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RESEARCH PAPER

Fish Oil Replacement with Virgin Coconut and Corn Oil: Impact on Growth Performance, Lipogenic and Digestive Enzyme Activity, and mRNA Expression of Genes Involved in Lipid Metabolism of Nile tilapia (*Oreochromis niloticus*)

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Abstract

In relation to mRNA expressions, digestive and hepatic enzyme activity, growth performances of *O. niloticus* (6.07±0.07) was evaluated after feeding 56 days. Fish were fed six (6) experimental diets where fish oil (FO) supplements dietary oils; virgin coconut oil (VO) {3%FO+3%VO; FVO}, and corn oil (CO) {3%FO+3%CO; FCO}, 6%VO (VO), 6%CO (CO) and 6%VCO {3%VO+3%CO; VCO}. Those fed FCO recorded higher WG (1334.10±5.53) and SGR (4.76±0.01). Muscle lipid was significantly higher in group VO than CO and VCO whereas, it was significantly lower in the liver than the control group ($P<0.05$). Dietary alternatives influenced digestive activities (amylase, lipase and trypsin) of which significant differences were not obtained between sections. Digestive enzymes lipase and pepsin were not significantly different at the posterior intestine. Liver receptors (FABP4 and PPAR-α) involved in fatty acid (FA) transport was not influenced, unlike CD36, G6PD and 6PGD. Although, the expression of PPAR-α gene was not different between groups, with the exception of group VCO, all other groups had no significant difference in expression levels of CD36, G6PD and 6PGD unlike FABP4. The study confirms that dietary vegetable lipids in partial replacement improve growth performances and maintain fish physiological functioning with preferences for utilization of 18: 3n-3 and 18: n-n9. Enzyme activity also positively correlates with growth rates and indicates good lipid metabolic activities and retention. As such, dietary alternatives influence liver enzyme activity and their mRNA expressions.

Introduction

Dietary lipids have a reflective effect on the expression of genes which leads to changes in cell differentiation, growth and metabolism apart from playing a role in membrane lipid composition and as energy source (Bravo-Ruiz et al., 2021; Martinez-Rubio et al., 2013). However, due to the higher demand of fish oil from various sectors and increase in aquaculture activities, it has become limited and as such other lipid sources need to be explored (Filipa-Silva et al., 2023; Kazemi et al., 2016).

Vegetable oils have been deemed as the best to replace fish oil as they have been investigated on most fishes and currently being incorporated in some commercial feeds with less effect on growth performance (Huang et al., 2016; Ng & Gibbon, 2010). However, these alternative lipid sources are generally deficient in some essential fatty acids (FA) (LC-PUFA: EPA, 20:5n-3; DHA, 22:6n-3), and as such alter the final

FA profile of the fillets (Trushenski et al., 2012; Szabó et al. 2011). Vegetable lipids are also assumed to be deposited mostly in the liver and other tissues. This phenomenon is linked to lipolysis and lipogenesis which involves the activities of some transcriptional and enzymatic factors (Saponaro et al., 2015; Zheng et al. 2013).

Key enzymes which account for fatty acids (FAs) transport are the hepatic lipase (HL) and lipoprotein lipase (LPL). The delivery process involves the hydrolysis of triglyceride (TG) on corresponding lipoproteins (Chen et al., 2024; Geng et al., 2021). These enzymes include peroxisome proliferator-activated receptor α (PPAR-α), Fatty acid translocate/cluster of differentiation 36 (CD36) and Fatty acid binding protein 4 (FABP4), etc. Hepatic gene expressions which are controlled by fatty acids are involved in the desaturation and elongation of the FAs (Geng et al., 2021; Kühn et al., 2018).

This study is hypothesizing that, difference in FA may lead to lipid accumulation and therefore replacing FO with dietary vegetable oils levels will have impact on the transport of lipids and Fatty acids in *O. niloticus*.

Tilapia (*Oreochromis niloticus*) is considered a suitable species for aquaculture worldwide owing to its ability to grow rapidly, high disease and stress resistance (Deng et al. 2010). Tilapia although have been noted to be capable of utilizing n-6 FAs, it still requires a certain number of n-3 FAs to enhance growth performance for maximum yield. This has resulted in several studies to establish its nutritional requirement especially on their FA (n6 FA) requirements and optimizing diet formulation. Although several studies have been conducted, the molecular mechanism responsible for changes in lipid deposition due to replacement of fish oil with virgin coconut oil (VO) and corn oil (CO) has not been studied extensively.

As such, an investigation into the correlation between gene expression and FA metabolism in the liver will present a deep understanding on the regulatory effects of vegetable oils in *O. niloticus*.

The aim of this study is therefore to assess the mechanism of hepatic lipid metabolism in *Oreochromis niloticus* juveniles. In this study, the effects of replacing fish oil with virgin coconut oil and corn oil on the growth performance, enzymatic activities and mRNA expressions was evaluated. The results will be beneficial for us to elucidate the molecular basis of FA transport in fish, with the prime aim being to determine mechanisms for optimizing the use of VO and CO in Nile tilapia culture.

Material and Methods

Feed and Feeding Trial

Six experimental diets (Table 1) were formulated for eight weeks feeding trial. Fish meal, soybean meal and rapeseed meal were used as the main protein sources, while virgin coconut oil (VO: rich in SFA), corn oil (CO: rich in PUFA-ALA and LA) and fish oil (FO: rich in LC-PUFA) were used as the sources of lipid as shown on Table 2. The alternative lipids replaced fish oil (control; FO) at 50% (FVO, FCO) and 100% (VO, CO), 50%VO+50%CO (VCO) and this was strategically chosen to generate distinct feed FA profiles for the purpose of testing the efficacy of their FA profile on the fish. All dry ingredients (Defatted Fish meal, soybean meal, rapeseed meal, wheat meal, mineral mix and vitamin mix) were mixed using the progressive enlargement method.

Table 1. Dietary ingredients and proximate composition of experimental diets with different lipid sources

Lipids/components (%)	FO	FVO	FCO	VO	CO	VCO
Fish meal	6.00	6.00	6.00	6.00	6.00	6.00
Soybean meal	30.00	30.00	30.00	30.00	30.00	30.00
Rapeseed meal	30.00	30.00	30.00	30.00	30.00	30.00
Wheat meal	22.95	22.95	22.95	22.95	22.95	22.95
Fish oil	6.00	3.00	3.00	-	-	-
Virgin coconut oil	-	3.00	-	6.00		3.00
Corn oil	-		3.00		6.00	3.00
Vitamin & mineral premix	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50
Inositol	0.05	0.05	0.05	0.05	0.05	0.05
Ca(H2PO4)	1.50	1.50	1.50	1.50	1.50	1.50
Soybean phospholipids	2.00	2.00	2.00	2.00	2.00	2.00
TOTAL	100	100	100	100	100	100
			Proximate Composition (%)			
Crude Protein	32.49	32.49	32.49	32.49	32.49	32.49
Crude Lipid	6.73±0.24	6.86±0.36	6.83±0.18	6.80±0.06	6.96±0.01	6.31±0.15
Feed Moisture	9.84±0.15	9.19±0.70	9.50±0.70	9.50±0.70	9.10±0.18	9.93±0.29

All values are similar to recommendations made by NRC, 2011.

Mineral mix (mg kg-1 dry diet): Cu (CuSO4), 2.0; Zn (ZnSO4), 34.4; Mn (MnSO4), 6.2; Fe (FeSO4), 21.1; I (Ca (IO3)2), 1.63; Se (Na2SeO3), 0.18; Co (CoCl2), 0.24; Mg (MgSO4.H2O), 52.7. Vitamin premix (IU or mg kg-1 diet): vitamin A, 16000 IU; vitamin D, 8000 IU; vitamin K, 14.72; thiamin, 17.8; riboflavin, 48; pyridoxine, 29.52; cynocobalamine, 0.24, tocopherols acetate, 160; ascorbic acid (35%), 800; niacinamide, 79.2; calcium-D-pantothenate,73.6; folic acid, 6.4; biotin, 0.64; inositol, 320; choline chloride, 1500; L-carnitine, 100.

