Effect of different dietary lipid sources on growth performance and nutrient utilization of + Nile tilapia (*Oreochromis niloticus*) fingerlings.

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Abstract

A feeding trial was carried out to investigate the use of plant oils in the feeding of *Oreochromis niloticus* fingerlings. Four iso-nitrogenous (30% crude protein) and iso-calorific (17 MJ kg^{-1} gross energy) diets including a fish oil control were formulated to contain 8%. A basal diet containing 100% FO was completely replaced by Soya oil, groundnut oil, and palm oil. A total of 120 *O. niloticus* fingerlings (1.20 ± 0.03 g, mean ± SE) were randomly allocated the diets after 7-day acclimation. There were 10 fish tank-1. Fish were hand-fed to apparent satiation twice for 63 days. The result obtained from the trial show that dietary supplementation of lipids significantly influenced growth performance and nutrient utilization of *O. niloticus* (P<0.05). The fish fed PO had the least weight gain (WG), final weight, protein efficiency ratio and highest compared to FO, SO and GO from d 7-28 (P<0.05). At d 35 the fish fed SO had the highest WG (P<0.05); no significant difference (P>0.05) in WG of fish fed FO, GO and PO diets was observed. The best weekly overall growth at the end of d 56 was in FO group; fish fed PO diet had the least WG, followed by GO. At d 63, WG of fish fed FO diets declined, while fish fed SO and PO diets showed a trend towards increase in growth. Result of ANOVA showed that there were significant differences (P<0.05) in FW (F=11.406, P=0.003), WG (F=11.428, P=0.003), FCR (F=7.274, P=0.011), and PER (F= 10.803, P=0.003) among the diets. At the end of the trial, the best growth in fish was recorded for FO group; however, there were no significant differences (P>0.05) in mean values of FW (P= 0.781), WG (P=0.999), SGR (P=0.991), PER (P=0.997), and FCR (P=0.879) between FO and SO-supplemented diets. The least WG was observed in fish fed PO diet followed by GO. Survival was 100% for all treatments. Whole body moisture and crude protein was least in PO group. FO had the highest (P<0.05) protein compared to all vegetable oils (VOs). However, whole-body protein of fish fed FO and SO were not significantly different (P>0.05). SO had the highest (P<0.5) lipid compared to FO, GO and PO. In conclusion, SO alone and a bend of PO and GO can be used as a alternative to FO without affecting growth performance of *O. niloticus*.

Keywords: Vegetable oils, Protein sparing, Feed utilization, Whole body composition, Tilapia.

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Introduction

Statistics have confirmed that world aquaculture production has been rising, making it one of the fastest growing food producing sector [1], with a reported increase of aquaculture fish food production from 66 million tonnes in 2012 to 70.5 million tonnes in 2013 [2]. In 2014, the contribution of the sector rose to 73.8 million tonnes, with China accounting for about 60% of global fish production [3]. However, the sector relies heavily on increasingly expensive and scarce fish meal and fish oil [4]. The success of any commercial aquaculture requires the provision of a balance mix of feed containing nutrients in the right proportion [5]. Of these nutrients, protein and lipid play essential roles as sources of protein, energy and essential fatty acid required for optimum fish growth [6-8]. These EFAs play vital roles in maintaining the structure and function of cell membranes [9]. Lipids are also important for coating pelleted feeds to reduce abrasiveness, resulting in minimal dustiness and improving the flavor and textural properties of the feed [10]. FO is the most important lipid source in aqua feeds due to the presence of long-chain polyunsaturated fatty acids, eicosapentanoic and docosahexanoic acid, which together can satisfy the essential fatty acid requirements of all fish species.

However, with the rising price of FO, there is limited prospect of FO production in the future, with an increased competition for small pelagic species, the source of this vital ingredient, for direct human consumption. The limiting supply of FO together with the rapid growth of aquaculture industry has resulted in increased feed costs [11,12], thus, it is necessary to find suitable alternative lipid sources to substitute FO in aqua feeds.

Therefore, in order to ensure the sustainability of the aquaculture sector, it is necessary to reduce the utilisation of FO in aqua feed formulations while ensuring that appropriate amounts of omega-3 (n^{-3}) LC-PUFA are present in the final fish product. Nogales et al. report that plant oils are readily available and have lower prices compared to FO [13]. Plant oils have been widely used in the feeding of several fish species such as turbot [14], Gilthead sea bream [15], Japanese sea bass *Lateolabrax japonicas* [16], sea bass *Dicentrachus labrax* [5], largemouth bass *Micropterus salmoides* [9], African catfish *Clarias gariepinus* [17], and Russian sturgeon *Acipenser*

gueldenstaedtii [8], with effects ranging from optimum response to deleterious effects.

