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Inclusion of duckweed (*Lemna minor*) in the diet improves flesh omega-3 long-chain polyunsaturated fatty acid profiles but not the growth of farmed Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

A 12-week experiment was conducted to determine the effect of dietary duckweed (*Lemna minor*) on growth performance, body composition and fatty acid profiles of juvenile Nile tilapia (*Oreochromis niloticus*). Five isonitrogenous (30 % crude protein) diets were prepared with the inclusion of dry ground *L. minor* at 0 %, 5 %, 10 %, 15 % and 20 % of diet dry weight. Each diet was randomly allocated to triplicate tanks stocked with juvenile tilapia that had an initial mean weight of 2.00 ± 0.01 g. The juvenile tilapia were reared in a flow-through system with each diet fed twice a day at a total ratio of 5 % body weight per day. While weight gain and specific growth rate (SGR) were generally higher, and food conversion ratio (FCR) generally lower in fish fed the control diet than fish fed *L. minor,* there were no significant differences in these performance parameters between tilapia fed *L. minor* at 15 % inclusion and fish fed the control diet. Survival ranged from 80 % to 96 % and was significantly higher in fish fed the control diet. Dietary *L. minor* significantly reduced whole-body total lipid contents and increased moisture contents. The dietary inclusion of *L. minor* significantly increased the proportions of omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) in muscle, with eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids increasing by over 10-fold from 0.5 % each of total fatty acids, respectively, in fish fed the control diet to 5 % each in fish fed 20 % *L. minor*. Since the inclusion of *L. minor* increased the contents of 18:3n-3, but not EPA and DHA in the diets, this indicates that the increased proportions of EPA and DHA in the muscle of tilapia fed *L. minor* was due to endogenous

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biosynthesis of LC-PUFA with the conversion of dietary 18:3n-3 to EPA and DHA. Therefore, the present study indicated that dietary inclusion of 15–20 % *L. minor* in feeds for Nile tilapia can significantly improve the omega-3 fatty acid profile of muscle for human consumers, with only minor effects on growth and feed utilization. These findings demonstrated the potential for the utilization of *L. minor* as an ingredient to increase omega-3 LC-PUFA, EPA and DHA, contents of farmed tilapia while reducing the use of fish oil and fish meal in tilapia feeds.

1. Introduction

Fish accounts for 16 % of animal protein consumed globally and provide more than 3.2 billion people with almost 20 % of the average per capita intake of animal protein (Little et al., 2016). In addition to protein, fish are rich sources of omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA) and highly bioavailable essential micronutrients such as vitamins D and B, and minerals including calcium, phosphorus, iodine, zinc, iron, and selenium (Kobayashi et al., 2015; FAO, 2018). The omega-3 LC-PUFA are essential dietary nutrients with well-established health benefits to humans (Arts et al., 2001; Gil et al., 2012). Specifically, the n-3 LC-PUFA, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) have key roles in neural development, immune and inflammatory responses, and dietary EPA and DHA can have beneficial effects in several pathological conditions including, cardiovascular and neurological diseases, and certain cancers (Gil et al., 2012; Delgado-Lista et al., 2012; Ambrozova et al., 2014; Laviano et al., 2013). The improvement of human nutrition in terms of dietary fatty acid composition is, therefore, an important goal since the supply of sufficient n-3 LC-PUFA for optimal human health is still low in most parts of the world (Salem and Eggersdorfer, 2015; De Roos et al., 2017).

Traditionally, humans have depended on fish and fish oil from marine capture fisheries as the primary dietary sources of EPA and DHA (Salem and Eggersdorfer, 2015; Tocher et al., 2019). However, with the increase in the global human population, there is considerable pressure on capture fisheries making aquaculture the primary source of fish for nutritionally deficient countries in the developing world (Fiogbé et al., 2004; Chakrabarti, 2018). Ironically, fish meal and fish oil have also been the main sources of protein, lipid and micronutrients in feeds for farmed fish (Sankian et al., 2019; Tocher et al., 2019). Due to increasing commodity prices, competition from other livestock feed production and unsustainable capture fisheries among other factors, significant research has been directed toward reducing the use of fish meal and fish oil in fish feeds (De Roos et al., 2017; Chakrabarti et al., 2018). These efforts have resulted in the increased utilization of various plant-derived protein and oil sources such as soybean meal and oil in fish feeds as alternatives to fish meal and fish oil (Sankian et al., 2019). However, the use of such alternative ingredients in aquafeeds has led to a significant reduction in the contents of n-3 LC-PUFA, micronutrients and vitamins in fish feeds and as a result, farmed fish fed on such ingredients contain low levels of n-3 LC-PUFA (Betancor et al., 2016; Sprague et al., 2016). However, α-linolenic acid (ALA; 18:3n-3), the precursor of the n-3 LC-PUFA, EPA and DHA, is abundant in certain terrestrial and freshwater aquatic plants (Salem and Eggersdorfer, 2015; Sankian et al., 2019) with most herbivorous fish, including tilapias and carps, having the metabolic capacity to convert dietary ALA to EPA and DHA (Tonial et al., 2009; You et al., 2019).

Duckweed (*Lemna minor*) is a free-floating freshwater macrophyte that is readily consumed by Nile tilapia (*Oreochromis niloticus*), common carp (*Cyprinus carpio)* and other herbivorous fish (Hassan and Edwards, 1992; Yılmaz and Günal, 2004). It is reported to contain up to 45 % crude protein (CP) of the plant's dry weight and is easily cultured in nutrient-rich water in tropical and subtropical countries (Hassan and Edwards, 1992; Hasan and Chakrabarti, 2009; Chakrabarti et al., 2018) where fish inhabiting these waters can access these nutrients. However, conditions for the growth of these macrophytes are not generally possible in intensive aquaculture systems, hence, it is important to incorporate these ingredients in the feeds for farmed fish to benefit from their nutrient content (Bag et al., 2011). Although previous studies have reported the use of *L. minor* as a dietary protein source for farmed tilapia (Wee, 1991; El-Shafai et al., 2004; Bag et al., 2011; Chakrabarti et al., 2018), no studies have focused on the impact of dietary *L. minor* on fatty acid profiles of farmed fish, and in particular their n-3 LC-PUFA enrichment. Therefore, this study was designed to evaluate the effects of feeding diets supplemented with different levels of *L. minor* on growth parameters and fatty acid composition of farmed Nile tilapia.

