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ORIGINAL ARTICLE

Does duckweed (*Lemna minor*) feed inclusion play a role on growth, feed conversion ratio and reproductive performance (fertilization, hatchability and survivability rates) in omnivorous fish? Evidence in Nile tilapia (*Oreochromis niloticus*-Linnaeus, 1758)

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Abstract

Fish feed production is fraught with high costs due to the inclusion of expensive fishmeal and animal proteins that can be sourced from aquatic macrophytes. Limited attempts have been made on use of the latter for fish feed production. Further, the quality of feed given to fish is known to affect its growth and reproductive performance. Role of feeds containing duckweed *(Lemna minor)* at 0%-control feed, 10%, 15%, 20% and 25% inclusion levels on growth and reproductive performance of *Oreochromis niloticus* of size 18 ± 1 g were evaluated for 12 weeks. The fish were fed twice daily at 10% body weight at 9.00 a.m. and 4.00 p.m. Length–weight measurements were done fortnightly using a measuring board and a weighing balance, respectively. Female mouth-brooding fish were used to evaluate reproductive performance indicators, namely %: fertilization, hatchability and survivability. Data were subjected to one-way analysis of variance followed by post hoc and polynomial orthogonal analysis to identify *L. minor* diets with significant differences ($p < 0.05$). Fish fed on a diet containing 10% *L. minor* inclusion showed significantly better growth performance and feed conversion ratio than those fed on the control diet. All *L. minor* diets gave good fish condition factors above 1.0. Fish fed on a diet containing 10% *L. minor* and those fed on the control gave reasonably high survival rates of 85.55% and 83.33%, respectively, whereas those fed on 20% *L. minor* produced the same growth performance as control. Orthogonal polynomial analysis for the final weights across the *L. minor* diets – 0%–25% – showed a cubic polynomial model ($p = 0.000$), whereas final lengths portrayed a linear inverse significant relationship ($p < 0.05$). Inclusion of *L. minor* in the diets resulted to slightly better fertilization, hatchability and survivability rates at 10%,

20% and 15%, respectively. In conclusion, inclusion of *L. minor* from 10% to 20% in fish feeds is recommended for enhancing growth and reproductive performance of *O. niloticus*.

KEYWORDS
fertilization and survivability rates, fish feeds, growth performance, hatchability, *Lemna minor*, *Oreochromis niloticus*, reproductive performance

1 | INTRODUCTION

Lemna minor (duckweed) is a small floating aquatic macrophyte found in tropical and subtropical ecosystems. Its distribution is determined by climatic characteristics, nutritional status of the water body, as well as inter- and intra-species interactions (Chakrabarti et al., 2018; Mandal et al., 2010; Sogbesan et al., 2015). The macrophyte reproduces rapidly under favourable growth conditions through budding, and within 10 days, an individual mother leaflet produces at least 10 daughter propagules (Bog et al., 2020; Skillicorn et al., 1993; Ziegler et al., 2015). Due to its rapid growth rates, *L. minor* mass culture can be conducted frequently at minimum cost throughout the year.

Nutritionally, *L. minor* has a variety of lipids which include polyunsaturated fatty acids (PUFAs), a moderate protein content of between 28% and 43% and an averagely low fibre content of 5.7% (Appenroth et al., 2017; Chakrabarti et al., 2018; Naseem et al., 2021; Yan et al., 2013; Yosef et al., 2022) which are appropriate for herbivorous and omnivorous fish such as *Oreochromis niloticus*, *Cyprinus carpio* and *Ctenopharyngodon idella* (Mandal et al., 2010; Opiyo et al., 2022; Orina et al., 2018; Yılmaz & Günal, 2005). *L. minor* is suitable for replacement of soybean and fish meals in formulation of fish feeds and can substitute up to 30% nutritional requirements of *O. niloticus* (Appenroth et al., 2018). However, these levels depend on the geographical region that *L. minor* inhabits and whether it is obtained from the wild or artificially produced. Recent research has focused on inclusion of *L. minor* in fish feed formulation. This is driven by the problem of finding a cheap protein source to replace expensive soybean and fishmeal from declining capture fisheries against a fast-growing animal feed industry.

