	16.6 ^b	16.02 ^b	5.92 ^a	6.3 ^a	19.4 ^d

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

monounsaturated fatty acid (MUFAs) in fish tissues (Table 4 and 7) and its content increased with increasing content of oleic acid in the diet (Table 3). Both Palmitic and oleic acid were preferentially retained in all the tissues for both fish species (Tables 4, 5, 6, 7)

Total MUFAs varied significantly (P<0.05) across the diets with the highest recorded in diet 5 which was attributed to the high olive oil inclusion in the diet (Table 1). Diet 1 recorded low MUFAs, however, it had the highest diet and tissue total polyunsaturated fatty acids (PUFA) and n-3 fatty acids such as docosahexaenoic acid, DHA, (C22.6). Tilapia muscle DHA composition was significantly (P<0.05) high ranging between 4.4% to 12.2% (Table 4) compared to tilapia liver DHA composition of between 3.4% to 10.4% (Table 5).

The tilapia DHA values were relatively higher than that in catfish where muscle DHA values were between 2.6% and 10.4% (Table 6) and catfish liver DHA values range were between 1.94% and 9.7%. The difference in relative composition of DHA in fish tissues probably relate to tissue specific selective retention of fatty acids whereas the species differences in DHA deposition may relate to differences in fatty acid metabolism in tilapia and catfish. Tissue n3/n6 ratios were relatively higher than in the diets and the tissue ratios increased relative to the increased linseed oil in the diet (Table 1 and 3).

The higher n3/n6 ratios in tilapia muscles ranged between 0.63, in diet 6 to 2.1 in diet 1 (Table 4. Diet 1 had significantly higher n3/n6 values. Tilapia liver n3/n6

values ranged between 0.6-2.24 (Table 5). Relatively lower n3/n6 values were observed in catfish tissues; muscles 0.5-1.3 (Table 6) and liver, 0.4-1.29 (Table 7). The tissue composition of linoleic acid (C18:2) and α-Linolenic acid C18:3) were relatively lower in the fish tissues (Table 4, 5, 6, 7) compared to diet (Table 3) but varied relative to the inclusion of linseed and sunflower oil in the diet (Table 1 and 3).

Linseed oil was predominantly α-Linolenic acid and sunflower oil was predominantly linoleic acid (Figure 1). Their proportional variation in diet determined variation in C18.3 and C18:2 compositions in the diet respectively (Table 3). Interestingly, the tissue concentration of arachidonic acid (C20:4) were low relative to the reduction of C18:2 in the fish tissues (Tables 4, 5, 6, 7) in all experimental diets. Nevertheless, arachidonic values were significantly higher in tissues of fish fed diet 6 in both catfish and tilapia.

DISCUSSION

From this study, it was evident that dietary lipid composition (Table 3) influenced tissue lipids composition (Tables 4, 5, 6 and 7), an observation made in earlier studies (Ng *et al.*, 2003 ; Vientiane *et al.*, 2005; Ji *et al.*, 2011). Tissue linolenic acid (C18:3) and oleic acid (C18:1) composition reduced with reduction of linseed oil and olive oil composition in the diet respectively (Tables

Table 4. Muscles fatty acid composition (%) of tilapia fed on diets containing varied concentrations of linseed and olive oil.