2. Materials and methods

2.1. Ethical statement

The experiment was conducted in compliance with the Prevention of cruelty to animals Act 1962, CAP 360 (Revised 2012) of the laws of Kenya and in accordance with EU regulation (EC Directive 86/609/EEC). Additionally, the study was carried out through a project that was subjected to and passed a thorough ethical review process carried out by the University of Stirling (UoS) Animal Welfare and Ethical Review Board (AWERB) before any work being approved. This was done using detailed ethical approval forms that require all aspects of the experimentation to be described including all animal health and welfare issues as well as other ethical considerations.

2.2. Feed formulation and preparation

The proximate composition of the main ingredients used in the experimental diet formulations is shown in Table 1. The diets used

during the trial were formulated to be isonitrogenous (30 % crude protein) (Table 2). In addition, and of key importance, the diets were specifically formulated to be entirely free of any marine or fish-based ingredients such as fish meal or fish oil. All diets were formulated using soybean meal as the primary protein source with the addition of *Lemna minor* balanced by appropriate reductions in the four main ingredients, soybean meal, maize germ, wheat bran and pollard, to maintain constant protein and energy levels. Dietary lipid was maintained between 4.2 % and 4.7 % and supplied by the plant meals and *L. minor*. *L. minor* was collected from ponds in Kisumu, Kenya and was subjected to minimum processing. The macrophyte was air-dried followed by oven drying at 40 °C before grinding to a meal prior to inclusion in feeds. Five diets were prepared with inclusion levels of *L. minor* of 0 % (Diet $L_{0;}$ control diet), 5 % (Diet L_5), 10 % (Diet L_{10}), 15 % (Diet L_{15}) and 20 % (Diet L_{20}) of diet dry weight. The dry feed ingredients were then thoroughly mixed with warm water using a feed mixer to make a dough, which was then pelletized to produce pellets ranging between 1 mm and 3 mm. The feeds were stored in airtight containers at room temperature prior to feeding.

2.3. Fish and experimental set-up

The feeding trial was carried out at the Mwea Aquafish Farm, Kimbimbi, Kirinyaga County, Kenya. Monosex Nile tilapia juveniles were obtained from the farm's hatchery and acclimated to the experimental conditions for seven days while being fed on the control diet. After acclimatization, 375 fish with an average body weight of 2.00 ± 0.01 g were randomly stocked in fifteen 50-L tanks at twenty-five (25) fish per tank. The tanks were supplied with fresh water in a flow-through system at a flow rate of 12 L/h, and each feed was allocated randomly to three tanks. The fish were fed each diet at a ratio of 5 % body weight daily split between two feeding times at 9.00 A.M. and 5.00 P.M. for 12 weeks. Feed adjustments were made for each tank at 3-week intervals after body weight measurements.

2.4. Water quality monitoring

Water quality parameters were measured weekly using a multi-parameter water quality meter model H19828 (Hanna Instruments Ltd., Chicago, USA). Nutrients were analyzed weekly using standard methods (Boyd and Tucker, 1998).

2.5. Fish sampling

Fish were assessed every 3 weeks for growth performance parameters. A day prior to sampling, fish were fasted for 24 h to allow for gut emptying. All the fish from each tank were sedated using tricaine methanesulfonate (MS-222) (2 g/l) and measured individually for weight using a digital balance (0.01 g) and total length measured to the nearest 0.1 cm using a measuring board according to Caspers (1969). Fish growth performance and feed utilization were calculated using standard formulae: Specific growth rate SGR (%) = 100 ($\ln W_t - \ln W_0/t$), where W_0 = initial weight (g), W_t = final weight (g) and t = period in days; Weight gain (WG) = final weight (g) − initial weight (g); feed conversion ratio (FCR) = feed given (g)/weight gain (g). At the end of the experiment, the fish in each tank were counted, and survival was calculated as: Survival (%) = (number of fish at harvest/number of fish stocked) × 100. At the end of the experimental period, a random sample of 8 fish were collected from each tank and were euthanized by putting fish in a container with ice water before culling (Lambooij et al., 2008). Five intact fish per tank were pooled to provide one sample per tank (n = 3 per diet). The fish were homogenized in a blender and the homogenates frozen immediately at − 20 °C prior to the determination of whole-body proximate composition. Similarly, muscle fillets were dissected from the other 3 fish per tank, pooled and homogenized to form one sample per tank (n = 3 per diet). The samples were immediately frozen at − 20 °C prior to determination of lipid content and fatty acid profiling.

2.6. Proximate composition analysis

Feeds and pooled whole fish homogenate samples were analyzed for crude protein, crude lipid, moisture and ash using standard methods of the Association of Official Analytical Chemists (AOAC, 1990). Dry matter content was measured by gravimetry. Moisture content was estimated by oven drying at 105 °C for 12 h to a constant weight and ash content was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, IO, USA) at 550 °C for 12 h. Protein content (N × 6.25) was determined using a micro-Kjeldahl apparatus (Labconco Corporation, Kansas City, USA) and, lipid content measured using a Soxhlet extractor (VELP Scientifica, Milano, Italy).

Table 1
Proximate compositions of the major ingredients used in the diet formulations.

Parameter	Ingredients (% of dry weight)				
	Soybean meal	Wheat bran	Wheat pollard	Maize germ	*Lemna minor*
Crude protein	42.30	17.10	12.95	12.02	26.58
Crude lipid	3.50	5.80	4.51	17.74	5.21
Carbohydrates	48.90	71.80	76.56	66.7	52.34
Ash	5.30	5.30	5.98	3.54	15.87

Table 2
Ingredients, formulations, and proximate compositions of the experimental diets.