All ingredients were purchased from Yuehai Feed Mill, Zhejiang, China. Virgin coconut oil was purchased from the Philippines.

The mixed dry ingredients were then homogenized with virgin coconut oil, corn oil and fish oil and distilled water in a Hobart mixer, and the resulting moist dough pelleted using a pellet extruder through a 1-mm die. The pelleted diets were wet extruded, air dried, broken up and sieved into proper pellet size. All experimental diets were stored frozen (-20°C) throughout the duration of the study (Ng et al., 2000).

Experimental Procedures

Seven hundred and fifty (750) fingerlings (6.07±0.07g) of *Oreochromis niloticus* were acquired and transported to an aquarium facility at Shanghai Ocean University, China. Fingerlings were acclimatized for a week and fed twice daily with a commercial diet to apparent satiation. The feed was obtained from Shanghai Jin Yuan Trade which contains 30% crude protein.

Fingerlings were starved for 24h prior to feeding trials, weighed and then distributed randomly into eighteen (18) cages (2.0m x 1.0m x 1.0m) at a stocking density of 40 fish per cage. Prior to the feeding trial, fifteen (15) fish from a general pool of fish were sampled randomly and stored at -80°C for determination of initial whole body proximate composition.

Triplicate groups of fish were fed their respective diets by hand to apparent satiation twice daily (08:00h and 16:00h) for 56 days feeding regime.

To allow for accurate measurements in weight gain, fishes were starved and fifteen (15) from each tank was randomly sampled on every two weeks (fifteenth day).

At the end of the feeding trial on the 56th day, all surviving fish were counted and weighed after starvation for 24h and sampled for various indices to be checked.

Sample Collection and Growth Performance

Five fish per tank at the end of the trial were randomly sampled, euthanized with an overdose of tricaine methane sulfonate (MS-222 at 200mg/L in culture water), weighed individually, pooled and stored at -80°C for subsequent determination of proximate composition. Digestive tract and liver samples were taken, pooled and stored at -80°C for subsequent lipogenic enzyme activity and mRNA expression.

The following growth parameters calculated were:

$$\text{Weight gain}, WG = [\frac{100(final\ weight - initial\ weight)}{initial\ weight}],$$

Feed conversion ratio, FCR defined as food ingested by generated biomass:

$$FCR = \frac{feed\ intake,\ \ FI(g)}{weight\ gain\ (g)}$$

Table 2. Fatty acid (FA) profiles (% total FA) of experimental diets

Fatty Acids (% total)	FO	FVO	FCO	VO	CO	VCO
8:0	0.00±0.00	0.59±0.06	0.00±0.00	1.32±0.05	0.00±0.00	0.60±0.00
10:0	0.00±0.00	0.76±0.05	0.00±0.00	1.69±0.05	0.00±0.00	0.75±0.00
12:0	0.00±0.00	8.24±0.37	0.00±0.00	18.02±0.32	0.06±0.06	8.07±0.02
14:0	0.61±0.00	4.35±0.07	0.43±0.02	9.04±0.09	0.27±0.01	4.03±0.01
16:0	10.40±0.02	11.02±0.06	10.25±0.19	12.30±0.29	9.99±0.02	10.68±0.06
18:0	3.02±0.05	3.10±0.04	2.41±0.04	2.97±0.07	1.68±0.00	2.16±0.02
20:0	0.29±0.01	0.24±0.01	0.31±0.00	0.00±0.00	0.32±0.00	0.25±0.00
22:0	0.23±0.00	0.20±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
∑ SFA's	14.54±0.05	28.49±0.06	13.44±0.04	45.35±0.06	12.34±0.04	26.59±0.04
16:1(n-7)	1.29±0.01	0.91±0.01	0.94±0.07	0.80±0.11	0.58±0.01	0.60±0.01
18:1(n-7)	3.18±0.02	2.83±0.07	3.00±0.04	2.92±0.20	2.88±0.01	2.98±0.06
18:1(n-9)	22.24±0.09	20.50±0.11	25.51±0.25	18.57±0.15	28.03±0.04	23.76±.0.02
20:1(n-9)	0.51±0.01	0.42±0.01	0.50±0.00	0.37±0.02	0.40±0.00	0.34±0.01
22:1(n-9)	0.32±0.01	0.23±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
∑MUFAs	27.48±0.10	24.89±0.19	29.95±0.22	22.68±0.43	31.88±0.02	27.69±0.04
18:2(n-6)	45.48±0.67	37.45±0.09	47.39±0.81	26.59±0.24	50.28±0.08	40.39±0.08
18:3(n-6)	6.49±0.12	5.14±0.12	4.39±0.07	3.15±0.02	2.80±0.00	2.95±0.00
Total n-6	51.97±0.79	42.59±0.21	51.78±0.88	29.74±0.26	53.08±0.08	43.34±0.08
18:3(n-3)	0.17±0.09	0.00±0.00	0.22±0.11	0.00±0.00	0.56±0.00	0.35±0.00
18:4(n-3)	0.18±0.09	0.25±0.02	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
20:4(n-3)	0.22±0.11	0.15±0.8	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
20:5(n-3) EPA	1.70±0.01	1.19±0.03	1.12±0.02	1.01±0.01	0.70±0.00	0.75±0.00
22:6(n-3) DHA	3.95±0.05	2.52±0.15	2.33±0.03	1.24±0.01	0.85±0.00	0.93±0.01
∑ n-3	6.23±0.30	4.10±0.23	3.67±0.10	2.25±0.03	2.11±0.01	2.03±0.01
∑PUFAs	58.19±0.31	46.69±0.28	55.45±0.81	31.98±0.28	55.19±0.08	45.37±0.09
∑LC-PUFAs	5.87±0.13	3.85±0.26	3.45±0.03	2.25±0.03	1.55±0.01	1.68±0.01
∑MC-PUFAs	52.32±0.43	42.84±0.06	52.01±0.78	29.73±0.26	53.63±0.08	43.70±0.08
n-3: n-6	0.12±0.01	0.10±0.01	0.07±0.00	0.08±0.00	0.04±0.00	0.05±0.00

Total saturated fatty acids (SFAs) includes all FAs without double bonds
Total monosaturated fatty acids (MUFAs) constitute all FAs with one double bonds
Total polyunsaturated fatty acids (PUFAs) are sum of all FAs with two or more double bonds
Total long-chain (LC) polyunsaturated fatty acids constitutes the sum of PUFAs with chain length of 20 or more carbon atoms and three or more double bonds
Total medium chain (MC) PUFAs is the sum of all PUFAs with 18 carbon atoms

Specific growth rate:

$$SGR = 100 * [\frac{In\ final\ weight\ (g) - In\ initial\ weight(g)}{number\ of\ experimental\ days\ (days)}]$$

Hepatosomatic index:

$$HSI = [\frac{liver\ weight\ (g)}{body\ weight(g)}] * 100$$

Serum Metabolites

Five (5) fish per cage were sampled in less than a minute of dip netting from the cages in groups and immediately anesthetized with MS-222 (1: 300 v/v) in water from experimental system. Using a 1ml syringe with a 22-gauge x 3.8cm (11/2 in) needle, blood was collected from the caudal vein from each fish and placed into a 1.5 ml heparinized micro centrifuge tubes. Some part of the heparinized blood was centrifuged for 15min at 3500rpm to obtain homogenized serum and stored at -80^{o}C for subsequent analysis. Plasma fractions for other analysis was extracted untreated with heparin from blood samples after allowing it to clot at 4^{o}C and centrifuged for 15min at 3500rpm. Serum levels of total cholesterol (TC), total protein (TP) and triglycerides (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) were measured using a biochemical analyzer (Mindary Chemistry Analyzer BS-200, Shenzhen, China). Kits used for the analysis were purchased from (Nanjing Jiancheng bio-engineering). In brief, 0.5–1.0 ml of serum was pipetted into 1.5ml eppendorf tubes and inserted into the biochemical analyzer. The analyzer was calibrated according to the manufacture instruction and results read on attached computer.