	Dietary treatments						Commercial
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	
C10:0	0.2 ^a ±0.01	0.2 ^a ±0.06	0.3 ^a ±0.06	0.3 ^b ±0.01	1.2 ^b ±0.01	1.2 ^b ±0.07	0.2 ^a ±0.05
C12:0	0.5 ^a ±0.07	0.6 ^a ±0.5	0.7 ^a ±0.21	0.9 ^{ab} ±0.03	1.4 ^{bc} ±0.21	1.6 ^c ±0.05	0.8 ^a ±0.17
C14:0	1.5 ^a ±0.64	1.7 ^a ±0.73	2.5 ^{bc} ±0.21	3.2 ^{de} ±0.44	3.6 ^e ±0.50	2.8 ^{cd} ±0.22	2.1 ^{ab} ±0.27
C16:0	10.7 ^a ±0.21	10.7 ^a ±0.67	11.3 ^b ±0.42	13.2 ^c ±0.57	15.2 ^e ±0.53	14.7 ^d ±0.53	12.9 ^c ±0.43
C18:0	5.9 ^a ±0.4	5.9 ^d ±0.97	4.7 ^c ±0.13	4.1 ^b ±0.07	4.2 ^{bc} ±0.32	2.15 ^a ±0.01	4.7 ^c ±0.02
C20:0	2.2 ^d ±0.01	1.7 ^{bcd} ±0.8	1.3 ^{ab} ±0.6	1.1 ^a ±0.9	0.9 ^a ±0.13	1.4 ^{abc} ±0.46	1.9 ^{cd} ±0.6
^a ∑SFA	21.3 ^a	21 ^a	21.1 ^a	23.03 ^a	26.8 ^b	24.05 ^{ab}	22.7 ^a
C14:1	0.7 ^a ±0.06	0.7 ^a ±0.36	0.65 ^a ±0.14	0.5 ^a ±0.11	0.7 ^a ±0.48	0.6 ^a ±0.72	0.7 ^a ±0.44
C16:1	0.9 ^a ±0.13	1.4 ^{ab} ±0.25	1.5 ^b ±0.02	1.7 ^b ±0.34	1.6 ^b ±0.9	1.4 ^{ab} ±0.54	1.3 ^{ab} ±0.25
C18:1	17.3 ^a ±0.5	18.5 ^b ±0.42	21.3 ^c ±0.24	23.1 ^d ±0.26	30.5 ^f ±0.27	27.1 ^e ±0.13	23.4 ^d ±0.5
^b ∑MUFA	19.1 ^a	20.78 ^b	23.5 ^c	25.34 ^d	32.8 ^f	29.19 ^e	25.5 ^d
C18:2	11.6 ^e ±0.3	11.1 ^d ±0.16	10.7 ^{bc} ±0.42	10.5 ^b ±0.18	9.8 ^a ±0.14	15.9 ^f ±0.23	10.6 ^{cd} ±0.34
C20:4	2.2 ^a ±0.3	2.4 ^{ab} ±0.21	2.4 ^{abc} ±0.17	2.8 ^{cd} ±0.18	3.1 ^d ±0.24	3.1 ^d ±0.22	2.1 ^a ±0.13
∑n6	13.9 ^d	13.5 ^c	13.2 ^{bc}	13.4 ^c	12.9 ^{ab}	19 ^e	12.7 ^a
C18:3	9.1 [±] 0.3	8.05 ^a ±0.18	7.7 ^{de} ±0.23	6.5 ^e ±0.13	2.2 [±] 0.52	2.9 ^b ±0.81	7.3 ^d ±1.1
C20:5	8.5 [±] 0.2	6.4 ^d ±0.13	6.2 ^d ±0.3	5.3 ^c ±0.24	2.9 [±] 0.6	3.6 ^b ±0.5	5.9 ^d ±0.4
C22:6	12.2 ^d ±0.8	11.3 ^c ±0.32	11.2 ^c ±0.51	11.3 ^c ±0.7	4.4 ^a ±0.94	5.4 ^b ±0.21	11.5 ^c ±0.61
^d ∑n3	29.7 ^e	25.8 ^d	25.2 ^d	23.4 ^c	9.6 ^a	12.1 ^b	24.8 ^d
^c ∑PUFA	43.7 ^f	39.3 ^e	37.4 ^d	36.6 ^c	22.5 ^a	31.1 ^b	37.5 ^d
n3/n6	2.1 ^f	1.9 ^e	1.8 ^d	1.7 ^c	0.7 ^b	0.6 ^a	1.9 ^e

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, C20:4 Arachidonic acid, C20:5 Eicosapentaenoic acid, C22:6 docosahexaenoic acid. ^a∑SFA: Total saturated fatty acids ^b∑MUFA: Total monounsaturated fatty acids ^c∑PUFA: Total Polyunsaturated fatty acids. ^d∑n3: Total omega-3 fatty acids, ∑n6: Total omega 6 fatty acids

4,5,6, and 7). Oleic acid (C18:1) was the dominant fatty acid in all tissues in both catfish and tilapia. Palmitic acid was the dominant saturated fatty acid in both diets and fish tissues.

This study confirms earlier studies that both C18:1 and C16:0 are preferentially retained in tissues and their retention may be related to energy storage as both of these two fatty acids are preferred substrates for β-oxidation in fish (Torstensen *et al.*, 2000, Kowalska *et al.*, 2010a).