Several growth studies have shown that VOs in fish diets produce comparable results to FO [18-21]. Thus, VOs have been considered as a suitable replacement for marine FO, especially for fish species such as tilapia, which has preference for n^{-6} Fas [17,18]. Lipid sources from plants are considered to be good alternatives to FO to support sustainable aquaculture production; production of plant oils is increasing and prices are lower than those for FO [22]. However, the source and levels of dietary lipids in fish diets affect growth performance and health of fish [19,12]. The most commonly studied VOs are SO, palm oil (PO), linseed oil (LO), rapeseed oils (RO), and groundnut oil GO[18,19,21].

Substantial research on the use of alternative plant oils (PO, LO, and SO) in juvenile hybrid tilapia [21,23]and in juvenile Nile tilapia *Oreochromis niloticus* fed diets with SFO and cotton seed oil (CSO) mixture [24], SO and GO mixture [25], SO and PO mixture [10], SO [26]; corn oil (CO), LO, beef tallow [17], show comparable result to FO. *O. niloticus* have been reported to utilize and require more of linoleic acid (n^{-6}) series ($18{:}2n^{-6}$ and $20{:}4n^{-6}$), with normal growth and reproduction, than omega-3 (n^{-3}) FA (National Research Council [12,27,28] The n^{-6} series of unsaturated fatty acids (UFA) are abundant in SO [21;9], LO [18] and GO [29]. While PO is known to contain large quantity of saturated fatty acids, SFA (16:0 and 18: $1n^{-9}$) and low n^{-3}/n^{-6}, which may reduce the nutritional value of PO [30], promising results in terms of growth improvement by dietary PO supplementation have been reported for large yellow croaker *Larmichthys crocea* [31], African giant catfish *Heterobranchus longifilis* fingerlings, [20] and hybrid tilapia raised from stocking to marketable size [32], with better growth and feed utilization achieved in fish fed diets supplemented with PO than FO or SO [31]. The total replacement of FO with GO and PO in Nile tilapia is yet to be reported.

Therefore, the study was carried out to evaluate the effect of replacing FO with plant sources of lipids on growth performance and nutrient utilization of *O. niloticus*) fingerlings.

Materials and Methods

Study site

The study was carried out at the wet lab of the Department of Fisheries, University of Port Harcourt, Nigeria. The duration of the trial was from 31st, June, 2017 to 1st of September, 2017. The study was conducted following standard procedure and guideline required for best practice in the use of laboratory animals.

Experimental design

A completely randomized design consisting of four treatments with three replications was used for the trial. Each replicate fish tank represented an experimental unit.

Experimental diets

Four iso-nitrogenous (30% crude protein) and iso-calorific diets (17 MJ kg^{-1}GE) diets including a control (Table 1) were formulated to contain 8%. A basal diet containing 100% FO, which was completely replaced by SO, GO, and PO. The lipid-supplemented diets were formulated to satisfy the optimum lipid [9,21,28] and protein-energy ratio (mg protein/kcal GE) [10]. About 1-2 kg of each feedstuff sourced from a feed dealer. FM was included as the main protein source. The soya bean and groundnut cake were ground with a locally-fabricated hammer mill and pelleted through a 3 mm die. Each diet was supplemented with 5% lipids from the different sources. Diet was mixed using a large bowl with clean cold water and air-dried to a moisture content of about 10-11%. After appropriate mixing, the diets were packed into bags and stored and stored at -200 prior to inclusion with other ingredients. Pearson's method [33] was used for feed formulation. The proximate composition of the diets is shown in Table 2.

Table 1. *Gross composition (g kg-1) of juvenile Nile tilapia experimental diets (30% CP) with different lipid sources included at 5%.*

Ingredient (g kg^{-1}, as-fed basis)	Control (FO)	Soya bean oil (SO)	Groundnut oil (GO)	Palm oil (PO)
Fish meal	182.8	182.8	182.8	182.8
Soya bean (solvent-extracted)	206.9	206.9	206.9	206.9
Groundnut cake	69	69	69	69
Maize	467.1	467.1	467.1	467.1
Fish oil	50	-	-	-
Soya bean oil	-	50	-	-
Groundnut oil	-	-	50	-
Palm oil	-	-	-	50
Methionine	4.2	4.2	4.2	4.2
Salt	5	5	5	5
Bone meal	5	5	5	5
Vit min premix	5	5	5	5
Starch	5	5	5	5
Total	1000 g	1000 g	1000 g	1000 g
Calculated composition (%)				
Crude Protein	29.99	29.99	29.99	29.99
Lipid	8.14	8.14	8.14	8.14

Fish meal (g 100 g^{-1}): crude protein (66.46), lipid (5.32), ash (4.33), CHO (13.79%), energy (15.91 MJ kg^{-1}); soya bean (g 100 g^{-1}): crude protein (48), lipid (2.5), ash (4.63), energy (16.32 MJ kg^{-1}); groundnut cake: crude protein (47), lipid (1.5), ash (6.56), energy (21.97 MJ kg^{-1}); Maize (g 100 g^{-1}): crude protein (10), lipid (3.32), ash (1.28), energy (15.49 MJ

kg-^{1}); Fish meal, soya bean and groundnut meal were included at a ratio of 2:3:1.