Proximate Composition and Fatty Acid Analysis

Fish composition was determined using standard methods (AOAC, 1995). Total lipid was measured following the method of Folch et al. (1957). FA methyl esters (FAME) was produced from total lipid aliquots and methylated with boron trifluoride (BF_3) in methanol. The FA composition of total lipid in the diets were determined using gas chromatography (GC-7890A, USA) according to the method of Huang et al. (2010). FA content was determined using the normalization method, while peaks obtained were also identified by comparing retention time with known fatty acid methyl ester standard (sigma-aldrich chemie). Reserved crude lipid samples were analyzed for FA composition according to the procedures described by Lane et al. (2006). All measurements were performed in triplicate and the fatty acids content expressed as area percentage.

Digestive Enzyme Activity

Digestive tract of fish that were previously bled was excised and divided into three portions: anterior (AI), mid (MI) and posterior (PI). The posterior intestine was distinguished from the mid intestine by increased diameter, darker mucosa, and annular rings. The remaining part was divided into two identical parts to obtain the anterior and mid portions. The samples were then frozen in liquid nitrogen and stored at -80^{0}C until measurement of enzyme activity. All enzyme activities were measured as previously described by Bowyer et al. (2012).

Hepatic Enzyme/Receptor Activities

Enzyme activities of PPAR-α, CD36, FABP4, G6PD and 6PGD were measured using enzyme linked immunosorbent assay (ELISA). A total of 0.5-1.0 g of liver was homogenized using a ground glass homogenizer on ice. The homogenates were then centrifuged (20,000 rpm, 50 min at 4^{0}C), and the clear upper phase was used for the analysis. Samples were analyzed in a 96-well plate by ELISA acquired from Shanghai MLBIO Biotechnology Co. Ltd, China. Optical Density (OD) was measured in an ELISA microplate reader (Bio Tek Synergy, USA) at 450nm. A standard curve was generated according to the manufacturer's instruction, and the standard diversity calculated with Excel 2003. Enzyme activity units (IU), defined as moles of substrate converted to product per minute at assay temperature, was expressed per mg of hepatic soluble protein specific activity or per gram of liver tissue wet weight.

RNA Extraction and Real-time Quantitative Polymerase Chain Reaction (RT-qPCR)

Measurement of RNA expression of target genes (PPAR-α, CD36, FABP4, G6PD, 6PGD) were performed using Real-time PCR. Trizol reagent (Invitrogen, China) was used to extract total RNA from liver samples that had been sampled and stored at -80°C. Through absorbance measurement, the concentration of RNA was determined. In brief, the RNA samples were digested by RNase-free DNase I (Takara) incubation for 15 minutes at 37°C. Next, 2ug of RNA was transcribed into cDNA using M-MLV reversed transcriptase. All cDNA samples were stored at -20°C until analysis. Samples were then transcribed to cDNA and stored at -20^{0}C until analysis as described by Kutluyer et al. (2017) and Montero et al. (2015). Reactions were performed using mini option Real-time PCR machine (Bio-Rad). The reaction was performed following protocols as previously described in Ayisi and Zhao (2017) and Livak & Schmittgen (2001). Primers of both target genes and referenced gene (B-actin) used for the real time PCR (Oku et al., 2006) are shown in Table 3.

Statistical Analysis

No significant interactions were obtained when multivariate and univariate GLM were employed in the data analysis and so only one-way analysis of variance (ANOVA) was used to compare treatments, where significant, Tukey's multiple test was used to compare all pairs of columns. Significant levels were set at 0.05 probability ($P<0.05$). Homogeneity of variance and data normality were tested using Bartlett's test and Shapiro-Wilk normality test respectively. All analysis was in triplicates and was also performed using the Graph Pad Prism V.5.03 and results presented as mean ± standard error of the mean (SEM).

Results

Growth Performances

Dietary alternatives had no effect on survival and feed conversion ratio. Weight gain (WG) and specific growth rate (SGR) were lowest in group fed diet VO. However, group fed FCO was observed to be significantly different from the other treatments ($P<0.05$). Also, fed intake was higher in supplemented diets (73.40-83.21) except those fed VO (70.82±1.44) as indicated in Table 4.

Proximate Composition

Dietary alternative lipids did not affect muscle moisture (75.09-77.90) and lipid (5.98-7.71) while whole body moisture in group VO recorded a high significant value (73.17±0.06) than groups FCO and CO (68.77±0.49 and 69.88±0.49 respectively). Whole body lipid was observed to be significantly high in group VO (10.69±0.43) than groups CO and VCO (8.67±0.14 and 8.98±0.19 respectively) whiles liver lipid in FO (6.81±0.40) was significantly different from VO (5.13±0.13).

Protein content in muscle was significantly higher in group VO (72.43±4.90) than VCO (85.64±2.63) although, both were not different from the other treatments. However, whole body protein content in FO, FVO and CO were significantly higher than recorded in groups VO and VCO but not different from FCO (Table 5).

Fatty Acids profiles

Muscle Fatty Acid

Significant differences between FAs analyzed in muscle was obtained as presented on Table 6. Increases in C12, C14 and C16 was observed in group fed VO diet which translated in the total SFA (38.01±0.18) among treatments whereas FO recorded the least significant value (17.95±0.83). Nominally, no differences were obtained in the total MUFAs, although diet VCO recorded a significantly higher value. ARA in vegetable diets was significantly higher than those blends with FO and its exclusive. While n-3 was higher in those fed FO (9.19±0.17), n-6 was higher in CO group (51.15±0.39) whereas VO group recorded the least amongst all. EPA and DHA was also significantly least in diets with vegetable lipid inclusive diets.

Table 3. Nucleotide sequence of primers used to assay gene expression for real-time quantitative polymerase reaction (qRT-PCR)

Target Genes	Forward (5'–3')	Reverse (5'–3')	GenBank accession no.
PPAR-α	TACGGTGTTTACGAAGCCCT	AGGAAGGTGTCATCTGGGTG	KF871430.1
FABP 4	ATTGCCGGAGACCTTACCAA	TGGTCTCTGGATGCCGATAC	KP025971.1
CD36	TCTCTCATTCTGACGCTCCC	CGCCCAGCTCTTTCATGTAC	XM_003452029.4
6GPD	GAGAGTCGTGGCCAGTAAGA	ACCAGCCGAACTCTTTAGCT	JX992745
G-6PD	TGCTCCTGTTTCTCTCTCCG	CATCCCAGCGTTCATTCCTG	JX992744
β-ACTIN	TAATAACAGAACGCAGCGCC	AGTGCGGCGATTTCATCTTC	EF026001.1

PPAR-α: Peroxisome proliferator-activated receptor α; Fabp4: Fatty acid binding protein 4; CD36: fatty acid translocase (cluster of differentiation); 6GPD: 6-Phosphogluconate dehydrogenase; G6PD: Glucose 6-phosphate dehydrogenase; β-Actin: Beta actin

Table 4. Growth Performance and feed utilization of *O. niloticus* fed different diets for 8 weeks

Group/ Growth performance	FO	FVO	FCO	VO	CO	VCO
Initial body weight (g)	6.19±0.16	6.00±0.31	5.88±0.09	5.98±0.07	6.08±0.18	6.28±0.25
Final weight (g)	$82.45±1.81^{a}$	$82.85±2.70^{a}$	$84.36±1.17^{a}$	$70.05±1.07^{b}$	$77.48±3.66^{ab}$	$83.61±3.74^{a}$
Feed intake (g)	$76.22±0.54^{b}$	$81.74±1.49^{a}$	$82.29±0.40^{a}$	$70.82±1.44^{c}$	$78.40±0.89^{ab}$	$83.21±1.29^{a}$
[a]WG (%)	$1234.00±56.55^{ab}$	$1284.00±33.30^{ab}$	$1334.00±5.53^{a}$	$1071.00±3.90^{b}$	$1180.00±87.29^{ab}$	$1232.00±25.88^{ab}$
[b]FCR	1.00±0.02	1.07±0.02	1.05±0.01	1.11±0.01	1.10±0.05	1.08±0.03
%[c]SGR (%)	$4.62±0.08^{ab}$	$4.68±0.03^{ab}$	$4.76±0.01^{a}$	$4.39±0.01^{b}$	$4.54±0.12^{ab}$	$4.62±0.03^{ab}$
[d]HSI	$1.32±0.03^{b}$	$1.60±0.03^{ab}$	$1.64±0.08^{a}$	$1.57±0.10^{ab}$	$1.68±0.06^{a}$	$1.60±0.44^{ab}$
[e]K	1.98±0.05	1.99±0.02	2.07±0.02	2.00±0.05	1.98±0.02	2.04±0.03

All values are mean ±SEM. Different superscript in each row represent significant differences ($P<0.05$) determined by one-way ANOVA.
[a]WG= weight gain; [b]FCR= feed conversion ratio; [c]SGR= specific growth rate; [d]HSI= hepatosomatic index; [e]K= condition factor.