The concentrations of saturated fatty acids (SFA) were lower than that of polyunsaturated fatty acid (Tables 4,5,6 and 7) in all experimental tissues partially confirming report by Menoyo *et al.*, (2005) that linseed oil offer low SFA concentration. The composition of omega-3 fatty acids, especially docosahexaenoic acid (DHA), C22:6, increased with the increased composition of linseed in the diet in all the tissues (Tables 4,5,6 and 7). There was a selective deposition and retention of DHA in muscles of both tilapia and catfish at a concentration higher than that in the diets (Table 3). The retention trend in this study partially confirm reports in Atlantic salmon (Bell *et al.*, 2003), rainbow trout (Caballero *et al.*, 2002), African catfish (Ng *et al.*, 2003) and turbot (Regost *et al.*, 2003). Findings in this study are also consistent with report by Li *et al.*, (2016) that increased inclusion of linseed in tilapia feed significantly increased DHA levels and that feeding diets

containing linseed oil can increase EPA/DHA content in tilapia (Justi, *et al.*, 2003; Toniai *et al.*, 2009). Bell (2001) suggested that the underlying mechanism for this selective retention of DHA include high specificity of fatty acyl transferases for DHA and relative resistance of DHA to beta-oxidation due to its complex metabolic pathway. Agata *et al.*, (2012) reported DHA composition of 17.1% with 6.7% inclusion of linseed oil in pikeperch fed for 56 days. Molnar *et al.*, (2012) observed 11.39% DHA composition in tilapia fillet fed 5% linseed diet inclusion for 42 days compared to 14.1% DHA composition in tilapia fed fish oil inclusion for the same period of time. In this study, DHA composition of 4.4-12.2% was recorded in tilapia muscle (Table 4), 3.4-10.8% in tilapia liver (Table 5), 2.6-10.4% in catfish muscles (Table 6) and 1.94-9.7% in catfish liver (Table 7) at linseed oil inclusion of 14% for a feeding period of 150 days. In addition, the DHA values from this study partially compare with reports by Menoyo *et al.*, (2005) who observed DHA value of 13.09% on replacement of linseed oil with fish oil at 100% inclusion in Atlantic salmon as well as study by Aguiar *et al.*, (2011) who reported a DHA value of 12.5% in tilapia head with linseed oil diet inclusion at 5% for a feeding period of 150 days. On the contrary, the aforementioned findings are inconsistent with report by Turchini *et al.*, (2011) that there is a decreased concentration of n3 PUFA when diets of greater linseed oil is fed to fish.

Table 5. Liver fatty acid composition (%) of tilapia fed on diets containing varied concentrations of linseed and olive oil.