Ingredients (%)	Diet[a]				
	L_0	L_5	L_{10}	L_{15}	L_{20}
Soybean meal	53.8	51.9	49.9	48.0	46.0
Wheat bran	11.0	8.3	5.5	2.8	0.0
Wheat pollard	9.2	9.0	8.9	8.7	8.5
Maize germ	20.0	19.9	19.8	19.6	19.5
Lemna minor	0.0	5.0	10.0	15.0	20.0
Vitamin premix	1.0	1.0	1.0	1.0	1.0
Mono calcium phosphate	3.0	3.0	3.0	3.0	3.0
L-Lysine	1.0	1.0	1.0	1.0	1.0
Methionine	1.0	1.0	1.0	1.0	1.0
Proximate composition (% of dry weight)					
Dry matter	89.1	89.2	89.3	89.2	89.5
Crude protein	30.0	30.2	30.7	31.0	31.1
Crude lipid	4.2	4.6	4.6	4.7	4.7
Carbohydrate	59.9	56.2	55.8	54.4	54.6
Potassium	0.7	0.7	0.7	0.7	0.7
Calcium	0.9	0.9	0.9	0.9	0.9
Ash	8.8	9.0	8.9	9.9	9.6

[a] L_0 (0 % *L. minor*); L_5 (5 % *L. minor*); L_{10} (10 % *L. minor*); L_{15} (15 % *L. minor*) and L_{20} (20 % *L. minor*).

2.7. Extraction of lipids and fatty acid analysis

Lipid extraction was performed according to the procedure of Bligh and Dyer (1959). Total lipid was extracted from 0.5 g samples of pooled fish muscle homogenates or finely ground experimental diets by homogenization in 10 ml chloroform/methanol (2:1, v/v) containing 0.01 % butylated hydroxytoluene (BHT) as an antioxidant and 2 ml cold isotonic saline, 0.9 % sodium chloride. Homogenates were mixed vigorously and allowed to stand for 20 min before being centrifuged at 3000 rpm for 10 min and the upper aqueous layer aspirated before the lower organic/chloroform layer was transferred to a 100 ml reflux flask and evaporated to dryness under vacuum. Fatty acid methyl esters (FAME) were prepared from the extracted total lipid and fatty acid standards by acid-catalyzed transmethylation. Briefly, 5 ml of 1 % H_2SO_4 (v/v) in methanol was mixed with 1 ml of extracted total lipid in a 50 ml reflux flask and refluxed at 70 °C for 3 h. FAME were extracted into 750 ml of distilled water and 10 ml of hexane then dehydrated using anhydrous sodium sulfate (Na_2SO_4). The extracted FAME were concentrated to 0.5 ml in a vacuum evaporator and transferred into gas chromatography (GC) vials prior to GC analysis.

Table 3
Fatty acid compositions of the *L. minor* meal and the experimental diets.

Fatty acid (%)	*L. minor*	Diet[a]				
	meal	L_0	L_5	L_{10}	L_{15}	L_{20}
14:0	1.3	0.1	0.2	0.2	0.3	0.3
15:0	0.5	0.1	0.1	0.1	0.2	0.2
16:0	27.1	15.7	19.9	19.7	20.0	20.1
18:0	4.8	2.8	2.1	3.2	3.2	3.1
20:0	0.6	0.4	0.5	0.5	0.2	0.2
Total SFA	36.6	19.1	22.8	23.7	23.9	24.0
16:1n-9	3.6	0.1	0.1	0.4	0.5	0.7
16:1n-7	1.7	0.2	0.2	0.2	0.3	0.3
18:1n-9	6.0	30.1	29.4	28.2	26.6	26.3
18:1n-7	2.6	0.5	0.4	0.3	0.3	0.3
20:1n-9	n.d	0.3	0.4	0.4	0.4	0.1
Total MUFA	15.0	31.1	30.4	29.5	28.1	27.7
18:2n-6	17.2	46.8	42.8	41.7	41.4	40.5
20:4n-6	n.d	n.d	n.d	n.d	n.d	n.d
Total n-6 PUFA	17.3	46.8	42.8	41.7	41.4	40.5
18:3n-3	29.0	3.0	4.1	5.1	6.6	7.8
20:5n-3 (EPA)	n.d	n.d	n.d	n.d	n.d	n.d
22:6n-3 (DHA)	n.d	n.d	n.d	n.d	n.d	n.d
Total n-3 PUFA	29.2	3.0	4.1	5.1	6.6	7.8
n-3 PUFA: n-6 PUFA	1.69	0.06	0.10	0.12	0.16	0.19
Total PUFA	46.5	49.8	46.8	46.8	48.0	48.3

[a] L_0 (0 % *L. minor*); L_5 (5 % *L. minor*); L_{10} (10 % *L. minor*); L_{15} (15 % *L. minor*) and L_{20} (20 % *L. minor*). MUFA, monounsaturated fatty acids; n.d., not detected; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

2.8. GC analysis

FAME were separated and quantified by GC (Shimadzu Model GC14B, Japan) fitted with on-column injection and equipped with a fused silica capillary column (SUPELCO Column Omega wax™ 530, 30 m × 0.5 mm × 0.5 μm) with nitrogen as carrier gas. Temperature programming was from 170 °C to 220 °C at 1.8 °C min^{-1}, then 220 °C for 47 min and a total run time of 75 min. Injection and detection temperatures were 240 °C and 260 °C, respectively. All GC analyses were performed under the same conditions. Individual methyl esters were identified by comparison with known FAME standards.

2.9. Data analysis

Data were expressed as means ± SE (n = 3). All data were analyzed using one-way analysis of variance (ANOVA) with differences among means for the dietary treatments determined using the Tukey HSD Test at $P < 0.05$. Percentage data were arcsine-transformed before statistical analysis. All statistical analyses were carried out using Statistical Package and Service Solutions (SPSS version 23).