Liver Fatty Acids

VO inclusive diets recorded the highest significant SFA values in the liver whereas, a low level of MUFA was obtained (26.86±2.06) as indicated on Table 7. Although significant differences were not obtained between treatments, DHA and EPA were higher in FO fed groups (7.21±0.78 and 0.16±0.08 respectively) than other treatments while VO diets was also higher than observed in CO. The same trend was observed for n-3 and n-3/n-6 ratio. However, n-6 was higher in FCO whereas VO fed diet was higher in ARA although, nominally no differences between treatments.

Serum Biochemical Properties and Their FA Interactions

Table 8 shows that, low levels of triglyceride (TG) was obtained in fish fed diets CO (1.06±0.04) and FVO (1.11±0.17) and higher levels in group FCO (1.70±0.14). Whereas, no differences were obtained between treatments when TP and TC were measured. Further, no significant differences were obtained when ALP, ALT, AST, LDL-C and HDL-C were measured.

Table 5. Biochemical compositions of feed and fish tissues after feeding alternative dietary lipids to *O. niloticus* for 8 weeks

Moisture	**FO**	**FVO**	**FCO**	**VO**	**CO**	**VCO**
Muscle	75.99±0.61	76.18±0.65	76.65±0.44	77.90±0.54	75.09±1.50	75.63±0.65
WB	70.98±1.06 *ab*	70.46±0.76 *ab*	69.88±0.49 *b*	73.17±0.60 *a*	68.96±0.77 *b*	71.01±0.57 *ab*
Lipid	**FO**	**FVO**	**FCO**	**VO**	**CO**	**VCO**
Liver	6.81±0.40 *a*	6.42±0.39 *ab*	5.69±0.13 *ab*	5.13±0.13 *b*	5.82±0.42 *ab*	5.52±0.55 *ab*
Muscle	6.44±0.55	7.17±0.36	6.65±0.41	6.41±0.22	6.14±0.31	5.98±0.41
WB	10.21±0.51 *ab*	10.22±0.40 *ab*	9.68±0.40 *ab*	10.69±0.43 *a*	8.67±0.14 *b*	8.98±0.19 *b*
Protein	**FO**	**FVO**	**FCO**	**VO**	**CO**	**VCO**
Liver	49.54±1.68	43.51±0.26	44.79±4.96	47.64±3.31	41.09±3.15	38.53±1.47
Muscle	74.35±1.96 *ab*	73.87±2.34 *ab*	81.62±62 *ab*	72.43±4.90 *a*	83.20±2.56 *ab*	85.64±2.63 *b*
WB	61.66±0.63 *a*	61.60±0.45 *a*	63.40±0.69 *ab*	66.50±1.03 *b*	60.86±0.73 *a*	65.18±1.09 *b*

*WB= whole body *Protein and lipid were all analyzed in dry matter

Table 6. Fatty acid (FA) profiles (% total FA) of muscle

Fatty Acids (% total)	FO	FVO	FCO	VO	CO	VCO
10:0	0.0±0.0^{c}	0.06±0.03^{b}	0.0±0.0^{c}	0.19±0.02^{a}	0.0±0.0^{c}	0.10±0.02^{b}
12:0	0.0±0.0^{c}	3.83±0.22^{b}	0.03±0.01^{c}	7.90±0.51^{a}	0.20±0.03^{c}	3.33±0.17^{b}
14:0	0.67±0.01^{c}	4.09±0.03^{b}	0.72±0.03^{c}	8.56±0.30^{a}	0.69±0.02^{c}	3.82±0.01^{b}
16:0	12.52±0.34^{cd}	13.45±0.13^{bc}	12.73±0.08^{cd}	16.96±0.53^{a}	11.84±0.09^{d}	14.64±0.17^{b}
18:0	4.47±0.41^{a}	3.77±0.07^{ab}	2.52±0.80^{b}	4.24±0.25^{ab}	3.02±0.16^{ab}	3.76±0.06^{ab}
20:0	0.23±0.02	0.16±0.01	0.20±0.01	0.16±0.02	0.22±0.02	0.17±0.01
22:0	0.05±0.02^{ab}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{ab}	0.05±0.01^{ab}	0.09±0.01^{a}
∑ SFA's	17.93±0.83^{c}	25.37±0.33^{b}	16.20±0.39^{b}	38.01±0.18^{a}	16.03±0.21^{c}	25.90±0.15^{b}
16:1(n-7)	1.36±0.15b^{c}	1.55±0.12b^{c}	1.54±0.04b^{c}	2.18±0.17^{a}	1.08±0.06^{c}	1.87±0.12^{ab}
18:1(n-9)	24.18±0.47^{b}	23.88±0.42^{b}	26.77±0.66^{a}	23.53±0.38^{b}	27.05±0.15^{a}	27.02±0.22^{a}
20:1(n-9)	1.03±0.08^{ab}	0.87±0.01^{b}	1.00±0.01^{ab}	1.14±0.04^{a}	1.06±0.02^{a}	1.03±0.02^{a}b
24:1(n-9)	0.02±0.02	0.02±0.01	0.02±0.02	0.0±0.0	0.04±0.02	0.0±0.0
∑ MUFAs	26.59±0.67^{bc}	26.31±0.44^{c}	29.33±1.01^{ab}	26.84±0.45^{bc}	29.22±0.28^{abc}	29.93±0.67^{a}
18:2(n-6)	37.56±3.48^{c}	37.82±0.40^{bc}	44.51±0.26^{ab}	27.12±0.53^{d}	45.63±0.25^{a}	35.45±0.32^{c}
18:3(n-6)	0.67±0.04b^{c}	0.57±0.01^{bc}	0.72±0.01^{ab}	0.54±0.04^{c}	0.84±0.06^{a}	0.67±0.01b^{c}
20:2(n-6)	1.60±0.12^{ab}	1.23±0.15^{bc}	1.51±0.17^{abc}	1.00±0.01^{c}	1.95±0.06^{a}	1.48±0.04^{abc}
20:3(n-6)	0.84±0.03^{abcd}	0.79±0.01^{bd}	0.81±0.00^{abcd}	0.56±0.15^{cd}	1.09±0.04^{ac}	1.09±0.00^{ab}
20:4(n-6) ARA	1.50±0.08^{b}	1.19±0.05^{c}	1.18±0.03^{c}	1.77±0.04^{ab}	1.65±0.09^{ab}	1.79±0.02^{a}
∑ n-6	42.16±3.90^{bc}	41.60±0.42^{bc}	48.74±1.32^{ab}	30.99±0.36^{d}	51.15±0.39^{a}	40.47±0.61^{c}
18:3(n-3)	2.82±0.28^{a}	2.68±0.04^{a}	2.37±0.02^{a}	1.31±0.16^{b}	1.32±0.06^{b}	1.32±0.01^{b}
20:3(n-3)	0.56±0.03^{a}	0.51±0.01^{a}	0.42±0.00^{b}	0.13±0.03^{d}	0.25±0.01^{c}	0.28±0.01^{c}
20:5(n-3) EPA	0.32±0.02^{a}	0.19±0.03^{b}	0.17±0.02b^{c}	0.18±0.00^{b}	0.10±0.01^{c}	0.12±0.00b^{c}
22:6(n-3) DHA	5.48±0.29^{a}	3.32±0.07^{b}	2.79±0.07^{bc}	2.56±0.09^{cd}	1.93±0.12^{d}	1.98±0.05^{d}
∑ n-3	9.19±0.17^{a}	6.69±0.28^{a}	5.74±0.13^{a}	4.16±0.30^{b}	3.59±0.10^{b}	3.70±0.11^{b}
∑PUFAs	51.35±3.73^{ab}	48.28±0.70^{ab}	54.47±1.39^{a}	35.15±0.40^{c}	54.75±0.44^{a}	44.17±0.72^{b}
∑LCPUFAs	8.70±0.32^{a}	5.99±0.39^{b}	5.36±0.02^{b}	5.18±0.22^{b}	5.02±0.27^{b}	5.26±0.11^{b}
∑ MCPUFA	41.05±3.79^{ab}	41.07±0.20^{ab}	47.60±1.25^{a}	28.97±0.32^{c}	47.78±0.39^{a}	37.44±0.58^{b}
n-3: n-6	0.22±0.03^{a}	0.16±0.01^{b}	0.12±0.00b^{cd}	0.13±0.01b^{c}	0.07±0.00^{d}	0.09±0.00^{cd}

