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Evaluation of blended virgin coconut oil and fish oil on growth performance and resistance to *Streptococcus iniae* challenge of Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Five isolipidic experimental diets (32% crude protein) were formulated to contain 3% fish oil (FO) and virgin coconut oil (3VCO) as sole lipids or blends of FO + VCO in ratios of 75:25% (0.75VCO), 50:50% (1.5VCO) and 25:75% (2.25VCO). Triplicate groups of *O. niloticus* were fed one of five diets to apparent satiation, twice daily for 8 weeks. It was observed that fish fed diet 3VCO exhibited the best performance with respect to feed intake (492.1 g), final weight (214.60 g) and weight gain (154.90 g). Significant effects of dietary fatty acid profile were reflected in fish fed the diets in whole body, muscle and liver C12:0 and C14:0. However, eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) were significantly different ($P \geq 0.05$) compared to their respective diets while liver n-3: n-6 ratio significantly increased and recorded low levels in whole body and muscle. Statistically, least values of mortality were recorded as VCO levels were elevated when fish were subjected to *Streptococcus iniae* infection while plasma metabolite indicators among treatments were not altered. The inclusion of VCO at 3% in the diet gave excellent performance, indicating that it could wholly replace FO and as such represents a better alternative lipid source for feeding *O. niloticus*.

1. Introduction

Dietary lipids are the only source of essential fatty acids and also provide highly digestible energy while facilitating the absorption of fat-soluble nutrients necessary for proper functioning of physiological processes and to some extent maintaining biological structure [30,55].

Fish oil and fish meal is considered as the main protein components of feed [36,57] in the aquaculture sector and as such influences the cost of production. It is therefore expected that, higher demand in these components will raise the cost of feed production and thereby affect production rate and the ability of the industry to maintain its pace and stability in growth. Also, the higher demand for fish oil has endangered some fish species (herring, sardine, anchovy, capelina, etc) which are considered to have low economic value and less for human consumption used in the production of the oil as they have been overexploited [10,17,49,58,75].

It is based on these facts that researchers have focused on reducing the cost of feed by producing practical diets that are available and cheaper at all times [24,33]. As such, dietary alternatives to FO has become the best option because they are currently incorporated at higher levels as FO is becoming costlier and less available [66].

Several studies have shown that sources and levels of dietary lipid affect the growth performance and also health of fish [31]. Other investigations have also shown the cost effectiveness in protein sparing effect of lipids to fish [22,60].

Vegetable oils have been successfully incorporated in aqua-feed for various fish species with similar or improved growth results as fish oil [9,41,47,62,63,72]. Vegetable oils have thus been considered as a suitable replacement of marine fish oil [11,25], especially for fish species with preference for n-6 fatty acids (FAs) unlike n-3 FAs [53] of which there are more to explore and enhance this substitution.

Nile tilapia (*O. niloticus*), a widely cultured fish species has been fed on a series of alternative lipids and blends with fish oil (FO) at various levels. These include soybean oil, sunflower oil, linseed oil and palm related oils, among many others [26,40–43] with a variety of desirable characteristics ranging from environmental tolerance to physiological changes [3]. Nile tilapia can accept higher levels of linoleic (n-6) series FAs (18:2n-6 and 20:4n-6) with normal growth and reproduction [41,71] than other warm-water

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fish that require more linolenic (n-3) FA series (18:3n-3; 20:5n-3 and 22:6n-3) in the diet [54].

Consumers' health in terms of immune response and disease resistance has been shown to be influenced by the choice of lipid FAs [10,44]. A deficiency in linolenic FA has been shown to have an adverse effect on antibody production and macrophage killing ability [27] while causing mortality in excessive amount [20].

Irrespective of widely available reports on vegetable oils (VO) as lipids in growth performance and disease resistance [2,7,12], few studies have evaluated virgin coconut oil as a dietary lipid for *O. niloticus* [4,34,46,35,70].

Virgin coconut oil (VCO) has the ability to increase antioxidant enzymes while reducing lipid peroxidation [39]. According to [15] and [73], the abundant MUFAs (65%) of VCO did not participate in the biosynthesis and transport of cholesterol and as such allowed for mobilization of protein for body protein synthesis. It has also been shown to maintain normal levels of lipid parameters in serum and tissues and inhibit LDL-oxidation [14,38] while having the ability to destroy pathogenic gram-negative bacteria with appropriate chelator [14–15].

This study was conducted to evaluate the effects of replacing fish oil with varying levels of virgin coconut oil on the growth performance, fatty acid composition and immune response to a *Streptococcus iniae* challenge in Nile tilapia (*Oreochromis niloticus*).

2. Materials and methods

2.1. Experimental diet preparation

Five isolipidic experimental diets were formulated to contain different lipid sources which included fish oil (FO) (Nonghao Feed Company, Shanghai, China), and Virgin Coconut oil (The Philippines) as the sole lipid source, blends of FO + VCO (50:50%), or in partial replacement of FO at increasing levels of VCO at 25 and 75% as represented by FO, 3VCO, 0.75VCO, 1.5VCO, and 2.25VCO respectively (Table 1).

Dietary ingredients were finely ground and sieved (40 mm mesh) before the addition of oil and approximately 200 ml of deionized water/kg diet. Extruded pellets were produced by an extrusion mill (SLP-45, Chinese Fishery Machinery and Instrument Research Institute of the Chinese Academy of Fishery Sciences, Shanghai, China) and air-dried at room temperature to a moisture content of 13%. Pellets were then sieved to obtain appropriate sizes and were stored frozen in air-tight plastic bags at −20 °C for subsequent use. Triplicate diet samples were analyzed to confirm the proximate composition according to standard methods for the determination of dry matter, protein and ash content of animal feeds [6]. Lipid content was determined following the method of [19] and the diets were analyzed for fatty acid composition (Table 2).

2.2. Fish and facilities

O. niloticus used in this experiment were obtained from Hainan Xinji Aquatic Science & Technology Co. Ltd, China, transported to concrete tank facilities at the Shanghai Ocean University Aquaculture Farm (Binhai, Shanghai, China), and acclimated for two weeks. Fish were fed commercial fish pellets (Tongwei Company Limited, Chengdu, China) during acclimation. 750 fish ranging between 53 and 56.5 g (55.35 ± 3.22 g; mean ± SD) were randomly stocked in 15 cages (2.0 × 1.0 × 1.0 m, L × W × D) in indoor concrete tanks at a density of 50 fish per cage. The tanks were supplied with a constant flow of well water and continuously aerated with air stones. Water samples were taken at 20 cm below the water surface. The water temperature monitored during the feeding trial ranged from 29.11 °C to 29.90 °C, pH from 7.37 to 7.55, dissolved oxygen from 7.07 to 7.70, nitrogen from 0.02 to 0.04 and ammonia ranging from 0.13 to 0.30 mg/l. Triplicate groups of fish per treatment were fed one of five experimental diets twice daily (008:30-009:00 and 16:00-16:30) to visual satiety with feed intake recorded by the difference in weight prior to and after feeding. Fish in each group were batch-weighed and counted to monitor growth, feed utilization survival once every two weeks whiles tanks were cleaned. Diet

Table 1
Ingredients and proximate composition of experimental diets with different lipid sources (% DM).