Availability of cheap fish feeds that provide required nutrients for optimal growth is a prerequisite to improved aquaculture yields (Mohapatra & Patra, 2013). Recent studies indicate that fish feeds of plant origin, such as aquatic macrophytes and algae, have certain beneficial fatty acids essential for optimal fish growth and reproduction (Chakrabarti et al., 2018; Opiyo et al., 2022; Yosef et al., 2022). For example, they contain PUFAs (Omega-3 and -6 fatty acids) which are useful in preventing and alleviating severity of various illnesses such as cancer, cardiovascular disease and psychological disorders in humans (Tocher et al., 2019). Hence, substantial research efforts are directed at evaluating nutritional value of different non-conventional fish food sources, such as aquatic macrophytes and terrestrial plants (Goswami et al., 2022; Omolo et al., 2017; Opiyo et al., 2022). Most terrestrial sources face competition in production of human and animal feeds. Current research is focused on use of aquatic macrophytes not utilized by humans as food, such as *L. minor*, *Azolla* spp. and *Ipomoea aquatica* as novel nutrient sources for fish feed production (Chepkirui et al., 2022; Opiyo et al., 2022).

Aquatic macrophytes are known to contain a wide range of nutrients not only suitable for optimal growth but also useful in fish reproduction. For example, Omega- 3 PUFAs including eicosapentaenoic acid and docosahexaenoic acid (DHA) found in *L. minor* can be useful in vitellogenesis. This is a vital stage in the female fish reproductive cycle which ensures subsequent hepatic production and distribution of lipoproteins, including vitellogenin. The latter alongside other lipids and vitamins are taken up by ova to provide energy stores for embryonic, larval and early life stage development (Lazzarotto et al., 2015). However, there is little research that has been conducted on the role that nutrients in *L. minor* play on growth and reproductive performance of cultured *O. niloticus*. For instance, there is a need to carry out investigations on the effect of *L. minor* feed inclusion on fish reproductive performance parameters such as the %: fertilization, hatchability and survivability.

Decreasing fish feed costs through the exploitation of novel, affordable, high-protein content sources is critical for successful aquaculture operations. Therefore, considerable research effort is required to determine nutritional requirements necessary to attain optimum reproductive performance and yields using the *L. minor* as a non-conventional feed ingredient. To formulate low-cost feeds, plant-based ingredients are used to fully or partially substitute expensive animal and fish meal protein sources (Mohapatra & Patra, 2013).

Most studies carried out on *L. minor* have demonstrated positive findings on the plant's capacity for use in the formulation of feeds for herbivorous and omnivorous species in India and Indonesia. Limited studies have adequately addressed the use of *L. minor* as a local fish feed ingredient for fresh warm water fish species such as *O. niloticus* in Kenya. This study will focus on the role of *L. minor* feed inclusion on growth and reproductive performance of *O. niloticus*. The species is chosen because it is the most abundantly pond cultured as well as nutritionally valued fish in Kenya.

2 | MATERIALS AND METHODS

2.1 | Culture of *L. minor*, Processing and its use in Fish Experimental Diets Formulation

Mass culture of *L. minor* was done using livestock manure in two outdoor earthen ponds measuring 600 m^2 for 6 months at the Kenya Marine Fisheries Research Institute (KMFRI) Sang'oro Aquaculture

FIGURE 1 Photographs showing, drying of harvested *Lemna minor*, its' formulated diets, pelletization and air drying of pellets: (a) harvested *L. minor*; (b and c) air and oven drying of *L. minor* respectively; (d) formulated diets 0%–25% *L. minor* feed inclusion; (e) pelletization of formulated *L. minor*; (f) drying of *L. minor* pellets.