Total saturated fatty acids (SFAs) includes all FAs without double bonds
Total monosaturated fatty acids (MUFAs) constitute all FAs with one double bonds
Total polyunsaturated fatty acids (PUFAs) are sum of all FAs with two or more double bonds
Total long-chain (LC) polyunsaturated fatty acids constitutes the sum of PUFAs with chain length of 20 or more carbon atoms and three or more double bonds
Total medium chain (MC) PUFAs is the sum of all PUFAs with 18 carbon atoms

Digestive Enzyme Activity

Table 9 shows that, no significant differences were obtained between groups and sections when amylase activities were measured. Trypsin activity in the anterior intestine (AI) was lowest in group fed diet FVO and significantly higher in group VO (4.150±0.0640). However, in the middle intestine (MI), FCO and CO recorded the least significant activities (4.030±0.0053 and 4.034±0.0071 respectively) while those fed VO recorded the highest activity (4.107±0.0209). Also, in the posterior intestine (PI), group VO recorded the highest significant activity.

Pepsin activity in the AI and PI sections of the intestine did not differ from each between groups. However, in the MI, groups FVO and VO recorded the

Table 7. Fatty acid (FA) profiles (% total FA) of liver

Fatty Acids (% total)	FO	FVO	FCO	VO	CO	VCO
10:0	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.09±0.01^{a}	0.00±0.00^{b}	0.00±0.00^{b}
12:0	0.00±0.00^{b}	0.99±0.21^{b}	0.00±0.00^{b}	4.04±0.63^{a}	0.00±0.00^{b}	1.29±0.27^{b}
14:0	0.99±0.15^{c}	2.93±0.22^{b}	1.03±0.14^{c}	6.93±0.54^{a}	0.98±0.03^{c}	3.74±0.07^{b}
16:0	11.64±1.57^{b}	16.41±0.3^{ab}	13.49±0.89^{b}	19.10±1.00^{a}	14.44±0.29^{ab}	18.55±1.33^{a}
18:0	7.00±1.13	9.22±0.06	6.41±0.66	7.61±0.54	6.81±0.02	8.25±0.50
20:0	0.20±0.03	0.13±0.07	0.50±0.31	0.14±0.01	0.10±0.01	0.05±0.00
22:0	0.31±0.15	0.13±0.02	0.05±0.05	0.18±0.02	0.22±0.02	0.15±0.02
∑ SFA's	20.14±2.58^{c}	29.81±0.0^{b}	21.48±1.93^{c}	38.09±1.06^{a}	22.55±0.27^{c}	32.02±1.45^{ab}
16:1(n-7)	1.70±0.16^{b}	2.32±0.07^{ab}	1.77±0.12^{b}	3.09±0.34^{a}	2.25±0.06^{ab}	2.84±0.26^{a}
18:1(n-9)	27.50±2.57^{ab}	31.42±0.89^{a}	27.89±1.37^{ab}	22.69±1.71^{b}	32.67±1.51^{a}	32.30±0.69^{a}
20:1(n-9)	1.11±0.16	1.19±0.09	0.88±0.12	0.98±0.06	1.37±0.11	1.06±0.11
22:1(n-9)	0.48±0.09^{a}	0.09±0.06^{bc}	0.22±0.03^{abc}	0.10±0.06^{b}	0.04±0.04^{c}	0.06±0.06^{c}
24:1(n-9)	0.00±0.00	0.01±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
∑ MUFAs	30.78±2.79^{ab}	35.04±1.08^{ab}	30.76±1.35^{ab}	26.86±2.06^{b}	36.33±1.63^{a}	36.26±1.01^{a}
18:2(n-6)	32.31±6.99^{a}	21.59±1.42^{ab}	35.16±1.92^{a}	16.62±0.99^{b}	26.96±1.86^{ab}	20.44±2.38^{ab}
18:3(n-6)	0.84±0.08^{ab}	0.58±0.02^{ab}	0.46±0.23^{a}	0.45±0.01^{a}	0.10±0.06^{b}	0.62±0.04^{ab}
20:2(n-6)	2.19±0.38^{a}	1.48±0.09^{ab}	1.56±0.09^{ab}	0.98±0.04^{b}	1.77±0.04^{ab}	1.14±0.05^{b}
20:3(n-6)	0.74±0.37	0.76±0.01	0.85±0.09	0.97±0.07	1.10±0.07	0.78±0.03
20:4(n-6) ARA	2.86±0.31^{ab}	2.50±0.16^{b}	2.88±0.37^{ab}	4.52±0.79^{a}	3.65±0.09^{ab}	2.75±0.01^{ab}
22:4(n-6)	0.52±0.09^{b}	0.46±0.05^{b}	0.50±0.07^{b}	1.01±0.11^{a}	0.74±0.01^{ab}	0.65±0.02^{b}
22:5(n-6)	0.68±0.09^{d}	0.94±0.13^{cd}	1.05±0.16^{bcd}	2.80±0.46^{a}	2.08±0.10^{ab}	1.77±0.11^{abc}
∑n-6	40.14±6.14^{ab}	28.29±0.98^{ab}	42.47±2.57^{a}	27.34±2.06^{b}	37.30±1.72^{ab}	28.16±2.43^{ab}
18:3(n-3)	1.57±0.12	1.54±0.54	0.47±0.47	0.89±0.12	0.47±0.01	0.62±0.10
20:3(n-3)	0.00±0.00^{b}	0.29±0.01^{a}	0.00±0.00^{b}	0.14±0.07^{ab}	0.07±0.04^{b}	0.08±0.04^{b}
20:5(n-3) EPA	0.16±0.08^{a}	0.15±0.01^{ab}	0.00±0.00^{ab}	0.10±0.05^{a}	0.06±0.03^{b}	0.05±0.03^{b}
22:6(n-3) DHA	7.21±0.78	5.45±0.11	4.93±0.75	6.58±1.11	3.19±0.23	2.82±0.10
∑ n-3	8.93±0.96^{a}	7.43±0.63^{ab}	5.40±0.69b^{c}	7.69±1.02^{ab}	3.80±0.23^{c}	3.57±0.12^{c}
∑ PUFAs	49.08±5.37^{ac}	35.73±0.46^{abcd}	47.87±3.21^{ab}	35.03±3.07^{abcd}	41.10±1.92^{bd}	31.73±2.41^{cd}
∑LCPUFAs	12.17±1.64a^{b}	10.55±0.44^{ab}	10.21±1.37^{ab}	16.10±2.44^{a}	10.90±0.30^{ab}	8.912±0.25^{b}
∑MCPUFAs	34.72±6.88^{a}	23.70±0.88^{ab}	36.10±2.51^{a}	17.95±1.10^{b}	28.43±1.81^{ab}	21.68±2.52^{ab}
n-3: n-6	0.24±0.07^{ab}	0.27±0.03^{a}	0.13±0.01^{ab}	0.28±0.02^{a}	0.10±0.00^{b}	0.13±0.01^{ab}

Total saturated fatty acids (SFAs) includes all FAs without double bonds

Total monosaturated fatty acids (MUFAs) constitute all FAs with one double bonds

Total polyunsaturated fatty acids (PUFAs) are sum of all FAs with two or more double bonds

Total long-chain (LC) polyunsaturated fatty acids constitutes the sum of PUFAs with chain length of 20 or more carbon atoms and three or more double bonds