Ingredients (g 100 g^{-1}) feed items	FO	0.75VCO	1.5VCO	2.25VCO	3VCO
Fish meal	10.00	10.00	10.00	10.00	10.00
Soybean meal	20.00	20.00	20.00	20.00	20.00
Wheat bran	20.00	20.00	20.00	20.00	20.00
Rape seed meal	24.26	24.26	24.26	24.26	24.26
Wheat middling	20.00	20.00	20.00	20.00	20.00
Fish oil	3.00	2.25	1.50	0.75	0.00
Coconut oil	0.00	0.75	1.50	2.25	3.00
Vitamin and mineral mix	0.65	0.65	0.65	0.65	0.65
Vitamin C	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.50	0.50	0.50	0.50	0.50
Inositol	0.04	0.04	0.04	0.04	0.04
$Ca(H_2PO_4)$	1.50	1.50	1.50	1.50	1.50
Total	100.00	100.00	100.00	100.00	100.00
Chemical analyses (%) on DM basis					
Crude protein	32.14 ± 0.29	32.03 ± 0.15	30.68 ± 0.09	31.25 ± 0.24	31.60 ± 0.23
Crude lipid	4.30 ± 0.42	4.22 ± 0.15	4.63 ± 0.21	4.27 ± 0.10	4.29 ± 0.08
Moisture	13.30 ± 2.20	13.16 ± 1.40	13.57 ± 2.41	14.35 ± 2.22	13.85 ± 0.87
Ash	3.27 ± 0.01	3.304 ± 0.01	3.31 ± 0.01	3.33 ± 0.01	3.48 ± 0.06

Proximate composition values represent Standard Error Means (±SEM) of triplicate samples.
Fish oil (FO) and all other ingredients was purchased from (Nonghao Feed Company (Shanghai, China), virgin coconut oil was obtained from the Philippines.
Vitamin premix (mg or IU/kg diet): vitamin A, 6000 IU; thiamine, 15 mg; riboflavin, 15 mg; nicotinic acid, 30 mg; pantothenic acid, 35 mg; pyridoxine HCl, 6 mg; cyanocobalamin, 0.03 mg; ascorbic acid, 200 mg; vitamin D3, 2000 IU; vitamin E, 50 mg; menadione, 5 mg; folic acid, 3 mg; biotin, 0.2 mg
Mineral premix (mg or g/kg diet): iodine, 0.4 mg; cobalt, 0.1 mg; copper, 4 mg; iron, 150 mg; zinc, 80 mg; manganese, 20 mg; selenium, 0.1 mg; magnesium, 100 mg; zeolite powder, 3.539 g.

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

Fatty acid (S)	FO	0.75VCO	1.5VCO	2.25VCO	3VCO
8:0	0.00 ± 0.00	0.90 ± 0.08	1.82 ± 0.05	2.34 ± 0.04	2.51 ± 0.64
10:0	0.00 ± 0.00^{a}	0.93 ± 0.03^{ab}	1.65 ± 0.04^{bc}	2.20 ± 0.06^{c}	2.40 ± 0.64^{c}
12:0	0.23 ± 0.04^{a}	5.51 ± 2.79^{ab}	14.87 ± 0.41^{b}	19.64 ± 0.36^{c}	20.97 ± 5.60^{c}
14:0	3.63 ± 0.13^{a}	5.56 ± 0.23^{ab}	6.84 ± 0.04^{ab}	8.31 ± 0.05^{b}	8.65 ± 1.10^{b}
16:0	15.33 ± 6.45	11.16 ± 1.18	11.15 ± 1.77	14.36 ± 0.78	15.22 ± 0.83
18:0	2.56 ± 1.38	1.55 ± 0.20	1.67 ± 0.38	2.52 ± 0.22	3.27 ± 0.41
20:0	0.11 ± 0.10	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.08 ± 0.08
22:0	0.04 ± 0.02	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.07 ± 0.07
Total SFA's	21.90 ± 8.12	25.64 ± 4.54	38.01 ± 2.70	49.37 ± 1.51	53.17 ± 9.37
16:1(n-7)	5.02 ± 0.48^{c}	4.19 ± 0.10^{c}	2.92 ± 0.10^{b}	1.84 ± 0.03^{a}	0.91 ± 0.02^{a}
16:1(n-9)	0.25 ± 0.99^{bc}	0.35 ± 0.04^{c}	0.14 ± 0.10^{b}	0.03 ± 0.03^{a}	0.02 ± 0.02^{a}
18:1(n-7)	11.25 ± 4.67	13.00 ± 6.60	5.01 ± 0.16	9.40 ± 4.75	9.81 ± 6.45
18:1(n-9)	19.98 ± 7.53	19.11 ± 6.26	21.65 ± 0.70	13.91 ± 4.50	10.71 ± 3.92
20:1(n-9)	1.19 ± 0.16^{c}	0.56 ± 0.28^{bc}	0.57 ± 0.02^{bc}	0.26 ± 0.28^{a}	0.10 ± 0.10^{a}
22:1(n-9)	0.73 ± 0.16^{cb}	0.44 ± 0.22^{ba}	0.31 ± 0.10^{ba}	0.00 ± 0.00^{a}	0.07 ± 0.07^{a}
Total	38.42 ± 13.99	37.65 ± 13.50	30.60 ± 1.18	25.44 ± 9.59	21.62 ± 10.58
MUFAs					
16:4(n-1)	0.15 ± 0.05	0.12 ± 0.06	0.13 ± 0.01	0.11 ± 0.01	0.07 ± 0.01
18:2(n-6)	25.22 ± 2.38	25.96 ± 0.81	24.14 ± 0.76	20.87 ± 0.26	23.12 ± 5.01
18:3(n-6)	0.02 ± 0.02	0.00 ± 0.00	0.04 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
20:4(n-6)ARA	0.49 ± 0.06^{b}	0.19 ± 0.10^{ba}	0.16 ± 0.08^{a}	0.11 ± 0.03^{a}	0.04 ± 0.02^{a}
Total n-6	25.73 ± 2.45	26.15 ± 0.91	24.34 ± 0.86	20.98 ± 0.29	23.16 ± 5.03
18:3(n-3)	0.46 ± 0.12^{b}	0.27 ± 0.03^{ba}	0.27 ± 0.06^{ba}	0.27 ± 0.05^{ba}	0.11 ± 0.05^{a}
18:4(n-3)	0.75 ± 0.08^{b}	0.48 ± 0.06^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.04 ± 0.04^{a}
20:4(n-3)	0.44 ± 0.39	0.17 ± 0.17	0.43 ± 0.04	0.27 ± 0.01	0.11 ± 0.01
20:5(n-3)EPA	*4.18 ± 0.40*^{c}	*3.56 ± 0.07*^{c}	*2.40 ± 0.05*^{b}	*1.57 ± 0.05*^{a}	*0.85 ± 0.01*^{a}
22:5(n-3)	0.07 ± 0.07	0.49 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22:6(n-3)DHA	*7.35 ± 0.81*^{b}	*5.22 ± 0.17*^{b}	*3.35 ± 0.07*^{b}	*1.87 ± 0.07*^{a}	*0.87 ± 0.06*^{a}
Total n-3	13.25 ± 1.87^{e}	10.19 ± 0.75^{d}	6.45 ± 0.22^{c}	3.98 ± 0.18^{b}	1.98 ± 0.16^{a}
Total PUFAs	*39.13 ± 4.37*^{b}	*36.46 ± 1.72*^{b}	*30.92 ± 1.09*^{b}	*25.07 ± 0.48*^{a}	*25.21 ± 5.20*^{a}
Total LC-PUFAs	12.53 ± 1.73^{e}	9.63 ± 0.76^{d}	6.34 ± 0.24^{c}	3.82 ± 0.16^{b}	1.87 ± 0.10^{a}
Total MC-PUFAs	*26.45 ± 2.60*^{c}	*26.71 ± 0.90*^{c}	*24.45 ± 0.84*^{b}	*21.14 ± 0.31*^{a}	*23.27 ± 5.19*^{b}
n-3:n-6	0.52 ± 0.76^{d}	0.39 ± 0.82^{dc}	0.27 ± 0.26^{cb}	0.19 ± 0.62^{ba}	0.09 ± 0.03^{a}