Table 2. *Analysed composition of experimental diets supplemented with different lipid sources for O .niloticus.*

Lipid	Moisture (%)	Ash (%)	Crude Protein (%)	Crude Lipid (%)	Crude fibre (%)	Carbohydrate (%)	Energy* (MJ kg-1)	Dry matter (%)
FO	11.57 ± 0.28	8.34 ± 0.15	30.16 ± 0.43	7.56 ± 0.01	4.18 ± 0.42	37.94 ± 0.17	16.90 ± 0.07	88.17 ± 0.01
SO	11.32 ± 0.06	8.61 ± 0.01	30.34 ± 0.80	8.06 ± 0.14	3.18 ± 0.00	38.50 ± 0.98	17.06 ± 1.00	88.69 ± 0.15
GO	10.28 ± 0.12	9.65 ± 0.64	30.48 ± 0.97	7.91 ± 0.01	5.45 ± 0.53	36.24 ± 0.13	16.81 ± 0.25	89.18 ± 0.14
PO	11.41 ± 0.03	8.73 ± 0.08	29.82 ± 0.25	7.96 ± 0.41	4.86 ± 0.88	37.23 ± 0.98	16.85 ± 0.07	88.60 ± 0.13

Values are means of triplicate samples (n=3, N=9). FO=Fish oil, SO=Soya oil, GO= Groundnut oil, PO=Palm oil. Gross energy (MJ Kg-^{1} diet) was calculated according to [27] using the following calorific values: 23.9, 39.8 and 17.6 MJ g-^{1} for protein, ether extract and nitrogen free extract, respectively.

Experimental fish

A total of 120 *O. niloticus* fingerlings (1.20 ± 0.03 g, mean ± SE) were used for the experiment. Fish were acclimated under laboratory conditions for seven days prior to experimental feeding, during which time they were fed commercial diet and held in five fish holding tanks (70 L) supplied with clean water from borehole. Water temperature was maintained at an optimum range of 250-32°C, dissolved oxygen (DO) level was kept above 5 mg/L to avoid stress. After acclimation, fish were left unfed for 24 hours prior to the start of the experiment. All fish (mean weight, 1.50 ± 0.02 g, mean ± SE) were randomly assigned to the diets. Each diet was randomly assigned to tanks. There were 10 fish tank-1. Fish were hand-fed to apparent satiation twice daily, in the morning (08:00 hours) and evening (16:00 hours) for 63 days. Faeces were siphoned 2-3 hours after each feeding period. About 80% water was renewed daily in a partial water exchange static renewal. Growth performance was monitored and measured weekly. Photoperiod was naturally set at 12h light: 12 h darkness (8:00-20:00 hour light). At the end of the experiments (after 63 days of culture), triplicate fish per diet (n=10, N=30) were sampled and body composition analysis carried out according to standard protocols and methods.

Proximate analysis

Proximate composition of feed and carcass samples was determined by standard method [11]. Triplicate feed (n=3, N=9) and carcass samples per diet (n=10, N=30) was homogenized, freeze-dried and analyzed for crude protein, moisture, crude lipid, ash, carbohydrate and gross energy. Moisture content of the sample was determined after oven-drying samples at temperature 130°C for 1 hour. Two grams of sample was weighed into already weighed moisture can and was sent into the oven for 1 hour after which it was brought out of the oven, cooled and weighed.

Moisture content (%) = Weight of moisture/sample weight × 100

Crude protein was analysed by kjedahl method. Briefly, the sample (0.5 g) was weighed into a filter paper and transferred into a digestion flask to which 3 g of sodium sulphate anhydrous, 0.3 g of copper sulphate and 12 mL of concentrated sulphuric acid (H_2SO_4) were added. The flask was heated on a heating mantle at 42°C for 1 hour. The sample was cooled and transferred into 100 mL measuring cylinder and was made up to volume using distilled water. 10 mL of the digested sample was pipetted into a micro-kjeldhal flask for distillation. About 5 mL of boric acid indicator was added into 100 mL conical flask and placed at the end of the condenser of the distillation apparatus so that the adapter is dipped into the liquid. About 10 mL of 45% sodium hydroxide (NaOH) was carefully poured into the kjeldahl flask, which was connected immediately. Steam was passed through alkaline liquid slowly until it is boiling; the liquid was trapped and distilled into the conical flask until 50mL of the distillate was collected and was titrated against 0.1N of hydrochloric acid (HCl). The nitrogen in the sample was determined from the formula:

% Nitrogen= titre value – blank x normality of acid (0.1N) × 1.4/ sample weight (g)

% Crude Protein = % nitrogen x protein factor (6.25).