3. Results

3.1. Fatty acid compositions of Lemna minor and experimental diets

Fatty acid compositions of the *L. minor* meal and the experimental diets are presented in Table 3. Over 50 % of total fatty acids in the control diet (L_0) were saturated (SFA) and monounsaturated (MUFA) fatty acids with about 19 % being SFA, predominantly 16:0 and, to a lesser extent, 18:0, and around 31 % being MUFA, predominantly 18:1n-9. Under 50 % of total fatty acids in the control diet were PUFA, predominantly 18:2n-6 (almost 47 %) and 3 % 18:3n-3 to give an n-3 PUFA: n-6 PUFA ratio of 0.06. Graded inclusion of *L. minor* increased the proportion of 18:3n-3 and decreased the proportion of 18:2n-6 so that values for these fatty acids were around 8 % and 40 % increasing the n-3 PUFA: n-6 PUFA ratio 3-fold to 0.19 in diet L_{20} which had the highest inclusion of *L.* minor. The diets were completely devoid of any long-chain polyunsaturated fatty acids (LC-PUFA) with EPA, DHA and arachidonic acid (ARA; 20:4n-6) not detected in the control diet or any of the diets containing *L. minor*. Saturated fatty acids increased while MUFA decreased with the graded inclusion of *L. minor*.

3.2. Water quality parameters

The results for water quality parameters are presented in Table 4. Water temperature was constant at around 25.9 °C, dissolved oxygen ranged between 4.01 and 4.42 mg L^{-1}, and pH varied from 7.50 to 7.53. Total phosphorus was 0.01 mg L^{-1}, nitrates around 0.19–0.20 mg L^{-1}, ammonia 0.01–0.02 mg L^{-1} and nitrites 0.00 mg L^{-1}. All water quality parameters were constant and within acceptable ranges during the experimental period.

3.3. Growth performance

Fish growth performance parameters are presented in Table 5. Fish fed the control diet (L_0) had the highest final weight and specific growth rate (SGR) that were significantly higher than fish fed on the *L. minor* diets. However, fish final weight and SGR of fish fed diet L_{15} containing 15 % *L. minor* were not significantly different to those of fish fed the control diet. Similarly, the feed conversion ratio (FCR) was lowest and not significantly different in fish fed the control and L_{15} diets, and both were significantly lower than the FCR in fish fed the other *L. minor* diets. Fish survival was highest in fish fed the control diet and lowest in fish-fed diet L_5. However, survival increased with increasing inclusion of *L. minor*; such that survival in fish fed on diet L_{20} was not statistically different to fish fed the control diet. Condition factor (K) was affected by *L. minor* inclusion and was significantly lowest in fish-fed diet L_{20}.

Table 4
Water quality parameters (mean values ± SE) during the experimental period.

Parameter	Diet[a]				
	L_0	L_5	L_{10}	L_{15}	L_{20}
Temperature (°C)	25.94 ± 0.25	25.84 ± 0.25	25.84 ± 0.25	25.86 ± 0.25	25.90 ± 0.24
Dissolved oxygen (mg L^{-1})	4.11 ± 0.15	4.08 ± 0.14	4.14 ± 0.14	4.01 ± 0.15	4.42 ± 0.12
pH	7.52 ± 0.01	7.51 ± 0.01	7.50 ± 0.01	7.50 ± 0.01	7.53 ± 0.01
Total phosphorus (mg L^{-1})	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Nitrates (mg L^{-1})	0.20 ± 0.02	0.19 ± 0.02	0.20 ± 0.02	0.19 ± 0.02	0.19 ± 0.02
Ammonia (mg L^{-1})	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
Nitrites (mg L^{-1})	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Data were collated to provide the average values in each tank for the experimental period and then presented as means ± SE for each dietary treatment (n = 3). There were no significant differences among mean values within a row.

[a] L_0 (0 % *L. minor*); L_5 (5 % *L. minor*); L_{10} (10 % *L. minor*); L_{15} (15 % *L. minor*) and L_{20} (20 % *L. minor*).

Table 5
Growth performance parameters of *O. niloticus* fed diets containing graded increased inclusion of *L. minor*.

Parameter	Diet[a]				
	L_0	L_5	L_{10}	L_{15}	L_{20}
Initial length (cm)	4.81 ± 0.04	4.83 ± 0.04	4.86 ± 0.04	4.91 ± 0.04	4.85 ± 0.04
Initial weight (g fish^{-1})	1.99 ± 0.01	2.00 ± 0.01	2.00 ± 0.01	2.00 ± 0.01	2.00 ± 0.01
Final length (cm)	9.01 ± 0.12^{a}	8.13 ± 0.15^{b}	8.12 ± 0.15^{b}	8.42 ± 0.11^{ab}	8.22 ± 0.14^{b}
Final weight (g fish^{-1})	13.03 ± 0.51^{a}	9.80 ± 0.51^{b}	9.96 ± 0.48^{b}	11.03 ± 0.43^{ab}	9.34 ± 0.42^{b}
SGR (% day^{-1})	2.17 ± 0.05^{a}	1.81 ± 0.06^{bc}	1.85 ± 0.06^{b}	1.98 ± 0.05^{ab}	1.76 ± 0.05^{c}
FCR	0.68 ± 0.04^{a}	0.77 ± 0.05^{b}	0.79 ± 0.06^{b}	0.67 ± 0.04^{a}	0.80 ± 0.05^{b}
Daily weight gain (g day^{-1})	0.13 ± 0.01^{a}	0.09 ± 0.01^{b}	0.10 ± 0.01^{ab}	0.11 ± 0.01^{ab}	0.09 ± 0.00^{b}
Weight gain (g fish^{-1})	11.04 ± 0.51^{a}	7.80 ± 0.51^{b}	7.99 ± 0.48^{b}	9.03 ± 0.43^{ab}	7.34 ± 0.42^{b}
Survival (%)	96.0 ± 2.3^{a}	80.7 ± 1.3^{b}	83.3 ± 4.8^{b}	88.7 ± 1.3^{b}	95.3 ± 2.7^{a}
Condition factor (K)	1.84 ± 0.01^{ab}	1.84 ± 0.01^{ab}	1.89 ± 0.02^{b}	1.90 ± 0.02^{b}	1.81 ± 0.01^{a}

Means within the same row with different superscript letters are significantly different at $P < 0.05$.
[a] L_0 (0 % *L. minor*); L_5 (5 % *L. minor*); L_{10} (10 % *L. minor*); L_{15} (15 % *L. minor*) and L_{20} (20 % *L. minor*).