SFA = saturated fatty acids.
MUFA = mono unsaturated fatty acid.
PUFA = polyunsaturated fatty acid.
LC-PUFA = long chain polyunsaturated fatty acid.
LC-MUFA = long chain mono unsaturated fatty acid.
Significant differences determined by one-way ANOVA ($P < 0.05$); means ± SEM.

was withheld 24 h prior to sampling days and offered once after sampling.

2.3. Harvest, sample collection and growth performance

Fish were starved for 24 h prior to harvest after completion of the trial period. All surviving fish within each tank were counted and batch-weighed. Fifteen fish at the initial stage of the experiment and 75 at the end of the trial were randomly sampled, euthanized with an overdose of tricaine methane sulfonate (MS-222 at 200 mg/L in culture water), weighed individually, pooled and stored at −20 °C for subsequent determination of proximate composition and analyses. Each sample was analyzed in triplicate for whole body proximate composition following standard methods [5]. A muscle sample (5 × 2 × 3 cm without skin) was taken from the left back, 3 cm below the dorsal fin, from three fish per tank [21]. Livers were dissected to calculate hepatosomatic index. The following parameters were calculated as such:

1. Total weight gain (WG)
   WG = FW (g) − IW (g).
   FW and IW for final weight and initial weight, respectively.
2. Specific growth rate (SGR) (%)
   SGR (%) = (ln FW (g) − ln IW) (g)/T × 100.
   T for total number of culture days.
3. Feed intake (FI) for the total feed consumed (g) during the entire trial.
4. Feed conversion ratio (FCR)
   FCR = FI (g)/WG (g).
5. Protein efficiency ratio (PER)
   PER = WG (g)/PI (g).
   WG and PI for body weight gain and protein intake, respectively.
6. The hepatosomatic index (HSI)
   HSI = [LW/BW] × 100
   LW and BW stand for liver weight (g) and total body weight (g), respectively.
7. Survival rate (SR)%
   SR = [TF/TFT] × 100
   TF and TFT for total number of fish stocked and total number of fish at termination point, respectively.
8. Condition factor (K)
   K = Total BW (g)/TL (cm) 3 × 100.
   TL for total length.

2.4. Assays of water, ash and protein content

The whole body, dorsal muscle and liver from all groups were analyzed in triplicate for moisture and protein content according to standard methods [6]: moisture was determined by oven drying

at 105 °C to constant weight; ash content was determined by incinerating dry matter samples in a muffle furnace at 550 °C for 12 h, crude protein was determined by the Kjeldahl method and by multiplying the nitrogen content by 6.25. Ash and water content were expressed as percentage content, and protein was expressed as% dry weight, (DW).

2.5. Analyses of lipid content and fatty acid composition

Dried tissues were ground individually to a powder before each assay was performed. The total lipid (TL) of each sample was extracted with chloroform–methanol (2:1, V/V), according to [19]. Fatty acid methyl esters (FAME) were prepared by transesterification with 0.4 M KOH-methanol and were then detected by gas chromatograph (GC-7890A, USA) following [23]. Fatty acid content was determined using the normalization method, while peaks obtained were identified by comparing retention time with a known fatty acid methyl ester standard (sigma-aldrichchemie). All measurements were performed in triplicate; the fatty acids content was expressed as area percentage.

2.6. Plasma metabolites

Fish were quickly (<1 min) dip netted from the experimental tanks in groups of five individuals and immediately anesthetized with 2-phenoxyethanol (1:300 v/v) in water. Blood samples from each fish were collected from the caudal vein using a 1-mL syringe with a 22-gauge × 3.8-cm (11/2 in) needle and were placed into 1.5-mL heparinized micro centrifuge tubes. The tubes were centrifuged (Eppendorf centrifuge 5417R) at 3500*g* for 15 min. after the blood had clotted. Serum samples were removed from the tubes and stored at −20 °C for the analysis. Serum levels of total cholesterol (TC), total protein (TP) and triglycerides (TG) (i.e., as triacylglycerol) were measured using a biochemical analyzer (Mindary Chemistry Analyzer BS-200, Shenzhen, China).

2.7. Bacterial challenge

Streptococcus iniae (*S. iniae* – ARS-98-60) frozen stock-culture obtained from an outbreak of streptococcal disease in Nile tilapia was grown in a tryptic soy broth (TSB) at 25 °C with shaking at 100 rpm for 24 h. The concentration of the culture was adjusted to an optimal density of 1.2, measured on a Biorad SmartSpec 3000 spectrophotometer (Bio-Rad laboratories, Hercules, Carlifornia, USA) at 540 nm to give an *S. iniae* concentration of 1×10^9 colony forming units (CFU)/mL. The desired bacterial concentration was prepared in a sterile medium by 1:10 serial dilutions. At the end of the feeding trial, 10 fish were randomly selected from each triplicate and were stocked in a 57-L aquarium containing 50 L of water and were kept at 28–29 °C throughout the challenge experiment. Fish were intra-peritoneally (IP) injected with 0.1 mL of 1×10^6 cfu/mL of *S. iniae* (10×10^5 cfu/fish) using a tuberculin syringe. They continued to receive their respective diets. Fish were monitored and mortality was recorded twice daily for 16 days following injection and dead fish were removed.