Centre. The *L. minor* was harvested, air and oven dried (Figure 1a–c), milled into *L. minor* powder and used to formulate fish experimental diets at 0%, 10%, 15%, 20% and 25% inclusion levels (Figure 1d).

The formulated *L. minor* diets were exclusively free of marine or fish-based ingredients such as fish meal or fish oil. Soybean meal was used in the formulation of the five diets as the major source of protein, and the rest was availed by *L. minor which* was progressively used to replace sunflower seedcake at 0%, 10%, 15%, 20% and 25% (Table 1). The WinFeed software was used to estimate the quantities of each ingredient in each of the five formulated experimental diets. Weights for the dry feed ingredients were taken using a Sartorius weighing balance. After thoroughly mixing the ingredients, they were ground using a power mill and then carefully mixed with warm water using a mixer to form a dough. This was used to produce 3 mm pellets (Figure 1e) using a manual pelletizer. After sun drying (Figure 1f) to constant weight, pellets were stored in air tight plastic bags at room temperature.

TABLE 1 Formulated experimental fish diets with *Lemna minor* inclusion at 0%–25%.

	Inclusion levels				
Feed ingredients (g kg^{-1})	**LM0**	**LM10**	**LM15**	**LM20**	**LM25**
Soy bean meal	48.00	49.00	49.50	50.00	50.00
Maize bran	12.60	11.60	11.10	10.60	10.60
Wheat pollard	12.00	12.00	12.00	12.00	12.00
Sunflower seed cake	25.00	15.00	10.00	5.00	0.00
Lemna minor	0.00	10.00	15.00	20.00	25.00
L-lysine	1.00	1.00	1.00	1.00	1.00
Methionine	1.00	1.00	1.00	1.00	1.00
Mycotoxin binder	0.20	0.20	0.20	0.20	0.20
Vitamin premix[1]	0.10	0.10	0.10	0.10	0.10
Mineral premix[2]	0.10	0.10	0.10	0.10	0.10
Proximate composition of the experimental diets (% of dry weight)					
Crude protein (%)					
Moisture	7.13	7.41	7.21	7.51	7.50
Crude lipid	4.17	4.63	4.75	4.82	5.51
Crude protein	29.52	29.69	29.78	29.87	29.87
Crude fibre	7.07	5.74	5.90	5.53	5.53
Ash	8.57	7.98	7.92	7.62	7.52
Carbohydrate	43.27	44.55	44.53	45.64	44.07

Note: LM0 (0% *L. minor)*; LM10 (10% *L. minor)*; LM15 (15% *L. minor)*; LM20 (20% *L. minor)* and LM25 (25%). Vitamin premix[1] containing per each 2.5 kg: vitamin A (6 million I.U.), vitamin D3 (1 million I.U.), vitamin E (30,000 I.U.), vitamin B1(2500 mg), vitamin B2 (6000 mg), vitamin B6 (5000 mg), vitamin B12 (11 mg), vitamin K (4200 mg), vitamin C (10,000 mg), nicotinic acid (25,000 mg), pantothenic acid (22,000 mg), folic acid (1500 mg) and biotin (10 mg). Mineral premix[2] containing copper (5000 mg), manganese (1500,000 mg), iodine (1400 mg), selenium (120 mg), cobalt (200 mg), chlorine chloride (150,000 mg), iron (40.000 mg), zinc (50.0 mg) and anti-oxidant (125.0 mg).

2.2 | Proximate Composition of *L. minor and* Experimental Diets

Triplicate samples of *L. minor* powder and its formulated diets: LM0, LM10%, LM15, LM20 and LM25 were analysed at Fletcher Limited Nairobi-Kenya for crude protein and lipids, moisture, fibre and ash contents using standard methods of the Association of Official Analytical Chemists (AOAC, 1995). Moisture content was determined by oven drying in a Gallenkamp oven at 105°C for 12 h to constant weight, whereas ash content was estimated through the combustion of dry samples in a Lenton muffle furnace at 550°C for 24 h. The % moisture content was calculated as the percentage of the difference between the weights of the sample before and after drying against the initial weight. In contrast, ash content was estimated as the percentage of the difference between the weight of the sample after and before heating and that of the initial sample. Protein content was determined using a micro-Kjeldahl apparatus. Its weight was estimated as indicated in the following equation:

$$\text{Crude protein}\,\% = \text{nitrogen} \times \text{protein factor} \quad (1)$$

where

$$\text{Nitrogen} = (V_1 - V_2) \times N \times F \times 0.014 \times \frac{100}{V} \times \frac{100}{S} \quad (2)$$

where V_1 is the titre for the sample (mL), V_2 is the titre for blank (mL), N is the normality of HCL standard solution (0.002), F is the standard HCL solution factor, V is the volume of diluted digest taken for distillation (10 mL), and S is the sample weight (g).

Lipid content was extracted using petroleum ether in a Soxhlet extractor. Percent crude lipid was calculated as the percentage of the difference between the weight of sample before and after extraction against that of the initial sample. Fibre content was established and expressed as the percentage of the difference between the weight of acid, and alkali digested sample and that of incinerated sample after acid and alkali digestion against that of the initial sample. Carbohydrate content was obtained by subtracting the sum of lipids, moisture, ash and protein contents from 100.

2.3 | Experimental Set-up for Fish Feeding Trials

Growth performance trials of *O. niloticus* juveniles were carried out at the KMFRI, Kegati Aquaculture Research Centre, Kisii, situated at latitude 00°42″S; 034°47″E and altitude 1700 m above sea level. The selectively bred fish were obtained from the centre's hatchery and acclimatized in two raised 16 m^3 outdoor wooden box ponds. During this time, fish were fed on regular commercial feeds for 2 weeks. The formulated diets were then tested on a completely randomized design

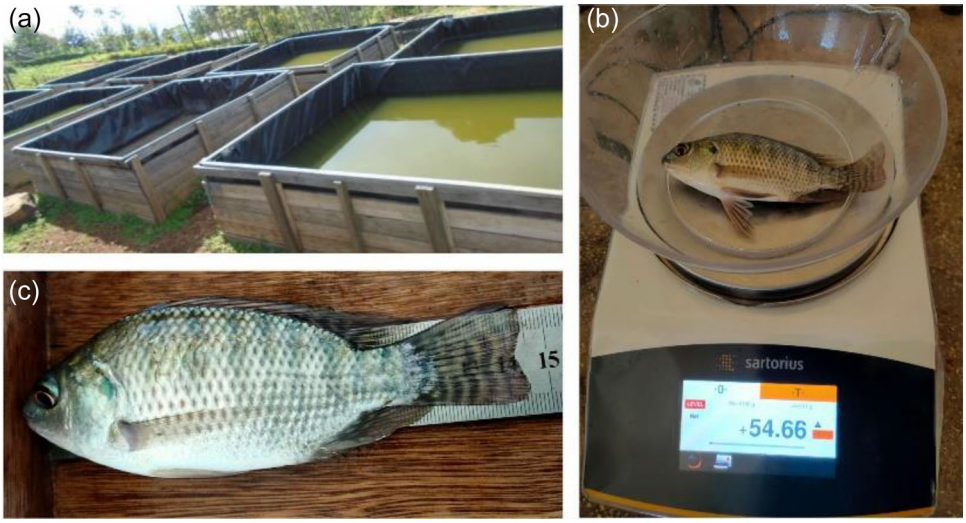

FIGURE 2 Photographs showing experimental set up comprising raised wooden box ponds, weighing and measuring of fish: (a) experimental raised wooden box ponds; (b) weighing fish using a Sartorius top pan balance; (c) measuring fish using a measuring board.

experiment containing triplicates of 30 fish (male to female ratio 1:3) per 9 m^3 raised wooden box pond (Figure 2a) at each treatment level for 12 weeks. Labels indicating diet treatment levels were affixed to the experimental ponds and feeding conducted daily at 9.00 a.m. and 4.00 p.m. at a ratio of 10% body weight.