For lipid analysis, one gram of the sample was weighed into thimble and placed in a soxhlet extractor. 150mL of hexane was measured in a previously weighed flash and mounted on the heating mantle for 6 hours and the sample extracted. The hexane extract was evaporated in an oven at 105°C for 1 hour. The flask was then allowed to cool in the desiccators and weighed.

Crude lipid (%) = weight of fat extracted/ sample weight × 100.

In the estimation of crude fibre, sample was defatted using methanol and chloroform in the ratio 1:1. The defatted sample was transferred into a beaker to which 25mL of 1.25% of sulphuric acid (H_2SO_4) was added. This was followed by stirring with rod. After stirring, the digested sample was heated on a heating mantle for 4-5 minutes. It was filtered immediately in the conical flask with filter paper and funnel and washed down with warm distilled water till it was acid free. The residue after filtering was removed from the filter paper into a beaker with 25 mL of 1.25% Sodium Hydroxide (NaOH) and was heated for 4-5 minutes. The filter paper was

dried in an oven at 105°C for one hour and cooled in the desiccators. The residue on the filter paper was washed down with warm distilled water till the sample was base-free. After complete filtration, the filter paper with residue was dried in the oven at 105°C for 1 hour, then cooled in the desiccator and weighed.

% crude fibre = weight of fibre/sample weight ×100

The analysis of ash was carried out by heating defatted sample obtained from crude fiber determination in a muffle furnace. One gram of the sample was weighed in a previously washed, dried and cooled crucible a desiccator. The sample was heated in the muffle furnace at 550°C for 1 hour, after which it was brought out, cooled in the desiccators before it was weighed. The percentage ash content was measured using the expression.

Ash content (%) = Weight of ash/sample weight × 100

Gross energy (MJ Kg^{-1} diet) was calculated according to NRC [27] using the following calorific values: 23.9, 39.8 and 17.6 MJ g^{-1} for protein, ether extract and nitrogen free extract, respectively.

Growth performance and nutrient utilization

Growth performance would be monitored biweekly with the following parameters measured:

Weight gain (g) = Final weight (g) –initial weight (g)

Feed intake, FI (g $100g^{-1}$BW day-1) = feed consumption/ [(initial weight + final weight/2) ×days] × 100

Feed conversion ratio (g g^{-1}): feed consumption (g)/fish weight gain (g)

Protein Efficiency Ratio (g g-1, PER): WG (g)/ Protein Intake (g)

Daily weight gain (g fish-1 day-1) = mean weight gain / 63 days.

Specific growth rate (% day-1): In (W2-W1)/ (t2-t1), where W2 and W1 are weights on day t2 and t1, respectively.

Protein retention (%) = (final body weight final body protein-initial body weight × initial body protein/ protein intake) × 100

Survival rate (%) = Initial number of fish –mortality/ initial number of fish × 100

Water quality analysis

Water quality was monitored daily and measured during and after the experiment using a combination of methods. Water parameters were analysed in triplicate samples per replicate tank for dissolved oxygen (DO), temperature, and pH. DO meter (Model Lab tech DO meter); pH (pen type) correct to ± 0.1, while temperature was determined using mercury-in-glass thermometer.

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) using SPSS 20. Normality and homogeneity of variance would be analysed using Levene's test (Levene, 1960). When significant differences were detected, multiple comparisons was carried out using Tukey Honestly Significant difference. Differences in the data analysed was considered significant at $P<0.05$. Mean values were reported as ± standard error.

Results

Water quality

The mean values of water quality parameters are shown in Table 3. The parameters were not significantly different among diets ($P>0.05$). DO varied from 4.57-5.13 mg/L, pH ranged from 6.73 to 7.53, temperature was 27.43-27.87 (°C). Total dissolved solids (TDS) ranged between 51.5 (mg/L) to 82.33 mg/L.