	Dietary treatment						
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Commercial
C10:0	0.7 ^a ±0.2	0.3 ^a ±0.1	0.4 ^a ±0.1	0.4 ^a ±0.1	1.8 ^b ±0.6	1.4 ^b ±0.1	0.2 ^a ±0.2
C12:0	0.4 ^a ±0.62	0.6 ^{ab} ±0.6	0.9 ^b ±0.21	1.1 ^b ±0.5	1.6 ^c ±0.1	2.0 ^c ±0.04	1.0 ^b ±0.1
C14:0	1.5 ^a ±0.3	1.7 ^a ±0.2	1.9 ^a ±0.24	3.7 ^d ±0.15	4.1 ^d ±0.7	3.1 ^c ±0.13	2.4 ^b ±0.34
C16:0	10.4 ^a ±0.56	11.05 ^a ±0.7	11.1 ^a ±0.37	13.8 ^b ±0.8	15.4 ^c ±0.6	14.9 ^c ±0.21	13.5 ^b ±0.3
C18:0	7.1 ^a ±0.31	5.4 ^e ±0.2	5.1 ^{de} ±0.2	3.5 ^b ±0.4	4.6 ^{cd} ±0.2	2.2 ^a ±0.73	4.2 ^c ±0.5
C20:0	2.1 ^c ±0.3	1.7 ^{bc} ±0.2	1.5 ^{bc} ±0.2	1.3 ^{ab} ±0.6	0.8 ^a ±0.6	1.3 ^{ab} ±0.41	1.6 ^{bc} ±0.9
^a ΣSFAs	21.76 ^a	20.95 ^a	20.8 ^a	24 ^{ab}	28.49 ^c	24.9 ^b	23.1 ^{ab}
C14:1	0.8 ^{ab} ±0.01	0.7 ^{ab} ±0.2	0.7 ^{ab} ±0.2	0.4 ^a ±0.13	0.8 ^{ab} ±0.6	0.9 ^{ab} ±0.19	1.1 ^b ±0.30
C16:1	0.8 ^a ±0.65	1.5 ^{bc} ±0.1	1.6 ^{bc} ±0.13	1.8 ^c ±0.04	2.4 ^d ±0.6	2.1 ^{cd} ±0.6	1.3 ^{ab} ±0.12
C18:1	16.2 ^a ±1.62	21.0 ^b ±0.1	22.3 ^c ±0.81	23.4 ^d ±0.18	31.1 ^e ±0.8	27.4 ^e ±0.18	23.2 ^d ±0.23
^b ΣMUFAS	17.9 ^a	23.3 ^b	24.6 ^{bc}	25.7 ^c	34.3 ^e	30.4 ^e	25.6 ^c
C18:2	10.4 ^d ±0.4	9.9 ^c ±0.14	9.7 ^b ±0.3	9.3 ^a ±0.4	7.4 ^a ±0.9	15.6 ^e ±0.51	8.8 ^b ±0.61
C20:4	3.0 ^b	3.3 ^b	3.7 ^c	4.0 ^d	2.4 ^a	4.5 ^e	2.6 ^a
Σn6	13.5 ^c	13.3 ^c	13.5 ^c	13.4 ^c	9.9 ^a	20.2 ^d	11.5 ^b
C18:3	8.6 ^d ±0.18	8.3 ^d ±0.4	7.6 ^d ±0.7	6.5 ^d ±0.16	2.1 ^a ±0.6	2.5 ^d ±0.8	7.6 ^c ±0.11
C20:5	7.7 ^e ±0.3	6.5 ^d ±0.12	6.4 ^d ±0.7	5.8 ^c ±0.19	3.1 ^a ±0.5	3.7 ^b ±0.17	6.4 ^d ±0.13
C22:6	10.8 ^c ±0.91	10.9 ^c ±0.4	10.5 ^c ±0.21	10.6 ^c ±0.6	3.4 ^a ±0.2	4.9 ^b ±0.31	10.6 ^c ±0.23
^d Σn3	27.2 ^d	25.8 ^{de}	24.6 ^d	23.1 ^c	8.5 ^a	11.2 ^b	24.7 ^d
^c ΣPUFAS	41.7 ^e	40.1 ^d	39.1 ^d	37.4 ^c	19.4 ^a	32.4 ^b	37.2 ^d
n3/n6	2.09 ^d	2.01 ^d	1.9 ^c	1.79 ^c	0.9 ^b	0.60 ^a	2.24 ^e

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, C20:4 Arachidonic acid, C20:5 Eicosapentaenoic acid, C22:6 docosahexaenoic acid. ^aΣSFAs: Total saturated fatty acids ^bΣMUFAs: Total monounsaturated fatty acids ^cΣPUFAs: Total Polyunsaturated fatty acids. ^dΣn3: Total omega-3 fatty acids, Σn6: Total omega 6 fatty acids

The total n-3 fatty acids were higher in tilapia muscles (9.6%-29.7%) than in catfish muscles (8.7%-22.6%) (Table 4 and 6) which indicate a probable more efficiency in conversion of C₁₈ PUFAS to HUFAS in tilapia. This finding is partially consistent with the study by Kwategyeka *et al.*, (2008), that wild tilapia had more n-3 PUFA (31.2%-32.0%) than catfish (24.0%-24.6%), however, the values found in this study were lower. The difference in n-3 composition between catfish and tilapia in this study is probably related to the difference in lipid storage capacity in their tissues and also preferences in selective retention and mobilization of specific fatty acids (Mourente and Bell, 2006).

Studies indicate that some fish species can bio-convert C18:3 and C18:2 into highly polyunsaturated fatty acids and that physiological accumulation of fatty acids in fish tissues occur when dietary supply of the same is high (Stubhaug *et al.*, 2005).