3.4. Fish body proximate composition

The whole-body proximate compositions of the experimental fish are presented in Table 6. Tilapia fed the control L_0 diet contained about 67 % moisture, 15 % protein, 12 % lipid and 3 % ash. The proportion of lipids in tilapia was significantly reduced by incorporating *L. minor* in the diet with concomitant significantly increased moisture content. There was a trend for protein content to be reduced in fish fed the diets containing *L. minor*, significantly so in fish fed diets L_5 and L_{15}. The ash content of fish was not significantly different among the treatments. The carbohydrate content of tilapia was low but generally increased with the inclusion of *L. minor* in the diets.

3.5. Fatty acid composition of the muscle

Fatty acid compositions of muscle (flesh) of tilapia fed the experimental diets are presented in Table 7. The total lipid of the muscle of fish fed the control diet contained SFA and MUFA at 39 % and 35 % of total fatty acids, respectively. SFA was predominantly 16:0 followed by 18:0, while MUFA was predominantly 18:1n-9 followed by 16:1. Over 26 % of total fatty acids in the muscle of fish fed the control diet were PUFA, predominantly n-6 PUFA with 23 % 18:2n-6 and over 1.5 % ARA, and a lesser amount of n-3 PUFA including 0.4–0.5 % each of 18:3n-3, EPA and DHA. This resulted in an n-3 PUFA: n-6 PUFA ratio of around 0.1. Graded inclusion of *L. minor* increased the proportion of 18:3n-3 but, very importantly, also significantly increased the proportions of LC-PUFA, EPA, DHA and ARA in a similarly graded manner. Specifically, the levels of EPA and DHA each reached around 5 % of total fatty acids in tilapia-fed diet L_{20} with the highest inclusion of *L. minor*. Therefore, total n-3 LC-PUFA increased significantly by 10-fold from around 1.0 % in the muscle of fish fed the control diet without *L. minor* to 10 % in fish-fed diet L_{20}. The proportion of the n-6 LC-PUFA, ARA, also increased in a graded manner with increasing inclusion of *L. minor*. Balancing the increased proportions of LC-PUFA, the inclusion of *L. minor* significantly decreased the proportion of 18:2n-6 and, consequently, the n-3 PUFA: n-6 PUFA ratio increased 10-fold from around 0.06 in fish fed the control diet to 0.63 in fish fed diet L_{20} with the highest inclusion of *L. minor*. Dietary *L. minor* had less impact on SFA and MUFA although there were trends for total SFA to increase and total MUFA to decrease, with some statistically significant differences in the proportions of the major fatty acids, 16:0, 18:0 and 18:1n-9.

4. Discussion

The advancement of aquaculture towards intensive systems and productivity demands innovative feed ingredient alternatives as substitutes for increasingly unsustainable sources of wild fish meal and fish oil. This has led to a decline in n-3 LC-PUFA, EPA and DHA levels for aquafeeds, consequently, reducing the levels of these key beneficial fatty acids in many farmed fish species (Sprague et al., 2016). Therefore, there is an urgent need to find novel ingredients that can sustainably meet the need of the fish feed industry as well as

Table 6
Whole body proximate composition of *O. niloticus* fed on fed on diets containing *L. minor* in flow through tanks.

Parameter (% wet weight)	Diet[a]					
	Initial	L_0	L_5	L_{10}	L_{15}	L_{20}
Moisture	74.09 ± 2.51	67.06 ± 2.61^{a}	74.29 ± 1.79^{b}	71.18 ± 2.56^{c}	72.93 ± 1.08^{b}	71.10 ± 2.64^{c}
Lipid	2.80 ± 0.10	12.34 ± 2.90^{a}	8.83 ± 1.28^{b}	10.45 ± 1.98^{c}	9.84 ± 2.20^{b}	10.33 ± 2.9^{c}
Protein	10.75 ± 0.10	15.16 ± 0.28^{a}	13.15 ± 0.96^{b}	14.57 ± 0.74^{ab}	13.59 ± 0.67^{b}	14.41 ± 0.34^{ab}
Ash	2.03 ± 0.33	3.08 ± 0.09^{a}	3.30 ± 0.15^{a}	3.15 ± 0.10^{a}	3.09 ± 0.43^{a}	3.10 ± 0.11^{a}
Carbohydrate	0.29 ± 0.07	0.37 ± 0.01^{a}	0.43 ± 0.03^{ab}	0.65 ± 0.01^{c}	0.85 ± 0.03^{d}	1.06 ± 0.06^{e}

Means within the same row with different superscript letters are significantly different at $P < 0.05$.
[a] L_0 (0 % *L. minor*); L_5 (5 % *L. minor*); L_{10} (10 % *L. minor*); L_{15} (15 % *L. minor*) and L_{20} (20 % *L. minor*).

Table 7
Fatty acid composition (percentage of total fatty acids) of the muscle of *O. niloticus* fed diets containing *L. minor*.