Table 3
Growth performance and feed utilization of *O. niloticus* after 8 weeks feeding trail.

Group/Growth performance	FO	0.75VCO	1.5VCO	2.25VCO	3VCO
Initial body weight	56.67 ± 2.67	55.84 ± 3.55	52.23 ± 3.25	54.83 ± 2.95	59.67 ± 3.70
Initial length	13.89 ± 0.33	14.03 ± 0.49	13.84 ± 0.09	14.00 ± 0.41	14.31 ± 0.19
Final weight	196.90 ± 7.22^{ab}	181.00 ± 5.72^{a}	205.20 ± 6.94^{ab}	187.00 ± 8.83^{ab}	214.60 ± 7.84^{b}
Final length	21.64 ± 0.28^{ab}	21.13 ± 0.24^{a}	21.93 ± 0.26^{ab}	21.37 ± 0.34^{ab}	22.31 ± 0.33^{b}
Feed intake	405.60 ± 20.67^{ab}	322.80 ± 19.22^{a}	410.70 ± 29.16^{ab}	373.70 ± 37.53^{b}	492.10 ± 29.90^{b}
[a]WG	140.20 ± 6.32	125.20 ± 32.48	153.00 ± 16.50	132.20 ± 12.79	154.90 ± 9.16
[b]FCR	2.90 ± 0.16	2.88 ± 0.60	2.72 ± 0.19	2.84 ± 0.19	3.19 ± 0.22
[c]SGR	2.24 ± 0.17	2.11 ± 0.50	2.44 ± 0.21	2.19 ± 0.08	2.29 ± 0.11
[d]PER	4.37 ± 0.22	3.95 ± 1.01	4.99 ± 0.55	4.23 ± 0.40	4.90 ± 0.27
[e]HIS	0.94 ± 0.12^{a}	1.62 ± 0.14^{b}	1.70 ± 0.133^{b}	1.47 ± 0.10^{b}	1.46 ± 0.12^{b}
[f]K	1.95 ± 0.38	1.90 ± 0.02	1.94 ± 0.01	1.91 ± 0.01	1.92 ± 0.03

All values are mean ± SEM.
[a] WG = weight gain.
[b] FCR = feed conversion ratio.
[c] SGR = specific growth rate.
[d] PER = protein efficiency ratio.
[e] HSI = hepatosomatic index.
[f] K = condition factor.

Table 4
Major nutrient composition (%DM) of moisture, crude lipid, protein and ash content of whole body and muscle of *Nile* tilapia fed different diets for 8 weeks.

Initial whole body		FO	0.75VCO	1.5VCO	2.25VCO	3VCO
		Final whole body				
Moisture	10.75 ± 1.20	11.40 ± 1.45	11.21 ± 0.90	11.18 ± 1.20	11.07 ± 0.31	11.09 ± 0.58
Protein	63.30 ± 1.42	58.40 ± 0.87	59.69 ± 0.33	61.00 ± 1.60	62.61 ± 2.46	64.06 ± 1.48
Lipid	7.04 ± 0.32^{bc}	2.74 ± 0.90^{a}	3.26 ± 0.33^{a}	4.67 ± 0.59^{ab}	7.44 ± 0.36^{c}	6.62 ± 0.15^{bc}
Ash	0.28 ± 0.00^{a}	0.80 ± 0.01^{c}	0.73 ± 0.01^{b}	0.72 ± 0.00^{b}	0.78 ± 001^{c}	0.72 ± 0.01^{b}
Muscle						
Moisture		10.45 ± 0.15^{a}	10.52 ± 0.86^{a}	10.42 ± 0.48^{a}	10.69 ± 0.18^{ab}	10.90 ± 0.90^{b}
Protein		94.33 ± 0.41^{a}	95.85 ± 0.20^{ab}	96.47 ± 0.32^{b}	95.63 ± 0.60^{ab}	96.48 ± 0.26^{b}
Lipid		2.22 ± 0.22^{c}	1.77 ± 0.18^{cb}	1.15 ± 0.04^{ba}	1.07 ± 0.17^{ba}	0.90 ± 0.13^{a}
Ash		0.28 ± 0.00^{ab}	0.26 ± 0.00^{ab}	0.27 ± 0.00^{ab}	0.25 ± 0.02^{a}	0.29 ± 0.00^{b}

Different superscript in each row represent significant differences ($P < 0.05$) determined by one-way ANOVA.

2.8. Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) to test the effect of the five experimental diets and by Tukey's multiple test to compare treatment means. Differences were considered significant at 0.05 probability level for all data. All analyses were performed using a Graph Pad Prism V.5.03 and the results presented as mean ± standard error of the mean (SEM).

3. Results

3.1. Growth performance

Growth performance and survival of *O. niloticus* were not impaired by either the partial or complete replacement of dietary fish oil (FO) with Virgin Coconut oil (VCO) during the 8-week experiment. There was no significance among groups for WG, FCR, specific growth rate (SGR) and PER. However, feed intake and final weight were significantly lower in fish fed diet 0.75VCO (322.80 ± 19.22 g; 181 ± 5.72 g) than diet 3VCO (492.10 ± 29.90 g; 214.60 ± 7.84 g), respectively (Table 3).

3.2. Proximate composition

Elevated levels of VCO did not significantly affect whole body moisture (11.21–11.40%) and crude protein (58.40–64.0%) levels in all treatment groups. On the contrary, lipid and ash contents were significantly different among treatment groups (Table 4).

Higher levels of moisture were observed among treatment groups at elevated levels of VCO (10.52–10.90%) with the exception of group 1.5VCO which decreased (10.42%) in muscle proximate composition. Lipid content decreased significantly with increasing levels of VCO (2.22–0.90 g). Crude protein of treatment FO was observed to be significantly different from treatments 1.5VCO and 3VCO. Ash content also differed between treatments 2.25VCO and 3VCO at 0.25 g and 0.29 g, respectively (Table 4).