In addition, the extent at which fish can desaturase/elongate C₁₈ polyunsaturated fatty acid, PUFA to highly polyunsaturated fatty acids, HUFA vary with species (Sargent *et al.*, 2002). Notably, most marine species are carnivorous and considered to have weak capacity to bio-convert C₁₈ PUFAS to HUFAS and thus require preformed HUFAs in their diet (NRC, 2011). This is inconsistent with findings in this study where catfish, a carnivorous fish species, recorded substantial DHA

composition (Tables 6 and 7). The length of feeding time determines the incorporation of n3 PUFA into fish fillet (Justi *et al.*, 2003). Toniai *et al.*, (2009), established that the shortest time period required for inclusion of linseed oil into adult fish muscle to raise n3 PUFA is 45 days. The feeding period in this study was 150days which was way above the established feeding period (Toniai *et al.*, 2009).

In this study, there was no significant difference ($p<0.05$) in the n3/n6 ratios between commercial diet and diet 1 in both catfish tissues (Tables 6 and 7) partially confirming a report by Molner *et al.*, (2012) that n3/n6 ratios in fish fed linseed oil are not significantly different from fish fed diets containing fish oil. The n3/n6 ratios between commercial diet and diet 1 varied significantly in the tilapia tissues (Tables 4 and 5). However, tilapia tissue n3/n6 ratios (Tables 4 and 5) reported in this study were lower than values reported in earlier studies on European sea bass, 3.26 (Mourente and Bell, 2006), Atlantic salmon, 4.59 neutral lipids and 6.72 polar lipids (Menoyo, 2005), and pike perch, 3 (Kowalska *et al.*, 2013). EPA composition was the lowest among the n-3 fatty acids in all the experimental subjects and diets. This finding is consistent with earlier study that EPA may selectively be used as a substrate for β -oxidation (Karapanogiotidits *et al.*, 2007) or converted into DHA (Sprecher, 2000) resulting into its apparent low composition in the tissues. Linoleic acid

Table 6. Muscles fatty acid composition (%) of catfish fed on diets containing varied concentrations of linseed and olive oil.

Diets	Dietary treatments						Commercial
	Diet1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	
C10:0	0.2 ^a ±0.16	0.3 ^a ±0.02	0.4 ^a ±0.03	1.1 ^b ±0.17	0.3 ^a ±0.03	0.3 ^a ±0.07	0.5 ^{ab} ±0.13
C12:0	0.5 ^a ±0.26	0.8 ^{ab} ±0.18	0.9 ^{ab} ±0.7	1.1 ^d ±0.3	1.6 ^c ±0.02	1.6 ^c ±0.08	0.5 ^a ±0.2
C14:0	1.12 ^a ±0.8	1.3 ^{ab} ±0.7	1.6 ^{bc} ±0.8	2.1 ^{cd} ±0.3	3.6 ^e ±0.25	3.5 ^e ±0.5	2.2 ^d ±0.4
C16:0	11.4 ^a ±0.49	12.3 ^b ±0.54	15.3 ^c ±0.4	16.3 ^d ±0.8	11.6 ^a ±0.6	16.1 ^d ±0.4	12.6 ^b ±0.31
C18:0	6.5 ^f ±0.3	6.3 ^f ±0.43	5.8 ^{de} ±0.1	5.5 ^d ±0.2	4.43 ^c ±0.14	3.3 ^b ±0.2	2.5 ^a ±0.06
C20:0	1.8 ^c ±0.01	1.4 ^{bc} ±0.6	1.3 ^{abc} ±0.9	1.3 ^{ab} ±0.4	1.3 ^{abc} ±0.3	1.1 ^{ab} ±0.08	0.8 ^a ±0.1
^a ∑SFAs	21.9 ^{ab}	22.5 ^{bc}	25.6 ^{cde}	27.5 ^e	23.12 ^{bcd}	26.02 ^{de}	19.35 ^a
C14:1	0.9 ^{abc} ±0.03	0.7 ^{abc} ±0.3	0.6 ^{ab} ±0.2	0.5 ^a ±0.12	1.1 ^{bc} ±0.25	1.2 ^c ±0.18	0.9 ^{abc} ±0.04
C16:1	1.4 ^c ±0.12	2.4 ^d ±0.02	2.6 ^d ±0.6	3.3 ^e ±0.14	1.1 ^{bc} ±0.25	0.2 ^a ±0.01	0.7 ^{ab} ±0.12
C18:1	17.4 ^a ±0.63	19.7 ^b ±0.5	26.1 ^e ±0.46	28.6 ^f ±0.21	32.43 ^g ±0.9	22.9 ^c ±0.6	24.8 ^d ±0.32
^b ∑MUFAS	19.81 ^a	22.9 ^b	29.2 ^e	32.5 ^f	34.8 ^g	24.4 ^c	26.5 ^d
C18:2	14.9 ^d ±1.4	14.8 ^d ±1.2	14.7 ^d ±1.57	12.8 ^b ±0.8	9.3 ^a ±0.12	17.9 ^e ±0.3	13.7 ^c ±0.8
C20:4	1.9 ^a ±0.32	2.2 ^b ±0.14	2.4 ^c ±0.27	2.4 ^c ±0.17	2.5 ^c ±0.23	3.1 ^d ±0.3	2.3 ^b ±0.21
∑n6	16.9 ^d	17.1 ^d	17.2 ^d	15.3 ^b	11.9 ^a	21.1 ^e	16.1 ^c
C18:3	7.9 ^e ±0.7	6.3 ^d ±1.13	6.1 ^d ±0.8	4.4 ^c ±0.13	2.6 ^a ±0.5	3.2 ^b ±0.12	6.4 ^d ±0.2
C20:5	4.2 ^{bc} ±0.46	4.5 ^{cd} ±0.83	4.4 ^{cd} ±0.4	3.8 ^{ab} ±0.4	3.4 ^a ±0.13	3.5 ^a ±0.6	4.9 ^f ±0.6
C22:6	10.4 ^f ±0.28	9.5 ^{de} ±0.58	9.1 ^d ±0.3	7.8 ^c ±0.7	2.6 ^a ±0.35	3.7 ^{ab} ±0.23	9.8 ^e ±0.53
^d ∑n3	22.63 ^f	20.41 ^{de}	19.5 ^d	16.6 ^c	8.72 ^a	10.51 ^b	21.21 ^e
^c ∑PUFAS	39.5 ^e	37.5 ^d	36.7 ^c	31.9 ^b	20.6 ^a	31.6 ^b	37.3 ^{cd}
n3/n6	1.34 ^e	1.19 ^d	1.13 ^c	1.08 ^c	0.73 ^b	0.5 ^a	1.32 ^e