Total medium chain (MC) PUFAs is the sum of all PUFAs with 18 carbon atoms

Table 8. Hematological and antioxidant profile of fish fed different experimental diets

Group/ Parameters	FO	FVO	FCO	VO	CO	VCO	P-value
TP (g/L)	69.39±10.16	71.13±2.43	69.78±7.75	71.02±3.32	84.97±6.74	69.57±2.58	0.4811
TG (mmol/L)	1.50±0.09^{ab}	1.11±0.17^{b}	1.70±0.14^{a}	1.29±0.05^{ab}	1.06±0.04^{b}	1.51±0.18^{ab}	0.0205
TC (mmol/L)	3.52±0.26	4.24±0.34	3.53±0.13	3.79±0.42	3.63±0.40	3.64±0.38	0.6735
LDL (mmol/L)	1.52±0.23	1.46±0.12	1.47±0.17	1.55±0.35	1.57±0.14	1.66±0.18	0.9839
HDL (mmol/L)	2.00±0.12	2.78±0.43	2.07±0.06	2.23±0.37	2.06±0.51	1.98±0.29	0.5572
HDL-C/LDL-C	1.31±0.15	2.01±0.36	1.38±0.29	1.43±0.19	1.43±0.36	1.19±0.08	0.3717
ALP (U/L)	119.5±7.62	92.12±3.22	106.8±8.88	136.0±8.96	88.27±15.14	97.11±20.21	0.1085
ALT (U/L)	24.26±5.14	29.93±5.26	24.89±1.36	22.47±2.22	34.92±5.78	26.13±4.03	0.3936
AST (U/L)	32.16±5.02	38.03±5.54	31.67±2.01	29.40±2.21	42.63±6.08	32.38±4.74	0.3722

Note* TP: Total protein; TG: Triglyceride; TC: Total cholesterol; LDL: Low-density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; ALP: Alkaline phosphatase; ALT: Alanine amino transferase; AST: Aspartate amino transferase;

highest significant activities (1.248±0.0020 and 1.253±0.0225 respectively) than FO, FCO and CO.

On the other hand, protease activity recorded no difference between groups and sections. Contrary, only groups VO and CO were significantly different (3.426±0.0011 and 3.426±0.0009 respectively) in the AI sections when lipase activity was measured. While only FVO (3.428±0.0010) and CO (3.424±0.0007) were significantly different in the MI, no differences in activity was obtained in the PI.

Lipogenic Enzyme Activity

As indicated on Table 10, the activities of PPAR-α and FABP4 did not differ significantly between groups when measured. However, group FVO recorded the least activity (2.884±0.0015) while group FCO was highest (2.898±0.0038) although both were not significantly different from other groups when CD36 was measured.

Also, G6PD activity in group FCO was highly significant different (1.484±0.0329) from other groups whereas the activities of 6GPD in VO was significantly higher (1.859±0.0215) from FCO (1.718±0.0319) which also was significantly higher from FO, FVO, CO and VCO.

Lipogenic Lipid Metabolic Genes and Principal Component Analysis

PPAR-α and G6PD expressions in groups did not record any significant differences as shown on Figure 1. VCO group recorded a significantly low expression in CD36, FABP45 and 6PGD regulations whiles groups FO, VO and CO were significantly upregulated, although they were nominally not different from each other.

However, the interaction between feed FAs and hepatic transport gene expressions were tested using principal component analysis (PCA). 94.61% variation of principal component 1 was obtained whiles 0.52% of variation data in principal component 2 was accounted as presented on Figure 2. FAs had no direct interaction with the hepatic transport genes, although, DHA, EPA, n3/n6 ratio and LC-PUFA were closely clustered whiles others were separated.

Discussions

Dietary alternative lipids had no negative influences on the general growth performances in *O. niloticus*. This indicates the viability of vegetable lipids to replace fish oil. Weight gain and specific growth rate

Table 9. Intestinal enzyme activities of *O. niloticus* fed different diets for 8 weeks

Parameters/Groups	FO	FVO	FCO	VO	CO	VCO
Anterior Intestine						
Amylase (mU/L)	2.798±0.0003	2.798±0.0002	2.798±0.0003	2.799±0.0005	2.799±0.0003	2.799±0.0005
Trypsin (IU/L)	4.051±0.0180^{ab}	4.039±0.0086^{b}	4.055±0.0094^{ab}	4.150±0.0640^{a}	4.095±0.0168^{ab}	4.047±0.0144^{ab}
Pepsin (U/L)	1.194±0.0265	1.188±0.0126	1.214±0.0170	1.180±0.0170	1.217±0.0148	1.187±0.0159
Protease (IU/L)	3.427±0.0012	3.424±0.0003	3.427±0.0008	3.426±0.0011	3.426±0.0009	3.424±0.0005
Lipase (mU/L)	4.882±0.0376^{ab}	4.869±0.0392^{ab}	5.010±0.0700^{ab}	4.829±0.0367^{b}	5.067±0.0543^{a}	4.869±0.0538^{ab}
Middle Intestine						
Amylase (mU/L)	2.798±0.0000^{b}	2.799±0.0002^{ab}	2.798±0.0001^{b}	2.799±0.0003^{ab}	2.800±0.0005^{a}	2.799±0.0003^{a}
Trypsin (IU/L)	4.027±0.042^{bc}	4.073±0.0125^{ab}	4.030±0.0053^{c}	4.107±0.0209^{a}	4.034±0.0071^{bc}	4.035±0.0045^{bc}
Pepsin (U/L)	1.156±0.0038^{b}	1.248±0.0231^{a}	1.171±0.0103^{b}	1.253±0.0225^{a}	1.177±0.0127^{b}	1.054±0.1320^{ab}
Protease (IU/L)	3.424±0.0001	3.428±0.0010	3.424±0.0004	3.428±0.0016	3.424±0.0007	3.425±0.0008
Lipase (mU/L)	4.895±0.0726^{ab}	5.086±0.0634^{a}	4.902±0.0542^{ab}	5.014±0.0821^{ab}	4.839±0.0416^{b}	4.893±0.0622^{ab}
Posterior Intestine						
Amylase (mU/L)	2.798±0.0001^{b}	2.798±0.0001^{b}	2.798±0.0002^{b}	2.800±0.0008^{ab}	2.800±0.0006^{ab}	2.801±0.0007^{a}
Trypsin (IU/L)	4.027±0.0065^{b}	4.051±0.0072^{b}	4.029±0.0105^{b}	4.143±0.0402^{a}	4.055±0.0080^{b}	4.054±0.0123^{b}
Pepsin (U/L)	1.159±0.0066	1.204±0.0113	1.193±0.0233	1.201±0.0300	1.209±0.0106	1.231±0.0220
Protease (IU/L)	3.424±0.0010	3.425±0.0005	3.425±0.0008	3.425±0.0006	3.426±0.0007	3.427±0.0010
Lipase (mU/L)	5.048±0.1900	4.937±0.0392	4.819±0.0410	4.843±0.0753	5.054±0.1085	5.123±0.0935

Values are mean ± SEM (n=3). Means not bearing the same superscript in the same row are significantly different (P<0.05).

Table 10. Lipogenic enzymes/receptors activities of *O. niloticus* fed six diets for 8 weeks

Parameters/Groups	FO	FVO	FCO	VO	CO	VCO
PPAR-α (U/L)	1.165±0.0002	1.165±0.0005	1.167±0.0007	1.167±0.0001	1.165±0.0004	1.166±0.0006
CD36 (ng/L)	2.889±0.0024^{ab}	2.884±0.0015^{b}	2.898±0.0038^{a}	2.886±0.0033^{ab}	2.888±0.0033^{ab}	2.891±0.0020^{ab}
FABP4 (ng/L)	3.766±0.008	3.768±0.0008	3.772±0.0024	3.768±0.0019	3.765±0.0013	3.767±0.0019
G-6-PD (U/ml)	1.301±0.0143^{b}	1.316±0.0401^{b}	1.484±0.0329^{a}	1.319±0.0318^{b}	1.303±0.0388^{b}	1.324±0.0286^{b}
6PGD (U/ml)	1.596±0.0088^{c}	1.701±0.0318b^{c}	1.718±0.0319^{b}	1.859±0.0215^{a}	1.612±0.0209^{bc}	1.677±0.0389^{bc}

PPAR-α: Peroxisome proliferator-activated receptor-α; CD36: Fatty acid translocase/ cluster of differences; FABP-4: Fatty acid binding protein-4; 6GPD: 6-Phosphogluconate dehydrogenase; G6PD: Glucose 6-phosphate dehydrogenase.