3.3. Whole body fatty acids

Fatty acid content of whole body among treatments was observed to differ ($P < 0.05$) at all levels (Table 5). A difference in single fatty acids was observed for C12 (0.00–8.93%) and C14 (3.68–8.67%). It was also observed that with every VCO addition, there was an increase of saturated fatty acid (SFA) (from 38.59% to 48.61%), with a decrease in mono-unsaturated fatty acid (MUFA) from diet FO to 3VCO ($P < 0.05$). Treatment 3VCO was therefore characterized by 48.41% SFA and 35.35% MUFA, while poly-unsaturated fatty acid, PUFA, showed significance across treatments. N-3 series MUFAs were affected to a great extent among treatments, decreasing from group FO (3.17%) to 2.25VCO (1.01%). The same trend was observed in DHA (2.03–0.40%), LC-PUFA (2.91–0.85%) and n-3: n-6 ratio (0.20–0.06%) (Table 5).

3.4. Muscle fatty acids

Fatty acids analyzed in the muscle (M) showed significant differences among treatments. C12 and C14 increased significantly among fish fed elevated levels of VCO (Table 6). However, total SFAs in FO and 0.75VCO did not differ significantly and the same trend was observed among 1.5VCO, 2.25VCO and 3VCO diets. Irrespective of different dietary feeds, the amount of long chain poly-unsaturated fatty acid (LC-PUFA) in muscle of FO, 0.75VCO and 1.5VCO did not differ ($P < 0.05$) although a significant difference in 22:6(n-3) was observed. The N-3 series in all treatments was affected with the inclusion of VCO, decreasing from 12.37% (FO) to 4.42% (3VCO) although, treatments FO-1.5VCO and 2.25VCO-3VCO differed statistically in comparison. DHA and PUFA

Table 5
Fatty acid (% total FA) of whole body of tilapia fed diets of elevated VCO levels for 8 weeks.

Fatty acid (S)	FO	0.75VCO	1.5VCO	2.25VCO	3VCO
10:0	0.000 ± 0.00^{a}	0.000 ± 0.00^{a}	0.11 ± 0.02^{ab}	0.18 ± 0.06^{b}	0.25 ± 0.05^{b}
12:0	0.000 ± 0.00^{a}	2.40 ± 0.07^{b}	5.27 ± 0.46^{b}	7.39 ± 0.59^{c}	8.93 ± 0.65^{c}
14:0	3.68 ± 0.07^{a}	4.18 ± 0.43^{a}	6.44 ± 0.31^{b}	7.61 ± 0.35^{c}	8.67 ± 0.31^{c}
16:0	27.41 ± 0.19^{b}	25.89 ± 0.19^{ba}	25.87 ± 0.41^{ba}	24.19 ± 0.62^{a}	24.19 ± 0.49^{a}
18:0	7.44 ± 0.14^{c}	7.16 ± 0.13^{cb}	6.90 ± 0.40^{b}	6.73 ± 0.21^{ab}	6.53 ± 0.22^{ab}
20:0	0.06 ± 0.06	0.01 ± 0.01	0.02 ± 0.02	0.04 ± 0.04	0.04 ± 0.02
Total SFA's	38.59 ± 0.46^{b}	39.64 ± 0.83^{b}	44.61 ± 1.62^{a}	46.14 ± 1.87^{a}	48.61 ± 1.74^{a}
16:1(n-7)	3.63 ± 1.01	3.91 ± 0.04	4.29 ± 0.22	2.82 ± 0.41	2.68 ± 0.39
16:1(n-9)	1.43 ± 1.15	0.21 ± 0.03	0.25 ± 0.02	0.54 ± 0.28	0.58 ± 0.39
18:1(n-7)	5.61 ± 0.15^{b}	4.99 ± 0.04^{ba}	5.24 ± 0.11^{ba}	4.97 ± 0.27^{ba}	4.67 ± 0.25^{a}
18:1(n-9)	30.60 ± 0.32^{c}	30.02 ± 0.35^{cb}	28.79 ± 0.73^{b}	27.64 ± 0.43^{ab}	26.91 ± 0.47^{a}
20:1(n-7)	0.52 ± 0.18	0.55 ± 0.05	0.38 ± 0.13	0.33 ± 0.03	0.51 ± 0.17
20:1(n-9)	0.10 ± 0.10	0.00 ± 0.00	0.05 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
Total MUFAs	41.89 ± 2.91^{b}	39.68 ± 0.51^{b}	39.00 ± 1.25^{b}	36.30 ± 1.42^{a}	35.35 ± 1.67^{a}
18:2(n-6)	16.11 ± 0.39^{ba}	18.25 ± 0.20^{b}	15.22 ± 1.00^{ba}	16.40 ± 0.90^{ba}	14.39 ± 0.59^{a}
20:2(n-6)	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
20:4(n-6)ARA	0.11 ± 0.06	0.17 ± 0.06	0.14 ± 0.05	0.08 ± 0.05	0.16 ± 0.14
Total n-6	16.22 ± 0.45	18.42 ± 0.26	15.39 ± 1.08	16.48 ± 0.95	14.55 ± 0.73
18:3(n-3)	0.27 ± 0.12	0.12 ± 0.02	0.19 ± 0.06	0.18 ± 0.04	0.11 ± 0.09
18:4(n-3)	0.10 ± 0.10	0.27 ± 0.13	0.06 ± 0.03	0.06 ± 0.04	0.10 ± 0.06
20:4(n-3)	0.64 ± 0.17^{b}	0.41 ± 0.12^{ba}	0.35 ± 0.00^{ba}	0.09 ± 0.03^{a}	0.30 ± 0.11^{ba}
20:5(n-3)EPA	0.13 ± 0.10	0.10 ± 0.02	0.12 ± 0.08	0.18 ± 0.05	0.13 ± 0.05
22:6(n-3)DHA	2.03 ± 0.06^{b}	1.28 ± 0.03^{b}	0.94 ± 0.03^{b}	0.50 ± 0.06^{a}	0.40 ± 0.03^{a}
Total n-3	3.17 ± 0.55^{c}	2.18 ± 0.32^{b}	1.66 ± 0.11^{ba}	1.01 ± 0.22^{a}	1.04 ± 0.34^{a}
Total PUFAs	19.39 ± 1.0^{c}	20.60 ± 0.58^{c}	17.05 ± 1.19^{b}	17.49 ± 1.17^{b}	15.59 ± 1.07^{a}
Total LC-PUFAs	2.91 ± 0.39^{b}	1.96 ± 0.23^{ba}	1.58 ± 0.19^{ba}	0.85 ± 0.19^{a}	0.99 ± 0.33^{a}
Total MC-PUFAs	16.48 ± 0.55^{b}	18.64 ± 0.35^{c}	15.47 ± 1.09^{ba}	16.64 ± 0.98^{b}	14.60 ± 0.74^{a}
n-3:n-6	0.20 ± 1.22^{b}	0.12 ± 1.23^{ba}	0.12 ± 0.10^{ba}	0.06 ± 0.23^{a}	0.07 ± 0.47^{a}

Values of different superscript on same row are significantly different ($P < 0.05$); mean ± SEM.
DHA = docosahexaenoic acid EPA = eicosapentaenoic acid.