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, C20:4 Arachidonic 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

(C18:2) and linolenic acid (C18:3) values in the fish tissues (Tables 4, 5, 6, 7) were lower than values in the experimental diets (Table 3) suggesting their selective utilization when present at high concentrations (Ng *et al.*, 2003). In this study, the concentrations of DHA, a metabolic product of C18:3, significantly increased in the fish tissue suggesting a possible conversion of C18:3 to DHA. However, we were not able to accurately account for low tissue concentration of arachidonic acid (C20:4), a desaturation product of C18:2 (Tables 4, 5, 6, 7). However, these low values of C20:4 relative to the dietary C18:2 was recently reported (Li *et al.*, 2016) and attributed to a number of roles played by C20:4 including its role in eicosanoids formation and resistance to stressors which are prevalent under intensive culture system (Bell and Sargent, 2003; Li *et al.*, 2016).

C18:2 and C18:3 are metabolized by same sequential desaturation and elongation enzymes (Visentainer, 2007) thus excessive dietary supply may create selective competition disrupting their bioconversion and resulting into low or physiological tissue levels of C20:4 (Ruyter *et*

al., 2006, Li *et al.*, 2016). In conclusion, linseed still remain the vegetable oil that can possibly replace fish oil in tilapia and catfish feeds. Increased linseed oil in diet improved fish tissue DHA and n3/n6 ratio pointing its relevance in fish feeds. Worth noting, is the dynamics of results obtained from different studies for same and/or different fish species. This suggest that factors influencing tissue fatty acids are not limited to dietary lipids and feeding period and that particular studies are unique in their own ways.

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Table 7. Liver fatty acid composition (%) of catfish fed on diets containing varied concentrations of linseed and olive oil.