Fatty acid	Diet				
	L_0	L_5	L_{10}	L_{15}	L_{20}
14:0	2.1 ± 0.14^{a}	2.3 ± 0.14^{ab}	2.9 ± 0.30^{b}	2.1 ± 0.05^{a}	2.0 ± 0.21^{a}
16:0	23.3 ± 0.75	23.0 ± 0.25	24.0 ± 0.70	25.2 ± 1.59	24.4 ± 0.18
18:0	12.0 ± 0.35^{cd}	13.4 ± 0.40^{d}	10.7 ± 0.90^{bc}	8.9 ± 0.39^{a}	9.7 ± 0.33^{ab}
20:0	0.5 ± 0.09^{a}	0.9 ± 0.20^{a}	2.5 ± 0.30^{c}	1.6 ± 0.09^{b}	1.5 ± 0.20^{b}
22:0	0.4 ± 0.03	0.4 ± 0.05	0.5 ± 0.05	1.8 ± 1.15	1.7 ± 0.64
24:0	0.2 ± 0.05^{a}	0.3 ± 0.02^{a}	0.5 ± 0.08^{a}	3.4 ± 0.37^{b}	3.7 ± 0.39^{b}
Total SFA[1]	39.2 ± 0.70^{a}	44.5 ± 1.26^{ab}	45.5 ± 1.57^{ab}	46.3 ± 2.14^{b}	42.8 ± 3.26^{ab}
16:1n-7[2]	4.6 ± 0.12^{b}	6.0 ± 0.13^{d}	2.7 ± 0.10^{a}	5.5 ± 0.20^{c}	4.5 ± 0.11^{b}
18:1n-9	29.72 ± 0.60^{b}	24.4 ± 0.97^{a}	28.5 ± 0.35^{b}	24.3 ± 0.22^{a}	22.4 ± 1.31^{a}
Total MUFA[3]	34.6 ± 0.81^{c}	36.73 ± 1.61^{c}	31.19 ± 0.30^{b}	29.79 ± 0.42^{b}	26.9 ± 1.41^{a}
18:2n-6	23.0 ± 0.25^{c}	14.2 ± 0.95^{a}	17.2 ± 0.45^{b}	15.9 ± 0.12^{b}	16.3 ± 0.57^{b}
20:4n-6 (ARA)	1.6 ± 0.09^{a}	2.1 ± 0.14^{ab}	2.2 ± 0.06^{b}	2.6 ± 0.09^{b}	2.3 ± 0.27^{b}
Total n-6 PUFA	24.6 ± 0.34^{c}	16.3 ± 0.87^{a}	19.4 ± 0.51^{b}	18.5 ± 0.21^{b}	18.6 ± 0.84^{b}
18:3n-3	0.5 ± 0.07^{a}	1.1 ± 0.19^{ab}	1.8 ± 0.42^{b}	0.9 ± 0.14^{a}	1.7 ± 0.22^{b}
20:5n-3 (EPA)	0.4 ± 0.08^{a}	0.5 ± 0.10^{a}	0.5 ± 0.06^{a}	2.2 ± 0.09^{b}	5.1 ± 0.53^{c}
22:6n-3 (DHA)	0.5 ± 0.05^{a}	1.0 ± 0.05^{ab}	1.7 ± 0.05^{ab}	2.3 ± 0.81^{b}	4.9 ± 0.55^{c}
Total n-3 PUFA	1.5 ± 0.14^{a}	2.6 ± 0.34^{a}	4.0 ± 0.53^{ab}	5.4 ± 1.03^{b}	11.7 ± 1.25^{c}
Total n-3 LC-PUFA	1.0 ± 0.09^{a}	1.5 ± 0.11^{a}	2.2 ± 0.07^{ab}	4.5 ± 0.83^{b}	10.0 ± 59^{c}
n-3 PUFA: n-6 PUFA	0.06 ± 0.01^{a}	0.16 ± 0.04^{ab}	0.21 ± 0.04^{bc}	0.29 ± 0.09^{c}	0.63 ± 0.06^{d}
Total PUFA	26.1 ± 0.46^{bc}	18.8 ± 0.69^{a}	23.3 ± 1.04^{b}	23.9 ± 1.23^{b}	30.3 ± 2.09^{c}

L_0 (0 % *L. minor*); L_5 (5 % *L. minor*); L_{10} (10 % *L. minor*); L_{15} (15 % *L. minor*) and L_{20} (20 % *L. minor*). MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; tr, trace (< 0.1).[1]Contains short-chain saturated fatty acids (< C14); [2]Contains 16:1n-9; [3]Contains 24:1n-9.

meet the nutritional requirements of cultured fish and ultimately enrich the n-3 LC-PUFA contents of farmed fish (Tocher et al., 2019) as highly needed by consumers.

Various studies have explored the use of aquatic plants such as duckweed (*Lemna* spp.) in fish feeds (Fasakin et al., 1999; Hassan and Edwards, 1992; El-Shafai et al., 2004). However, the above studies have focused on using freshwater macrophytes like *Lemna* species as protein sources to replace fish meal. In contrast, the present study has been designed to explore the use of *L. minor* as a potential lipid source and, more specifically, as an ingredient to promote levels of the n-3 LC-PUFA, EPA and DHA, in cultured freshwater fish that have endogenous capability for the production of EPA and DHA from the short-chain precursor, ALA (18:3n-3) (Tocher, 2003).

Despite having a relatively low lipid content, the fatty acids in *L. minor* are rich in 18:3n-3 and can therefore be regarded as a potential source of ALA for the endogenous synthesis of n-3 LC-PUFA in fish like Nile tilapia. The batch of *L. minor* used in the present study was collected from irrigation canals in Western Kenya and contained just over 5 % lipid, with ALA accounting for up to 30 % of total fatty acids. However, the cultivation of *L. minor* with the addition of organic fertilizers can result in higher lipid (up to 8–9 % of dry weight) and ALA (up to 46 % of total fatty acids) levels, improving its nutritional quality (Chakrabarti et al., 2018). The addition of *L. minor* to feeds in the present study replaced the other plant ingredients, soybean meal, maize germ and wheat bran and pollard, which are all derived from seeds where n-6 PUFA, specifically 18:2n-6, dominate the lipid content. Therefore, in the present study, increasing the dietary inclusion of *L. minor* had a substantial impact on the fatty acid profiles of the cultured fish. The levels of 18:3n-3 doubled while n-6 PUFA, specifically 18:2n-6, was reduced due to the lower inclusion of terrestrial plant-based products. Consequently, the n-3 PUFA: n-6 PUFA ratio in the feeds increased by over 3-fold in the feed with the highest inclusion of *L. minor*. These data have, for the first time, demonstrated that the inclusion of products derived from aquatic plants like duckweed *L. minor* can have a substantial beneficial impact on the fatty acid composition of sustainable, marine-free fish feeds, despite these ingredients having a relatively low lipid content and not previously being regarded as major lipid sources in feeds (Chakrabarti et al., 2018).