Table 6
Fatty acid composition of muscle (% total FA) of tilapia fed different diets for 8 weeks.

Fatty acid (S)	FO	0.75VCO	1.5VCO	2.25VCO	3VCO
10:0	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03	0.03 ± 0.16	0.07 ± 0.03
12:0	0.00 ± 0.00^{a}	1.44 ± 0.43^{ab}	2.81 ± 0.05^{ab}	6.03 ± 1.28^{bc}	7.61 ± 1.86^{c}
14:0	2.48 ± 0.14^{a}	2.70 ± 0.19^{a}	3.97 ± 0.37^{ab}	4.78 ± 1.09^{ab}	6.26 ± 1.11^{b}
16:0	28.20 ± 0.13^{a}	29.74 ± 0.69^{a}	32.67 ± 0.43^{b}	29.03 ± 0.36^{a}	29.47 ± 0.53^{a}
18:0	9.83 ± 1.05	12.23 ± 0.48	12.85 ± 0.51	9.89 ± 1.08	9.79 ± 1.27
20:0	0.05 ± 0.04	0.16 ± 0.11	0.07 ± 0.07	0.24 ± 0.10	0.00 ± 0.00
Total SFA's	*40.56 ± 1.36*^{a}	*46.27 ± 1.90*^{ab}	*52.33 ± 1.46*^{bc}	*50.00 ± 4.07*^{bc}	*53.20 ± 4.80*^{c}
16:1(n-7)	2.41 ± 0.25	1.92 ± 0.20	1.66 ± 0.31	1.70 ± 0.44	2.14 ± 0.21
16:1(n-9)	0.68 ± 0.36	0.08 ± 0.04	0.25 ± 0.25	0.45 ± 0.24	0.00 ± 0.00
18:1(n-7)	5.37 ± 0.05^{b}	5.15 ± 0.09^{ba}	5.46 ± 0.07^{b}	4.68 ± 0.20^{a}	4.91 ± 0.15^{ba}
18:1(n-9)	22.71 ± 0.68^{ba}	22.00 ± 0.28^{ba}	21.65 ± 0.18^{ba}	23.24 ± 0.77^{b}	20.93 ± 0.25^{a}
20:1(n-9)	0.13 ± 0.13	0.00 ± 0.00	0.04 ± 0.04	0.04 ± 0.04	0.01 ± 0.01
20:1(n-7)	0.56 ± 0.35	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total	31.86 ± 1.82^{c}	29.15 ± 0.61^{b}	29.06 ± 0.85^{b}	30.11 ± 1.69^{c}	27.99 ± 0.62^{a}
MUFAs					
18:2(n-6)	13.31 ± 0.15^{ab}	14.81 ± 0.71^{b}	10.92 ± 0.14^{a}	13.90 ± 0.60^{b}	13.24 ± 0.70^{ab}
20:2(n-6)	0.23 ± 0.14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:4(n-6)ARA	*1.93 ± 0.33*	*1.37 ± 0.30*	*1.08 ± 0.17*	*1.52 ± 0.62*	*0.11 ± 0.23*
Total n-6	15.47 ± 0.62	16.18 ± 1.01	12.00 ± 0.31	15.42 ± 1.22	13.35 ± 0.93
18:3(n-3)	0.15 ± 0.06	0.18 ± 0.13	0.16 ± 0.09	0.55 ± 0.14	0.37 ± 0.03
20:4(n-3)	1.62 ± 0.43	1.32 ± 0.23	1.32 ± 0.21	0.50 ± 0.27	0.83 ± 0.12
20:5(n-3)EPA	*0.06 ± 0.06*	*0.02 ± 0.02*	*0.03 ± 0.02*	*0.06 ± 0.03*	*0.08 ± 0.01*
22:5(n-3)	1.52 ± 0.65^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
22:6(n-3)DHA	9.02 ± 0.14^{d}	6.53 ± 0.23^{c}	5.04 ± 0.69^{cb}	2.31 ± 0.38^{a}	3.14 ± 0.54^{ba}
Total n-3	12.37 ± 1.34^{b}	8.05 ± 0.61^{b}	6.55 ± 1.01^{b}	3.42 ± 0.82^{a}	4.42 ± 0.70^{a}
Total PUFAs	27.84 ± 1.96^{b}	24.23 ± 1.62^{b}	18.55 ± 1.32^{a}	18.84 ± 2.04^{a}	17.77 ± 1.63^{a}
Total LC-PUFAs	14.38 ± 1.75^{b}	9.24 ± 0.78^{b}	7.47 ± 1.09^{b}	4.39 ± 1.30^{a}	4.16 ± 0.90^{a}
Total MC-PUFAs	13.46 ± 0.21^{b}	14.99 ± 0.84^{b}	11.08 ± 0.23^{a}	14.45 ± 0.74^{b}	13.61 ± 0.73^{b}
n-3:n-6	0.70 ± 2.16^{c}	0.50 ± 0.60^{b}	0.55 ± 3.26^{b}	0.22 ± 0.67^{a}	0.33 ± 0.75^{a}

Values of different superscript on same row are significantly different ($P < 0.05$); mean ± SEM; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid.

Table 7
Liver fatty acid composition of *O. niloticus* fed elevated levels VCO for 8 weeks.