Values are mean ± SEM (n=3). Means not bearing the same superscript in the same row are significantly different (P<0.05).

were higher in those fed diet FCO which suggest that the physiological functioning of *O. niloticus* will be maintained when FO is replaced with vegetable oils at a 50% level. The study results agree with previous study reports when vegetable oils replaced FO at different levels in Juvenile Nile and Mozambique tilapia and common carp (Erondu et al., 2021; Peng et al., 2015; Demir, Tüker et al., 2014). Also, the performance of FCO can be as a result of an enhanced metabolic activities due to the preference for 18: n-9 content in vegetable oils which is a substrate for energy production (Tian et al., 2023; Mazzocchi et al., 2021; Peng et al., 2016). Further, synthesis of FA is essentially obtained from 18: 3n-3 (α-linoleic acid, ALA) which is in abundance in FCO.

Improved growth rates are likely related to higher digestive activities which correlates to an improved intestinal morphology (Gopalraaj et al., 2024; Yin et al., 2021). Different fishes have been noted to be influenced by amylase activity which in turn is also influenced by the nature of their diet (Jiao et al., 2023; Murtaza et al.,

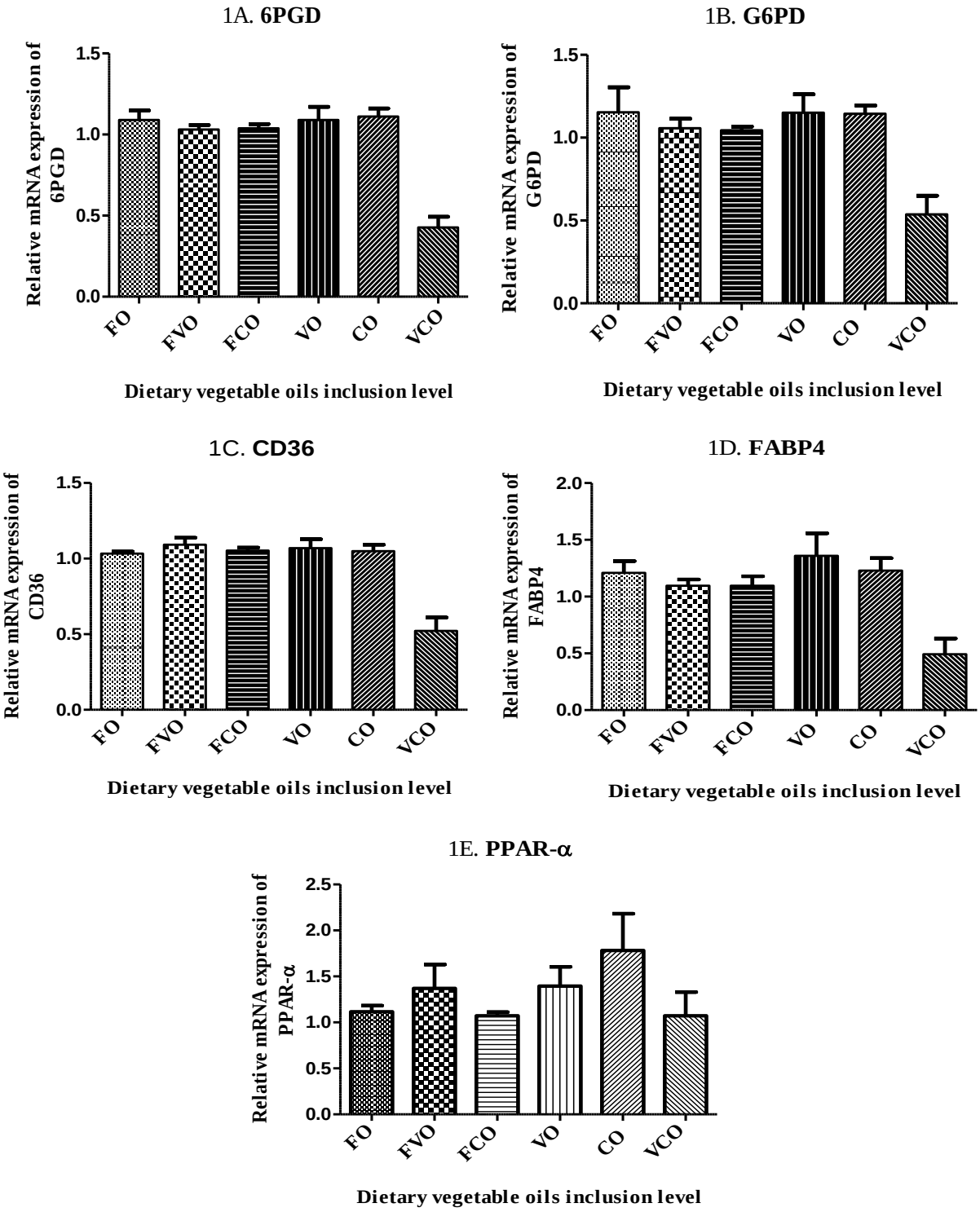

Figure 1. Relative mRNA expression of (A) 6PGD, (B) G6PD (C) CD36, (D) FABP4 and (E) PPAR-α of fish fed different experimental diets.

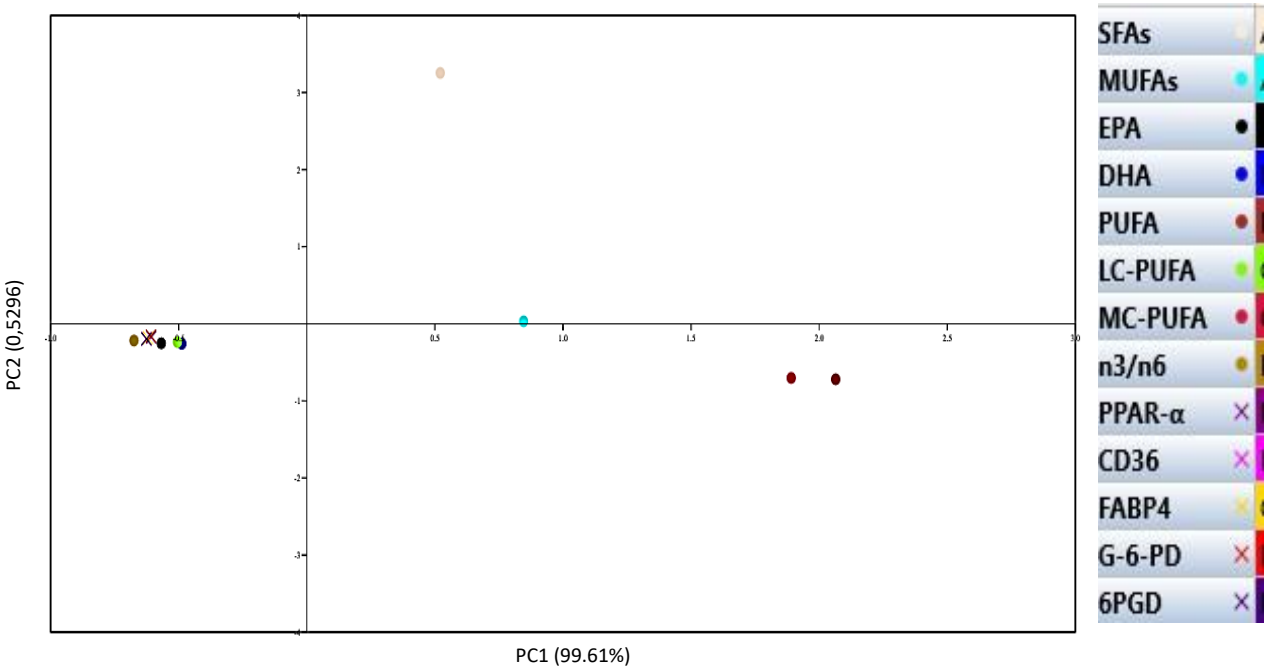

Figure 2. Principal component analysis of selected FAs with gene expressions involved in lipid metabolisms.