The second important outcome of the present study was that the inclusion of *L. minor* in the feeds, and the consequent increase in dietary ALA levels, resulted in increased muscle levels of the n-3 LC-PUFA, EPA and DHA, indicating a direct dietary influence on tissue fatty acid compositions. In particular, this result clearly indicated active bioconversion of the dietary C_{18} n-3 PUFA, ALA, to LC-PUFA in the tilapia with the subsequent accumulation of EPA and DHA in the flesh. This was consistent with previous studies that reported increased n-3 PUFA, EPA and DHA in fish tissue in response to increased n-3 PUFA in diets (Kolditz et al., 2010; Stoneham et al., 2018; Li et al., 2019). While previous studies have also reported that farmed fish like Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) (Olsen et al., 1990; Betancor et al., 2016) and tilapia (Tocher et al., 2001; Maina et al., 2003) can convert ALA to EPA and DHA, the data in the present study using feeds that were free of fish meal and fish oil and so contained no n-3 LC-PUFA, provided clear evidence that the increased level of EPA and DHA in fish fed the diets containing *L. minor* over that found in fish fed the control diet were derived from the biosynthetic conversion of dietary ALA. Interestingly, muscle ALA levels were low in fish fed all the diets supporting an efficient biosynthetic pathway, albeit it is also well known that ALA is also efficiently oxidized for energy in fish (Bell et al., 2001).

As mentioned previously, Nile tilapia possess desaturation and elongation enzymes that can efficiently convert shorter chain C_{18} PUFA, including n-6 PUFA, to longer chain PUFA (Olsen et al., 1990; Tonial et al., 2009). This was further supported in the present

study by the graded increased levels of ARA in the muscle of tilapia fed the feeds including *L. minor*, while the muscle levels of 18:2n-6 decreased in fish fed the dietary *L. minor* consistent with active desaturation and elongation of 18:2n-6 to ARA, although the level of 18:2n-6 also decreased due to the lower dietary levels in the feeds containing *L. minor*. Similar results have been reported by Pinandoyo et al. (2019) in Nile tilapia fed a fermented *L. minor* meal. Li et al. (2019) reported that the levels of SFA and MUFA were significantly influenced by the levels of the respective fatty acids in the diet of the fish. Total SFA was significantly higher in the fish-fed diet L_{20} while fish-fed the control diet had the lowest level of saturated fats, consistent with a study on Nile tilapia-fed water hyacinth and *L. minor* (Bag et al., 2011). Similarly, total MUFA levels in muscle were within the range reported for Nile tilapia-fed *L. minor* and *Azolla* meals (Bag et al., 2011). However, in the present study, dietary inclusion of *L. minor* generally had a little biologically significant effect on the levels of SFA and MUFA in tilapia muscle. Therefore, the overall conclusion on the impact of dietary *L. minor* on the fatty acid composition of the edible part of tilapia was a ten-fold increase in the levels of the health-beneficial LC-PUFA, EPA and DHA, substantially enhancing the nutritional quality of the farmed fish for human consumers.

The nutritional quality of farmed fish is, of course, dependent upon more than just fatty acid composition. In the present study, the overall body composition was affected by dietary *L. minor*. Specifically, total lipid content was reduced in tilapia fed *L. minor* compared to fish fed the control diet without *Lemna*., This is in agreement with the study of El-Shafai et al. (2004) that reported lower lipid content in tilapia-fed diets containing *L. minor*. The fact that the body lipid content of tilapia can depend on the size of the fish (Van Trung et al., 2011) may be a factor in the present study as fish fed the control diet were generally larger with higher weights compared to fish fed the *L. minor*. Therefore, the lower lipid content may partly reflect lower growth albeit the lipid content was not well correlated with size and so other metabolic factors are likely involved. Notably, there was no clear dose-response effect on lipid level due to *Lemna* inclusion. As expected, moisture content showed an inverse relationship with lipid content but was higher in fish fed *L. minor* than can be explained simply by lower lipid content. Thus, protein content was also lower in fish-fed *Lemna*, which was consistent with previous studies showing lower protein content in tilapia-fed *L. minor* (Hassan and Edwards, 1992) and in the muscle of tilapia-fed diets with *Azolla pinnata* (Abou et al., 2011). In contrast, ash content was not significantly affected by dietary *L. minor*, contrary to the results of El-Shafai et al. (2004). However, studies on tilapia-fed duckweed have reported varying results, with some showing an increase in ash with increased dietary *L. minor* while others have noted a reduction in ash content (Fasakin et al., 1999; Solomon and Okomoda, 2012).

The impact of alternative feed ingredients on growth is also crucial. In the present study, we found a non-linear relationship between the level of *L. minor* inclusion and the growth performance of the tilapia. In general, fish fed *L. minor* showed lower final weights and SGR than fish fed the control diet. Similarly, feed efficiency was lower (higher FCR) in fish fed *Lemna* compared to fish fed the control diet. However, it was interesting that final weight, SGR and FCR in fish fed 15 % *L. minor* (diet L_{15}) were similar to fish fed the control diet. Therefore, there was an optimal level (15 %) for the inclusion of *L. minor* in terms of growth performance and feed efficiency. Fish survival was also impacted by *L. minor* with significantly higher survival in tilapia fed the control diet than in fish fed 5 % *L. minor*. This was contrary to the results of Fasakin et al. (1999) who reported the highest survival rates in Nile tilapia fed 5 % and 10 % *Spirodela polyrrhiza*. However, in the present study, survival increased with increasing inclusion of *L. minor* and survival in fish fed the highest inclusion level of *L. minor* (L_{20}) was not significantly different to that in fish fed the control diet.