Table 3. *Water quality parameters of the culture tanks of O. niloticus fingerlings fed different lipid supplemented diets.*

Parameters	FO	SO	GO	PO	SEM*	ANOVA P value
DO+ (mg L^{-1})	5.13	4.4	4.6	4.57	0.315	0.415
pH	6.73	7.03	7.53	7.33	0.125	0.817
Temperature (°C)	27.87	27.6	27.63	27.43	0.029	0.297
TDSb (mg L^{-1})	82.33	61.33	70.67	51.5	3.254	0.063
*SEM= Standard error of mean; TDS= Total dissolved solids; +Dissolved oxygen						

Growth performance and nutrient utilization

The measurement of growth data of Nile tilapia fed experimental diets during the 63-d trial (Figure 1) showed that dietary supplementation of lipids significantly influenced growth performance and nutrient utilization of *O. niloticus* ($P<0.05$). The fish fed PO had the least WG compared to FO, SO and GO from d 7-28 ($P<0.05$). At d 35, the fish fed SO diet had the highest WG ($P<0.05$). There was no significant difference in WG of fish among FO, GO and PO diets ($P>0.05$). FO diet had the highest weekly overall growth at the end of d 56; fish fed PO diet had the least WG, followed by SO and GO. At d 63, WG of fish fed FO diets declined, while fish fed diets supplemented with SO and PO showed a trend towards increase in growth.

The mean values of growth and nutrient utilization parameters measured at the end of the study are presented in Table 4. Survival was 100% for all treatments investigated. There were significant differences in FW ($F=11.406$, $P=0.003$), WG ($F=11.428$, $P=0.003$), FCR ($F=7.274$, $P=0.011$), PER ($F=10.803$, $P=0.003$) and SGR among the diets. The best growth in fish was obtained in FO group. There were no significant differences in mean values of FW ($P= 0.781$), WG ($P=0.999$),

SGR (P=0.991), PER (P=0.997), and FCR (P=0.879) between FO and SO-supplemented diets (P>0.05).

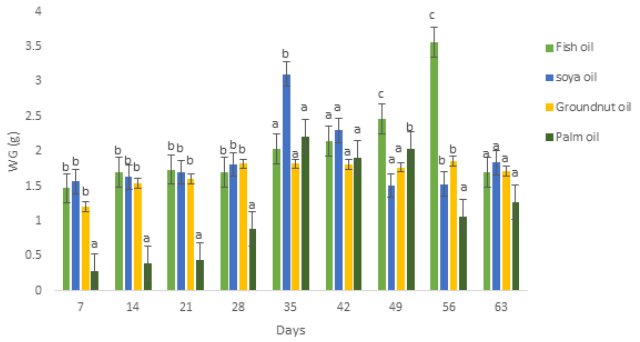

Figure 1. *WG of Nile tilapia fingerlings fed dietary lipid sources for 63 days. Values (means ± SE) with different superscripts are significant (P<0.05). Bars are standard errors.*

Table 4. *Effect of dietary lipid sources on growth performance and nutrient utilization of O. niloticus fingerlings.*

Parameters	FO	SO	GO	PO	P value
IW(g)	15.10 ± 0.21a	14.83 ± 0.23 a	14.93 ± 0.13 a	15.03 ± 0.13 a	0.745
FW (g)	34.78 ± 1.49 b	32.94 ± 0.85b	29.68 ± 1.90 ab	24.19 ± 1.00 a	0.03
WG (g)	18.35 ± 1.34 c	18.10 ± 1.66 c	14.74 ± 3.38 b	9.15 ± 1.82 a	0.03
FI (g day^{-1})	2.53 ± 0.13 a	2.54 ± 0.05 a	2.84 ± 0.09 a	2.46 ± 0.21 a	0.286
FCR (g g^{-1})	1.90 ± 0.04 a	1.89 ± 0.06 a	2.45 ± 0.30 ab	2.84 ± 0.15 b	0.011
SGR (% day-1)	1.47 ± 0.07b	1.43 ± 0.07b	1.25 ± 0.15ab	0.84 ± 0.13 a	0.012
PER (g g^{-1})	1.76 ± 0.05 b	1.76 ± 0.08 b	1.39 ± 0.16 ab	1.13 ± 0.01 a	0.003
DWG (g day-1)	0.34 ± 0.01 b	0.31 ± 0.05 b	0.26 ± 0.03 ab	0.16 ± 0.02 a	0.006
Survival (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	-

Data are means ± SE of three replicates (n=3 tanks per diet). FO = Fish oil, SO = Soya oil, GO = Groundnut oil, PO = Palm oil

Values with different superscripts in the same row are significant (P<0.05).

IW = initial weight (g), FW = final weight, WG (g) = weight gain, FCR = Feed conversion ratio, SGR = Specific growth rate, PER = Protein efficiency ratio, DWG = Daily weight gain

Whole-body composition of *O. niloticus* fingerlings fed dietary lipid sources

Analysis of fish whole-body presented in Table 5 showed that lipid sources significantly influence moisture, protein, lipid and ash (P<0.05). Whole body moisture and crude protein was lowest in PO group. However, FO, SO and GO did not significantly affect the moisture content (P<0.05). FO had the highest protein compared to VOs. However, whole-body protein of fish fed FO and SO were not significantly different (P>0.05). SO had the highest (P<0.5) lipid and least body ash compared to FO, GO and PO.