2016; Zahran et al., 2014). Amylase activity in fish was affected by replacing fish oil in the MI and PI. The exclusive replacement of FO by VCO in PI recorded the highest activity, although those fed FO, FVO and FCO were not influenced whilst amylase activity in MI was higher in VCO and CO groups. This phenomenon is suggested to be influenced by the lipid sources and their intestinal morphology of various treatment groups (Castro et al., 2016; Long et al., 2019). Likewise, in the AI, the balanced activities of amylase indicate fish were not affected by lipid sources in diets which could be related to an effective and efficient utilization of carbohydrate in diets which corresponds in their growth performance. Protease activity was not affected in all sections (AI, MI and PI). This led to a better protein efficiency ratio in all groups because, protease activities were not affected.

Dietary alternatives influenced the activities of lipase secretion which disagrees with other studies when FO was substituted with palm and canola oils in *O. niloticus* and yellowtail kingfish (*Seriola lalandi*) respectively (Turchini et al., 2020; Ayisi et al., 2018). VO had a limiting effect on lipase activity in AI indicating digestion of VO and its utilization as an efficient energy source although it was nominally lower. However, in the MI, VO seemed to stimulate higher lipase activities between groups. Although, nutrient digestion usually takes place in the AI and MI to some extent, it also plays a major role (Magalhães et al., 2015). This could explain the high activity of lipase in the VO group. The possible drag of secreted mucous to this part in the VO group could also be a reason for this activity. In general, lipase activity was limited in vegetable diets which agrees with Tengjaroenkul et al. (2000) indicating the limiting effects of low fats that are broken down and leads to triacylglycerol and digestion (Nolasco-Soria et al., 2024; Castro et al., 2016).

Digestion and absorption of proteins involved in growth are enhanced by trypsin (Dong et al., 2018). In this study, trypsin was affected by dietary alternatives. Treatment group fed diet VO exhibited higher activities of trypsin. This may be related to the MUFA property of VO which spare protein and boost energy for metabolic activities (Long et al., 2019; Strobel et al., 2022; Apraku et al., 2017). As such protein intake was higher in this regard than other treatments although, no significant differences were obtained.

Pepsin activity on the other hand was not affected in AI and PI. Pepsin activity in MI was lower in CO diets inclusive. This result is suggested to be unusual as it did not correlate with its growth performance. This could be attributed to the properties of CO which might have altered the morphology of their intestine. However, in general, dietary alternatives did not influence negatively the digestive enzyme activities of *O. niloticus.*

Plasma cholesterol was not influenced by the increases in DHA/EPA ratios as it had a stimulating effect on TAG (Shibabaw, 2021; Jin et al., 2017). Vegetable oils are often associated to lower cholesterol (Turchini et al., 2020) as indicated from the study results. Thus, non-significant differences in TC levels of the other treatments from FO group (Ayisi et al., 2018; Long et al., 2019; Zhou et al., 2015; Jin et al., 2017). Fish across treatments can be suggested to have good capacity in retaining protein and maintaining a balanced nutritional status. This can speculatively be due to the insignificant differences in results obtained for TP (Rombenso et al., 2022; Mozanzadeh et al., 2016).

In agreement (Peng et al., 2016), AST and ALT in this study were not influenced by lipid sources. This indicate the blood stream of fish was not contaminated and shows the liver was in good functional status as opposed to Lin & Shiau (2007). Also, metabolic transport and associated activities were not influenced as the results for HDL-C and LDL-C shows. As such, cholesterol transport can be said to be efficient and thereby present a quality and healthy fish (Sankian et al., 2019; Ringø et al., 2022; Fei et al., 2015).

To our knowledge, studies on hepatic enzymes activities (PPAR-α, FABP4, CD36, G6PD and 6GPD) have not been done and this result basically presents the first-hand information on these activities. FABP4 and PPAR-α enzymatic activities in the liver was not influenced by dietary alternatives. These are key enzymes in the transport of FAs (Lei et al., 2021; Zhong et al., 2023). As such, this result could be explained that, dietary alternatives do not influence or impact the transfer of FAs in regard to these enzymes. However, FA translocase/cluster of differentiation 36 (CD36), G6PD and 6GPD were all affected between treatments. Lipid metabolism was observed to be lower in FVO group. This is indicated in TG which shows that partial replacement of FO affected the uptake of FA once free FAs were released from the TG on lipoproteins (Zhong et al., 2023). The results of this activities are similar to other report which suggest this is related to the nutrient/lipid sources other than growth rate (Lei et al., 2021; Zhong et al., 2023).

One of the key transcription factors involved in lipid metabolism is PPAR-α. It plays a role in FA catabolism by regulating expressions of major enzymes involved in FA oxidation (Zhang et al., 2024; Bougarne et al., 2018; Li et al., 2015a; Li et al., 2016). In this study, PPAR-α was not influenced by dietary alternatives. This indicates the intake of LA and n-6 PUFA was at a balanced level and as such, this gene was not expressed differently between treatments and agrees with other studies (Zhang et al., 2024; Lu et al., 2014). This however disagrees with reports when fish was fed different levels of soybean oil and phospholipid rich in LA (Zhang et al., 2024; Bougarne et al., 2018; Li et al., 2015b; Li et al., 2016). This result could be suggested to represent a good lipid retention as opposed to what was reported in blunt snout bream (Li et al., 2016).

FABP4 in liver of group VCO was downregulated, although it was not different from those fed FCO and FVO. This indicates that a blend of different dietary lipids affects the expression FABP4. The result of this study correlates with the hepatic PUFA levels. The result disagrees with higher mRNA expression of FABP4 when fish were fed levels of soybean oil. It has been noted intracellular trafficking and solubility of LC-FAs and other hydrophobic ligands are provided by FABPs. As such, the results indicate that, low levels of LC-PUFAs in dietary vegetable alternatives affect the regulation of FABP4 gene expressions when fed to fish (Lei et al., 2021; Zhong et al., 2023; Li et al., 2016).

The lowest mRNA expression levels of 6GPD was obtained in fish fed VCO, which indicates blends of dietary vegetable oils might affect lipid synthesis by reducing NADPH supply which is essential for lipid synthesis. However, with the exception of VCO effect, a positive relationship between G6PD and 6GPD was obtained. This suggest a synergistic effect on pentose phosphate pathways (Wang et al., 2024; Ayisi et al., 2018).

The uptake of FAs in the VCO group was lower as shown in the mRNA expression levels of CD36. This correlates with the results obtained in the expression of 6PGD mRNA levels where lower supply of NADPH affects the synthesis of lipid. As such, this could be speculatively suggested to be the effect of vegetable oils in high lipid accumulation in liver (Zhao et al., 2023; Li et al., 2016). This agrees with results reported when CD36 was also downregulated upon feeding *O. niloticus* with vegetable oils. It has been suggested that, the lower expression of CD36 indicate a good FA transport and uptake activity (Zhao et al., 2023).

Separation between n3 and n6 FAs in this study from the PCA analysis agrees to report from Hixson et al. (2017). It however, disagrees with result obtained by Ayisi et al. (2018) when these FAs were clustered, although, it is in line with the separations of FAs from gene expressions. The results from this study can be due to the differences in fish species and sizes, the selected genes and dietary FAs which were considered as suggested by Strobel et al. (2022), Rombenso et al. (2022) and Ayisi et al. (2018).

It can be concluded that, dietary lipid supplementation affected the mRNA expression levels of G6PD, 6PGD and CD36. Fish had the affinity to utilize more of 18: 3n-3 and 18: n-9 FAs as indicated from those fed FCO. This indicated that, LA and n-6 PUFA was at balanced levels and both lipids are efficient for fish growth. Alternative lipids had little effect on the digestive and hepatic enzyme activities, and also fish growth performances. However, since the hepatic enzyme activities is the first to be measured, further studies are needed to understand how they correlate to lipid and liver functioning to understand their effect to growth performances and nutrient retention.

Ethical Statement

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Funding Information

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Author Contribution

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Conflict of Interest

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