The generally poorer growth performance of the fish fed the diets with *L. minor* could be associated with palatability that may have been affected by the inclusion of *L. minor* with an associated impact on feed digestibility. Indeed lower feed intake, reduced digestibility and nutrient utilization associated with dietary duckweed have been reported in tilapia (El-Shafai et al., 2004) and grass carp (*Ctenopharyngodon idella*) (Dyke and Sutton, 1977), as well as the presence of antinutritional factors in *L. minor* as observed by Solomon and Okomoda (2012). Diets with reduced nutrient digestibility and utilization have been reported to result in lower growth of tilapia (Hlophe and Moyo, 2011). Similar results have been reported for common carp fed duckweed (Yılmaz and Günal, 2004) and Silver barb (*Barbodes gonionotus*) fed *L. minor* where growth was lower than that in fish fed the control diets (Noor et al., 2000). However, levels of key micronutrients may be a further factor impacting growth, feed efficiency and, possibly, survival in tilapia-fed *L. minor* in the present trial. While diets were balanced for gross nutrients and thus were isoproteic, isolipidic and isoenergetic, the replacement of several other ingredients by *L. minor* likely also affected amino acid and micronutrient compositions (Chakrabarti et al., 2018). Therefore, impacts on growth could be related to levels of essential amino acids and/or other micronutrients such as specific vitamins or minerals. Therefore, balancing amino acid composition and/or ensuring all micronutrients are in adequate supply may be able to mitigate or even prevent negative impacts on growth and survival. This is an important focus for future research.

It is also important to acknowledge that, when it comes to the use of processed Lemnaceae (duckweeds) in diets for fish, it is often difficult to make meaningful comparisons between studies. This is due to the different ways the studies have been performed and, in particular, what the diets containing dietary Lemnaceae have been compared to, with control feeds often based on fish meal with the macrophyte meal replacing this (Fasakin et al., 1999; Olaniyi and Oladunjoye, 2012; Velásquez, 2016). Similar to the present study, some used fish meal-free feeds although the plant-based ingredients replaced by the Lemnaceae meals varied between studies (Bag et al., 2011; Solomon and Okomoda, 2012). Precise processing of the meals also varied and, while most were harvested from the wild, dried and milled, the temperature and process of drying varied (Fasakin et al., 1999; Chareontesprasit and Jiwyam, 2001; Bag et al., 2011; Olaniyi and Oladunjoye, 2012; Solomon and Okomoda, 2012) and some studies have used additional processing such as fermentation (Velásquez, 2016). These differences between studies can impact the effects that the dietary Lemnaceae can have, and how they are reported, on growth and fish composition limiting the value of direct comparisons between different studies.

5. Conclusions

The current study has confirmed that Nile tilapia can effectively convert dietary ALA into the LC-PUFA, EPA and DHA, and thus has

clearly indicated the possibility of replacing dietary marine ingredients, fish meal (and fish oil), with duckweed (*L. minor*) in feeds for tilapia. Specifically, the levels of EPA, DHA and the n-3 PUFA: n-6 PUFA ratio were all significantly increased in a graded manner with the graded increase in dietary *L. minor*, which indicated the potential of *L. minor* meal to improve the nutritional quality of farmed tilapia. As the feeds used in the study were all plant-based without fish meal or any marine ingredients, this demonstrated that *L. minor* could replace marine sources of n-3 LC-PUFA in feeds while enabling tilapia to endogenously biosynthesize and accumulate EPA and DHA. The study further demonstrated that inclusion of *L. minor* meal at 15 % in the diet of tilapia resulted in similar growth to control feeds without *Lemna* and better growth than feeds with 5 %, 10 % and 20 % inclusion, suggesting an optimal level was likely. Overall, plant-based diets containing *L. minor* could improve the sustainability of feeds while maintaining and/or improving the levels of n-3 LC-PUFA that are important to human health in farmed tilapia. Further studies are required to explore possibilities for improving palatability, feed intake and bioavailability of nutrients, balancing micronutrients, and minimizing impacts of anti-nutritional factors and feeding strategies for maintaining efficient fish growth. Additionally, further research should be carried out to assess the economics of the commercial culture of duckweed for use in fish feeds.

CRediT authorship contribution statement

Mary A. Opiyo: Conceptualization, Data collection, Data analysis, Writing – original draft, Revision of the manuscript. **Patricia Muendo:** Conceptualization, Data collection, Project administration, Writing – original draft, Revision of the manuscript. **Kevin Mbogo:** Data curation, Data analysis, Writing – original draft, Revision of the manuscript. **Charles C. Ngugi:** Methodology, data collection, Writing – original draft, Revision of the manuscript. **Harrison Charo-Karisa:** Conceptualization, Writing – original draft, Revision of the manuscript. **Paul Orina:** Conceptualization, data collection, Writing – original draft, Revision of the manuscript. **William Leschen:** Conceptualization, Project administration, Writing – original draft, Revision of the manuscript. **Brett D. Glencross:** Conceptualization, Funding acquisition, Writing – original draft, Revision of the manuscript. **Douglas R. Tocher:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing, Revision of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contribution

Mary Opiyo, Patricia Muendo and Kevin Mbogo contributed to the conceptualization, data collection, data analysis, writing of the draft and revision of the manuscript; Charles Ngugi, Harrison Charo-Karisa and Paul Orina participated in the conceptualization, writing of the draft, revision of the manuscript; William Leschen, Brett Glencross and Douglas Tocher contributed to conceptualization, funding acquisition, project supervision, review of the draft and revision of the manuscript. All authors read and approved the final manuscript.

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