Table 5. *Effect of dietary lipids on whole-body composition (% of wet weight) of O. nilotucus fingerlings.*

Parameters	FO	SO	GO	PO	SEM*	ANOVA P value
Moisture (%)	64.59a	71.83 b	73.69b	71.15b	0.003	0.005
Crude protein (%)	16.99 b	16.83b	13.96a	14.02a	0.03	0.001
Ash (%)	6.03b	4.02 a	3.44a	3.93 a	0.122	0.06
Crude lipid (%)	4.77a	5.67 b	4.44 a	4.78 a	0.071	0.014
Carbohydrate (%)	7.63 c	1.66 a	4.41	4.12 b	0.139	0.001

Values are means of triplicate samples per diet (n=10, N=30). FO= Fish oil, SO=Soya oil, GO= Groundnut oil, PO= Palm

Discussion

Growth performance and nutrient utilization

Water quality parameters analysed were within the requirement for Nile tilapia [34-36]. While the partial replacement of FO with VOs did not affect growth and feed utilization in some fish species [14,37], in other species the total replacement of dietary FO with VOs significantly impacted negatively on growth and fillet quality resulting in growth retardation, abdominal and hepatic fat deposition [22,29]. Studies on *Catla catla* fingerlings [38], Rohu carp *Labeo rohita* [39], sea bass Dicentrachus babrax [5], juvenile African catfish Clarias gariepinus [17], largemouth bass Micropterus salmoides [9], *Russian sturgeon Acipenser gueldenstaedtii* [8] and large *yellow croaker* [29] showed comparable growth performance of fish fed FO-supplemented diet to fish fed 100% VOs (SO, GO, PO, SFO, poultry fat) or blend of VOs.

However, few have reported the effect of complete replacement of FO with PO, SO and GO in the diet of Nile tilapia. In this study, total replacement of FO with SO had variable effect on WG, FCR, PER and FI, with SO diet with SO showing comparable growth with FO diet. This is in line with previous studies which evaluated the effect of VOs on growth of hybrid tilapia [21,28] and *O. niloticus* [17,40]. In contrast to the result obtained in this study, [18] found that SO had the poorest WG, PER, FCR and SGR in juvenile *O. niloticus* (9 g) compared to FO, RO, LO and PL. The difference between the results obtained by the authors and in this study may be due to differences in diet formulation, fish species, fish size and dietary lipid level. The comparable growth of SO to FO fish group could be due to the presence of the linoleic (n^{-6}) series of EFAs (18:2n^{-6} and 20:4n^{-6}) which is required growth of Nile tilapia [12]. Tilapia is capable of converting EFA (18:2n^{-6}) to LC-PUFA), which leads to growth

enhancement. The n^{-6} LC-PUFA bioconversion pathway is similar, beginning with Δ 6 desaturation to transform 18:2n^{-6}into 18:3n^{-6}, followed by elongation to form 20:3n^{-6}, and Δ 5 desaturation to form 20:4n^{-6} (arachidonic acid, ARA) [41]. National Research Council reported that n^{-3} series FAs as well as (n^{-6}) series FAs are essential for tilapia growth [42]. However, the growth-promoting effects of the n^{-6} series FAs were superior to those of the n^{-3} series for *O. niloticus* [26]. This showed that SO can completely replace FO in diet of tilapia. The reduction in WG of FO from d 56 to 63 (Figure 3), suggests that *O. niloticus* does not depend on FO to satisfy requirement for EFA to supply energy which reduces protein turnover. The continued growth of fish fed SO at d 63 supports reports of protein-sparing in fish diet supplemented with VO, which influenced overall growth of *O. niloticus*.

Previous study on red tilapia [21,32] and hybrid tilapia [21,23] fed FO-supplemented diets showed no significant difference compared to those fed diets with PO as the sole lipid source, owing to the ability of tilapia to bio-convert EPA and most SFAs to maintain levels of LC-PUFAs [27,43]. This might explain the increase in WG from d 7 to 35 for PO, and a tendency for improvement at the end of d 63. However, compared to FO, SO and GO, result in the present study showed significant reduction in WG, PER SGR and least FI in fish fed PO. This is line with the findings of Ochang et al., who reported significant reduction in mean WG in juvenile Nile tilapia (9.09 g) fed diet in which CLO was either partially (66.67%) or completely (100%) replaced with PO [44]. In the present study, poor growth performance of fish fed PO diet may be as a result of the limited nutritional value of PO [30]. The nutritional value of lipid sources depends on the component of FA especially of the EFA profiles [9]. Takeuchi et al. reported that SFAs with C 8–18 are not suitable lipid sources for tilapias [26]. SFAs are far more abundant in PO than linoleic acids found in SO and GO [21]. Although FI did not showed significant differences among the diets as observed in other studies in Nile tilapia fed lipid sources [18], the highest FI was observed in fish fed GO which recorded better growth and nutrient utilization than PO. According to De Silva et al. fish make dietary selections based on their 'nutritional wisdom' to compose a balanced diet that best meet their nutritional demands [45].