	Dietary treatments						
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Commercial
C10:0	0.2 ^a ±0.03	0.3 ^a ±0.03	0.4 ^{ab} ±0.1	0.8 ^b ±0.13	0.2 ^a ±0.01	0.4 ^{ab} ±0.04	0.4 ^{ab} ±0.11
C12:0	0.5 ^{ab} ±0.03	0.6 ^{abc} ±0.4	0.8 ^{bcd} ±0.8	1.1 ^{cd} ±0.12	1.7 ^e ±0.01	1.3 ^{de} ±0.09	0.2 ^a ±0.03
C14:0	0.9 ^a ±0.2	1.2 ^a ±0.3	1.3 ^{ab} ±0.07	1.7 ^{bc} ±0.02	1.8 ^c ±0.25	1.7 ^{bc} ±0.04	2.8 ^d ±0.26
C16:0	10.6 ^a ±2.2	11.8 ^b ±0.8	14.8 ^d ±0.28	15.4e±0.81	10.6a±0.26	15.6 ^e ±0.73	13.4 ^c ±0.13
C18:0	6.8 ^d ±0.3	6.4 ^{cd} ±0.1	6.1 ^{bc} ±0.17	5.9 ^{bc} ±0.02	5.5 ^b ±0.3	5.5 ^b ±0.63	1.6 ^a ±0.12
C20:0	2.1 ^c ±0.3	1.7 ^{bc} ±0.03	1.3 ^b ±0.8	1.7 ^{bc} ±0.2	1.4 ^b ±0.4	0.7 ^{bc} ±0.01	0.4 ^a ±0.01
^a ΣSFAs	21.3 ^a	22.2 ^{ab}	24.9 ^{bc}	26.8 ^c	21.5 ^a	25.4 ^c	19.12 ^a
C14:1	1.1 ^{ab} ±0.6	0.7 ^a ±0.04	0.6 ^a ±0.12	0.5 ^a ±0.06	0.7 ^a ±0.28	0.7 ^a ±0.34	1.4 ^b ±0.23
C16:1	1.3 ^{bc} ±0.21	2.1 ^d ±0.25	2.5 ^{de} ±0.72	2.7 ^e ±0.51	0.9 ^b ±0.48	0.2 ^a ±0.02	1.4 ^{bc} ±0.21
C18:1	16.3 ^a ±0.15	19.1 ^b ±0.75	25.1 ^d ±0.92	27.2 ^e ±0.21	33.8 ^a ±0.13	20.5 ^c ±0.31	24.6 ^d ±0.61
^b ΣMUFAS	18.8 ^a	21.8 ^b	28.3 ^c	30.5 ^d	35.5 ^e	21.6 ^b	27.4 ^c
C18:2	15.5 ^d ±0.25	14.6 ^c ±0.33	14.4 ^c ±0.72	13.7 ^b ±0.4	11.2 ^a ±0.34	21.5 ^e ±0.12	15.3 ^d ±0.31
C20:4	1.9 ^a ±0.21	2.1 ^{ab} ±0.13	2.3 ^b ±0.3	2.6 ^b ±0.26	2.8 ^c ±0.17	3.2 ^d ±0.23	1.7 ^a ±0.11
Σn6	17.5 ^c	16.7 ^b	16.8 ^{bc}	16.4 ^b	14.3 ^a	24.8 ^d	17.1 ^c
C18:3	7.4 ^e ±0.12	6.7 ^d ±0.63	5.2 ^d ±0.15	4.8 ^d ±0.8	2.2 ^d ±0.12	3.5 ^d ±0.9	7.1 ^{de} ±0.4
C20:5	4.4 ^b ±0.32	4.3 ^b ±0.51	4.2 ^b ±0.3	4.3 ^b ±0.7	3.5 ^a ±0.17	3.1 ^a ±0.51	4.3 ^b ±0.21
C22:6	9.7 ^f ±0.34	8.2 ^{de} ±0.81	7.7 ^{cd} ±0.21	7.5 ^c ±0.37	1.9 ^a ±0.47	3.1 ^b ±0.91	8.3 ^e ±0.31
^d Σn3	21.6 ^e	19.3 ^d	17.2 ^c	16.7 ^c	7.7 ^a	9.7 ^b	19.7 ^d
^c ΣPUFAS	40.1 ^e	37.1 ^d	35.1 ^c	34.1 ^b	23.1 ^a	35.5 ^c	37.8 ^d
n3/n6	1.29 ^e	1.22 ^d	1.08 ^c	1.08 ^c	0.61 ^b	0.43 ^a	1.21 ^d

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, C20:4 Arachidonic acid, C20:5 Eicosapentaenoic acid, C22:6 docosahexaenoic acid. ^aΣSFAs: Total saturated fatty acids^bΣMUFAs: Total monounsaturated fatty acids ^cΣPUFAs: Total Polyunsaturated fatty acids. ^dΣn3: Total omega-3 fatty acids, Σn6: Total omega 6 fatty acids

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