Fatty acid (S)	FO	0.75VCO	1.5VCO	2.25VCO	3VCO
10:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.04
12:0	0.00 ± 0.00^{a}	0.34 ± 0.17^{a}	3.02 ± 0.76^{b}	4.15 ± 0.89^{bc}	6.30 ± 0.21^{c}
14:0	3.32 ± 0.24 ^{a}	4.59 ± 0.15 ^{a}	6.05 ± 0.10^{b}	6.64 ± 0.28^{b}	8.32 ± 0.08^{c}
16:0	22.56 ± 2.76	28.87 ± 1.90	30.72 ± 0.67	27.37 ± 3.56	25.53 ± 3.58
18:0	13.35 ± 1.03	12.27 ± 1.34	12.25 ± 0.76	10.69 ± 2.07	11.65 ± 0.65
20:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.39 ± 0.39
22:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.32 ± 0.32
Total SFA's	39.23 ± 4.03^{a}	50.10 ± 3.52^{a}	52.04 ± 1.53^{b}	48.85 ± 6.80^{a}	52.59 ± 5.27^{b}
16:1(n-7)	4.18 ± 0.31^{b}	2.20 ± 0.59^{ba}	2.63 ± 0.19^{ba}	2.20 ± 0.58^{ba}	1.49 ± 0.68^{a}
16:1(n-9)	0.85 ± 0.28	1.33 ± 0.19	0.57 ± 0.35	0.68 ± 0.34	0.85 ± 0.44
18:1(n-7)	6.07 ± 0.16^{b}	5.63 ± 0.09^{b}	4.55 ± 0.19^{a}	4.69 ± 0.16^{a}	4.14 ± 0.04^{a}
18:1(n-9)	27.41 ± 0.29^{a}	31.38 ± 1.72^{ba}	29.58 ± 1.13^{ba}	33.08 ± 0.92^{b}	26.52 ± 0.74^{a}
20:1(n-7)	1.12 ± 0.09^{b}	0.30 ± 0.30^{a}	0.09 ± 0.09^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
20:1(n-9)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.39 ± 0.39
Total MUFAs	39.61 ± 1.13^{dc}	40.84 ± 2.89^{d}	37.421.95^{b}	40.65 ± 2.00^{c}	33.39 ± 2.29^{a}
18:2(n-6)	11.45 ± 1.26	9.27 ± 0.78	7.15 ± 0.07	8.82 ± 1.96	9.36 ± 0.45
20:3(n-6)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.39 ± 0.39
20:4(n-6)ARA	*0.08 ± 0.08*	*0.19 ± 0.19*	*0.00 ± 0.00*	*0.00 ± 0.00*	*0.49 ± 0.49*
Total n-6	11.53 ± 1.34	9.46 ± 0.97	7.15 ± 0.07	8.82 ± 1.96	10.24 ± 1.33
16:3(n-4)	0.81 ± 0.51	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:3(n-3)	0.16 ± 0.16	0.00 ± 0.00	0.25 ± 0.13	0.00 ± 0.00	0.00 ± 0.00
20:4(n-3)	0.85 ± 0.34^{cb}	1.06 ± 0.05^{c}	0.15 ± 0.08^{ba}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
20:5(n-3)EPA	*0.16 ± 0.16*^{a}	*3.06 ± 1.17*^{b}	*0.07 ± 0.07*^{a}	*0.00 ± 0.00*^{a}	*0.08 ± 0.08*^{a}
22:5(n-3)	1.07 ± 0.22^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.32 ± 0.32^{ba}
22:6(n-3)DHA	*6.25 ± 0.38*^{c}	*1.05 ± 0.39*^{a}	*3.20 ± 0.27*^{b}	*1.06 ± 0.12*^{a}	*2.48 ± 0.78*^{ba}
Total n-3	8.49 ± 1.26^{c}	5.17 ± 1.61^{c}	3.67 ± 0.55^{b}	1.60 ± 0.12^{a}	2.88 ± 1.18^{ba}
Total PUFAs	*20.83 ± 3.11*^{c}	*14.63 ± 2.58*^{b}	*10.82 ± 0.62*^{a}	*10.42 ± 2.08*^{a}	*13.12 ± 2.51*^{b}
Total LC-PUFAs	8.41 ± 1.18^{c}	5.36 ± 1.80^{c}	03.42 ± 0.42^{b}	1.60 ± 0.12^{a}	2.88 ± 1.18^{ba}
Total MC-PUFAs	*11.61 ± 1.42*^{c}	*9.27 ± 0.78*^{b}	*7.40 ± 0.20*^{a}	*8.82 ± 1.96*^{ab}	*9.36 ± 0.45*^{b}
n-3:n-6	0.74 ± 0.94 ^{c}	0.55 ± 1.66^{b}	0.51 ± 7.86^{b}	0.18 ± 0.06^{a}	0.28 ± 0.89^{a}

Values of different superscript on same row are significantly different ($P < 0.05$); mean ± SEM.
DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid.

Table 8
Plasma metabolites *O. niloticus* fed on elevated levels of VCO for 8 weeks.

Parameters/Lipid sources	FO	0.75VCO	1.5VCO	2.25VCO	3VCO
HDL-C	2.85 ± 0.12^{b}	2.04 ± 0.30^{a}	2.60 ± 0.10^{ba}	2.35 ± 0.07^{ba}	2.51 ± 0.10^{ba}
LDL-C	0.43 ± 0.03	0.32 ± 0.06	0.27 ± 0.02	0.38 ± 0.04	0.51 ± 0.09
HDL-C/LDL-C	6.63 ± 4.00^{b}	6.38 ± 5.00^{ba}	9.63 ± 5.00^{c}	6.18 ± 1.75^{ba}	4.92 ± 1.11^{a}
TC	5.03 ± 0.63	4.80 ± 0.59	4.03 ± 0.22	3.91 ± 0.41	3.11 ± 0.48
TP	39.64 ± 1.96	37.36 ± 1.97	34.28 ± 0.75	34.94 ± 0.85	37.12 ± 1.07
TG	1.72 ± 0.16	1.74 ± 0.78	1.85 ± 0.12	1.98 ± 0.11	2.16 ± 1.27

HDL-C: High density lipoprotein cholesterol (mmol/L).
LDL-C: Low density lipoprotein cholesterol (mmol/L).
TC: Total cholesterol (mmol/L).
TP: Total protein (mmol/L).
TG: Triglyceride (mmol/L).

Table 9
Mortality (%) of *O. niloticus* 16 day post *S. iniae* challenge.

Lipid sources	FO	0.75VCO	1.5VCO	2.25VCO	3VCO
Mortality	53.33 ± 0.67	40.0 ± 2.31	33.33 ± 1.33	46.67 ± 1.76	26.67 ± 1.76

Data mean ± SEM. Differences were determined by one-way ANOVA ($P < 0.05$).

decreased in all five treatments with an increasing rate of VCO in the diets, although the EPA was observed to be at similar levels in all treatments (Table 6).

3.5. Liver fatty acids

The main difference in fatty acids observed in all treatments with an increasing rate of VCO in diets was C12 (0.00–6.30%). The elevated levels of VCO resulted in a significant increase in the total SFAs of liver (L) from diet FO (39%) to diet 3VCO (52%). Although 16:1(n-7), 18:1(n-7) and 20:1(n-7) decreased significantly, the total amount of MUFAs and PUFAs showed inconsistent differences ($P < 0.05$) among treatment groups, but similar trends were observed in DHA and EPA and n-3 series (Table 7).

3.6. Plasma metabolites

High density lipoprotein cholesterol (HDL-C) was the main difference observed in this analysis, with fish fed diet FO recording a higher value of 2.85 ± 0.12 mmol/L and fish fed diet 3VCO recording 2.51 ± 0.10 mmol/L. The ratio of HDL-C/low density lipoprotein cholesterol (LDL-C) differed significantly among all treatments with fish fed diet 1.5VCO recording the highest value (9.63 ± 5.0). However, there was no significant difference ($P < 0.05$) in other parameters measured, including TC, TP and TG (Table 8).

3.7. Bacterial challenge

The cumulative percent mortality of the Nile tilapia 16-day post challenge with *S. iniae* was lower in treatment E compared to treatment A, although, no significant difference ($P \geq 0.005$) was observed among all treatments (Table 9).