Whole-body composition and protein retention

The evaluation of whole-body composition of fish allows assessing the efficiency of transfer of nutrients from feed to fish and also helps predict the overall nutritional status [30]. There are conflicting reports concerning the effects of FO substitution by other lipid sources on muscle proximal composition. Results obtained from several studies suggest that there are no significant effects of FO substitution on fish whole body [17,46]. In other reports, there were significant changes in proximate composition in whole body of fish fed different lipids as replacement for FO [29] Moreover, the type of dietary lipid affects body composition of fish [12]. In our study, the different sources of lipids from plants affected the body composition of *O. niloticus*. While [25] demonstrated that partial or total replacement of FO with VO mixture (SO and GO) in diet of *O. niloticus* (1.43 g) influenced body composition. Owafeir et al. did not find significant effect on body composition of 5.5 g *O. niloticus* fed diet in which FO was partially or completely replaced with SO and PO mix [10]. In this study, the complete replacement of FO with VOs (SO, GO and PO) influenced whole-body protein of *O. niloticus* fingerlings with FO and SO group recording comparably similar whole body protein. Previous study showed that the complete replacement of FO with SO in the diet of *O. niloticus* significantly affected whole body composition, with fish fed SO diet having the lowest body protein compared to FO, RO, LO and PL [18]. The difference between the results obtained by the authors and in this study could be due to differences in the levels of dietary crude protein (32 *vs* 30%), energy (15 *vs* 17 MJ kg-1 gross energy), lipid (4 *vs* 8%) and fish size (9 *vs* 1.5g). In line with this study, [44] found that the least body protein in whole-body of *O. niloticus* (9.09 g) fed PO supplemented diet. The result obtained could be related to the high content of SFA in both lipids with reduced n^{-3} and n^{-6} FA compared to SO. Previous studies have shown that the inclusion of PO in tilapia diets lowers the content of EFAs such as EPA and DHA, and reduce the n^{-3}/n^{-6} FA; [21,30,47]. The n^{-3}/n^{-6} FA ratio can alter protein and lipid contents in fish muscle [48-50]. This may explain lower lipid content in body of *O. niloticus* fed diet supplemented with PO than SO recorded in our study; this contrasted the report that SFAs are easily accumulated in fish muscle [18]. However, body lipid in PO group were similar to GO, suggesting a blend of both lipid sources in the diet of *O. niloticus* can improve the lipid profile (n^{-3}/n^{-6} ratio) and performance.

The highest body lipid was recorded in the group fed SO (Figure 2), which contains substantial amount of linoleic (18:2n^{-6}) as reported by several authors [9,21]that can adequately satisfy the lipid and energy requirement for lipid deposition and growth. According to Ferreira et al. when animal metabolism experiences an energy influx above its needs, the excess energy is stored in tissue lipid depots; the formation of lipid depots relies on the transport of lipids, either ingested or synthesized de novo, to peripheral tissues as lipoproteins, and finally, the release of FAs from lipoproteins by lipoprotein lipase (LPL) for tissue metabolism and nutrient deposition (Figure 3) [51]. The higher body lipid in the group that received PO (4.78%) than FO (4.77%) and GO (4.44%) might be related to the deposition of SFA in the fish. Qiu et al. reported that SFAs are easier to deposit in tissues than monounsaturated fatty acid (MUFA) and PUFA [29]. The reduced lipid deposition in the group fed FO diet could be due to the lower activity of lipogenic enzymes in n^{-3} PUFA lipid sources. Studies in fish show that n^{-3} HUFAs, which are highly concentrated in FO, reduce fat cell development and lipid accumulation in cultured pre-adipocytes [49], reduce fat content in Atlantic salmon white adipose tissue, and increase FA β-oxidation activity in comparison with fish fed RO-supplemented diet [50]. FA synthetase is the main lipogenic enzyme that produces FA in fish [30,51].

As observed in a study by [51], the highest body protein and least body lipid were obtained in *O. niloticus* fed FO diet. [52] also found a positive relationship between dietary n^{-3} HUFA and protein deposition in muscle of *O. niloticus*. The high protein in FO group may be due to increased retention of protein in the fish. The protein in SO (16.83%) group in this study was higher than in 5.5 g *O. niloticus* (14.7%) fed 30% CP diet supplemented with mixed oils (SO and PO) [10] and 1.2-1.3 g Nile tilapia fed diet supplemented with FO (15.8%), SFO (15.2%) and CO (0.56%) [24]. The difference between the results obtained by these authors and the present study could be due to the restricted feeding approach they adopted in their study, although the dietary crude protein used was similar in both studies. In our experiment fish were fed to satiation without restriction. The result obtained might suggest that protein sparing effect of SO be dependent on the type of feeding used. Robinson et al. reported that for fish fed ad libitum, FI is largely regulated by dietary energy.

In this study, the mean values of whole-body protein in fish were higher than values reported by [2] for 2.85g Nile tilapia fed FO (15.3%), LO (15.2%), CO (15.3%); 9 g *O. niloticus* fed SO (15.7%), RO (15.8%), LO (16.0%) and FO (16.1%) [19]; and by [51] for Nile tilapia fed SO (16.52%), CO (16.58%), LO (16.24%), and olive oil (16.28%). The discrepancy in the mean protein values may be due to differences in diet composition (32% crude protein for all studies vs 30% in this study), fish size, and culture environment. The high protein in whole body of hybrid tilapia fed FO (18.69%), PO (18.58%) and SO (18.70%) [21] Compared to result obtained in this study could be as a result of differences in species and duration of study. The authors reported a longer duration of feeding (140 days) compared to that used in this study.

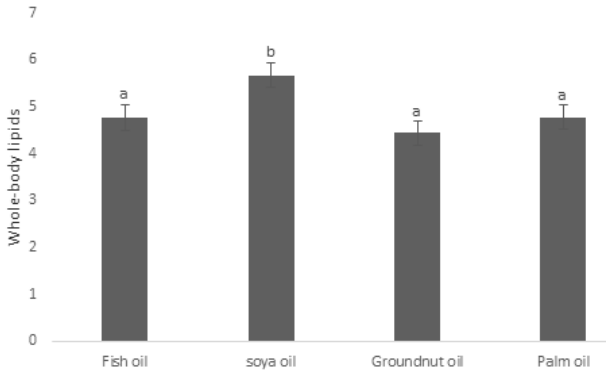

Figure 2. *Effect of dietary lipids on body lipid of Nile tilapia fingerlings Values of triplicate fish (means ± SE) with different superscripts are significant (P<0.05).*

In conclusion, while previous studies did not finding significant effect on growth and body composition in Nile tilapia fed diets in which FO was partially or totally replaced with VOs, this study confirms that significant growth performance and body protein as well as protein retention was achieved with the use of SO as an alternative lipid to FO in the diet of *O niloticus*, suggesting complete replacement of FO with SO in the feeding of Nile tilapia fingerlings is possible. There were no significant changes in most growth parameters between GO and PO.

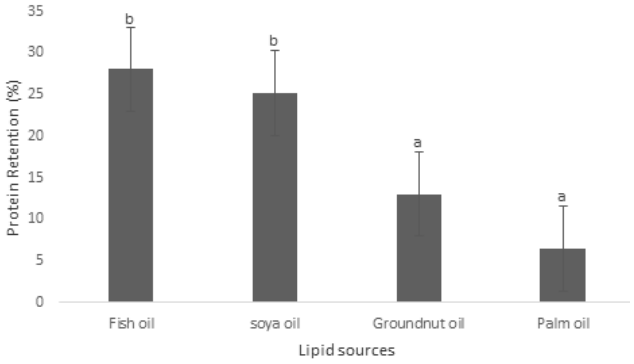

Figure 3. *Effect of dietary lipids on body protein retention (d) of Nile tilapia fingerlings.*

Values of triplicate fish (means ± SE) with different superscripts are significant (P<0.05).

This finding indicates the possibility of combining the use of both lipid sources with as cheap substitute to FO in fish diet. However, this needs further investigation in future research. Dietary modification, such as the use of low FM and use of SO, in combination with appropriate protein/energy ratio in Nile tilapia diet will ensure maximum protein sparing and environmentally sustainable aquaculture.

Conclusion

Finally, experiments should be conducted to evaluate protein retention, nutrient utilisation and growth of Nile tilapia from fry to marketable size to evaluate the use of VOs in pond environment. The use of finishing diets in which FO is supplemented and fed to fish receiving VOs as wash-out should be evaluated to ensure the appropriate amount of EPA, DHA, and n^{-3}/n^{-6} ratio are present in the final product for human health and well-being.

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