4. Discussion

Acceptable growth performances of fish have been reported using a wide range of alternative lipids [63,64] with vegetable oil shown to be an outstanding source for dietary improvement or replacement [45,50]. The increase in weight gain (WG), final weight (FW) and feed intake (FI) in fish fed diet 3VCO indicated the satisfactory acceptance of vegetable oil in freshwater fish growth. Earlier studies indicated that the linoleic (n-6) series FAs are essential FAs requirement for maximal growth of Nile tilapia [36,71]. It was also speculated that the performance of fish fed diet 3VCO in this study could be attributed to the properties of the VCO MUFAs which are easily absorbed for metabolic activities and thereby sparing protein. According to [10], coconut oil is supplied to increase diet flavor (aroma) and thus to serve as an attractant to enhance feed intake [69]. Graded levels of plant oils have also been shown to improve growth rate and enhance feed intake [8]. [1] reported a higher feed intake when coconut oil was fed to African mud catfish (*Clarias gariepinus*). This study confirms the efficiency of coconut oil as reported by [29].

The present study showed that, the growth rate of fish fed dietary 3VCO was associated with high feed intake, indicating the ability to digest and absorb lipid [36] at 3% dietary inclusion.

A correlation between elevated VCO levels and whole body lipid content was observed at increasing levels. The quantity of lipid used in the experiment enhanced the utilization of protein for growth which reflected in the decrease in muscle lipid and increase in whole body and muscle crude protein [1].

Elevated VCO was observed to stimulate the secretion of serum total triglyceride (TG) while lowering plasma cholesterol [39,68]. Vegetable oils have been noted to possess a lower content of cholesterol [66] while the ineffectiveness of VCO MUFAs in biosynthesis and cholesterol transfer may contribute to this effect [15]. This conforms with reports from previous studies on other fish species [52,71]. The levels of LDL-C indicated good lipid metabolic activities in liver and tissues [18], and which also reflected in the whole body lipid [13]. This shows the beneficial effects of VCO in lowering lipid levels in serum and tissue components and LDL-C oxidation [37]. The ratio of HDL-C/LDL-C indicated good transport activity of cholesterol [74] whereas a good indicator of fish health was reflected in HDL-C levels.

The inconsistent trend in total protein as VCO increased could probably be due to differences in nutritional status and other factors [51]; although it was not significantly different, it may indicate good retention of protein.

Tissue FA profile in fish is generally a reflection of dietary FA composition [67]. The results of this study were largely supportive of this concept. However, the paradigm of the FA profiles conformed more with the characterized deviation in the generalized

patterns of FA profile of fish. This effect is characteristically referred to as the indirect effect of saturated FA intake on tissues FA profile [61,63]. N-6 FAs in the fish profile were higher than the n-3 FA, whereas a higher amount of EPA in the diet failed to be reflected in the fish FA profiles. However, DHA was observed to increase in tissue FA with similar trends in both whole body and liver FA profiles. It has been noted that tilapia has the ability to bio-convert EPA and most SFAs in an attempt to maintain LC-PUFAs [59]. Thus, increasing the intake of SFA results in a disproportionate increase of SFA content regardless of dietary intake. Similar reported results have been suggested to indicate a preference in fish for utilizing EPA as an energy source (β-oxidation) and/or in synthesis of DHA [56]. This hypothesis has been suggested to be a function of selectivity for specific FAs, including unsaturated FAs in their body lipid, unlike SFAs. The results obtained in this study with fish fed 3VCO support this although fish fed FO maintained the highest LC-PUFAs in muscle and whole body lipid.

However, it has been suggested that fish cannot effectively incorporate LC-PUFAs in muscle lipid because it cannot explicitly differentiate between LC-PUFAs, MUFAs, and MC-PUFAs [61,63]. Thus, less desirable unsaturated FAs may compete with MC-PUFAs and interfere with attempts to maximize tissue nutritional value. The results of this study showed good retention of LC-PUFAs, greater amounts of MUFAs and MC-PUFAs, which correspond with the performance of fish fed other diets rich in SFA and MC-PUFA [65]. It has also been noted that disproportionate enrichment of 18:2(n-6) in lean fleshed fish may be most directly related to preferential incorporation of this FA into polar lipids. This hypothesis also explains the lower LC-PUFAs content observed in this study [65].

The experiment confirmed that the FA profile of O. niloticus is affected by dietary lipid sources.

Contradictory information on the effect of dietary essential fatty acids on immune response and disease has been reported in recent years. Abnormalities observed in Nile tilapia RBC and WBC counts were attributed to excessively high dietary preformed LC-PUFAs levels after feeding fish for 12 weeks with 7% menhaden oil as the sole lipid source unlike those fed other oils [71]. However, no significant effects were observed in the RBC and WBC when fish were fed menhaden oil, soybean oil, beef tallow, or corn oil, although those fed menhaden oil were lower than the others [28]. It has been documented that excessive levels of n-3 FAs decrease antibody titers [16] and increase mortality of fish [27]. No significant difference was observed in cumulative mortality against *S. iniae* 16-day post challenge in this study. However, it is worth noting that fish fed 3VCO had the lowest mortality.

Also, fish fed 0.75VCO showed lower mortality than those fed FO, 1.5VCO, and 2.25VCO. This observation confirms that n-3 FAs at moderate levels in diets can be beneficial to disease resistance as reported by other investigations [27,31].

The FA composition of virgin coconut oil demonstrated its antibacterial, antiprotozoal and antiviral properties [48] in the survival of fish fed solely 3VCO. [14] indicated that monoglycerides and FAs ranging between C6 to C14 have the ability to inactivate all members of herpes simplex virus (HSV). Lauric acid has also been shown to destroy pathogenic gram-negative bacteria with an appropriate chelator. The results obtained in this study therefore confirm that the possession of these active FAs components destroys pathogenic bacteria and therefore agrees with the report that indicated monolaurin as an additional property of VCO FA which enhances the destruction of gram-negative bacteria [32]. Thus, VCO demonstrates to have medicinal and therapeutic abilities to boost the immune system of fish fed VCO without compromising growth performance.

5. Conclusion

The performance of 3VCO has ascertained the importance of n-6 FA as required by tilapia although, the different inclusion levels did not impair growth while the FA profiles were significantly altered. The study has demonstrated that manipulation of dietary lipids sources can alter FA composition of fish to yield a desirable FA composition. Tilapia resistance to S. iniae was improved with VCO inclusion indicating the antibiotic properties of VCO can replace various antibiotics and other therapeutic treatments with reduced or no adverse environmental consequences.

The incorporation of VCO at 3% in diets gave excellent performance and therefore detailed studies on its sources, essential FA's ratios and levels in relation to growth performance and disease control should be further investigated to enable completely replace the use of FO in Nile tilapia diets.

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