2.4 | Growth Performance, FCR and Survival Estimation

Fortnight measurements of fish were carried out to assess growth performance using lengths and weights of all fish in the experimental set up. Total body length (TL cm) and body weights (Wg) were measured using a measuring board (Figure 2c) and a Sartorius digital top pan balance (Figure 2b) to the nearest 0.1 cm and 0.01 g, respectively. Before measurements, water was siphoned from individual wooden box ponds to a depth of 20 cm using a hosepipe with its openings secured by netting material to prevent fish loss. Fish were collected from the pond using scoop nets and put in 50-L buckets containing water supported with portable mini aerators. All the fish from each pond were anaesthetized using 30 ppm clove oil (Eugenol, 120 ppm to avoid handling mortalities and reduce stress; Charoendat et al., 2009).

Growth performance and feed utilization were assessed in terms of weight gain, average daily growth, specific growth rates (SGRs) and condition factors as follows:

$$\text{SGR}\ (\%) = 100\ (\ln(W_t) - \ln(W_0))\ /t \quad (3)$$

where W_0 is the natural logarithm of initial weight (g), W_t is the natural logarithm of final weight (g), and t is the period in days.

$$\text{Weight gain (WG)} = \text{final weight (g)} - \text{initial weight (g)} \quad (4)$$

$$\text{Average daily growth} = \text{mean weight gain/number of feeding days} \quad (5)$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Average feed given (g)}}{\text{weight gain (g)}} \quad (6)$$

At the end of the experiment, the fish in each raised wooden box pond were counted, and the survival rates were calculated as follows:

$$\text{Survival}\ (\%) = \frac{\text{number of fish at end of experiment}}{\text{number of fish stocked}} \quad (7)$$

2.5 | Estimation of Selected Reproductive Performance Parameters in Female *O. niloticus*

After the 10th week of the experiment, the frequency of spawning was determined by counting the number of female mouth brooders at each subsequent sampling. Mouth-brooding spawners (Figure 3a) were identified and transferred using labelled buckets containing clean water to the KMFRI hatchery. At the laboratory, eggs were removed from brooders by opening their mouths under water to let go of the eggs (Figure 3b). They were then filtered off the water using a nylon mosquito net of 0.5 mm mesh size. The eggs were put in a petri dish (Figure 3c), and the fertilized eggs which appeared yellow were separated from pale yellow unfertilized (Figure 3d) ones using a feather. These together with the hatched fry obtained from the buccal cavity of the brooders were counted. The unhatched eggs were transferred into labelled glass aquaria (Figure 3e) fitted with air pump aerators and thermostats for continual monitoring for determination of hatching rate. Water temperatures in all aquaria were maintained at 24.5°C. Daily light period was the normal tropical 12 h light–dark cycle.

FIGURE 3 Photographs showing activities involving egg collection from female mouth-brooding *Oreochromis niloticus*: (a) eggs in a female *O. niloticus* mouth brooder; (b) extraction of eggs from a female mouth brooder; (c) eggs contained in a petri dish; (d) a pale yellow unfertilized egg; (e) labelled glass aquarium fitted with air pumps aerators and thermostats.

The collected data were used to estimate the reproductive performance parameters, namely % fertilization, % hatchability and % survivability.

To determine the % fertilization, all the eggs that were collected from the brooder's buccal cavity were weighed and their total count estimated by weighing 1 g of eggs in a petri dish and then counted using a feather. Total egg count was estimated as

$$\text{Total egg count} = \text{total egg weight (g)} \times \text{no. of eggs in 1 (g)} + \text{No. of eggs in 1 g} \quad (8)$$

Unfertilized eggs which appeared pale yellow-white were counted and subtracted from the total egg count to obtain the number of fertilized eggs. % Fertilization was estimated as

$$\%\text{Fertilization} = \frac{\text{fertilized egg count}}{\text{total egg count}} \times 100 \quad (9)$$

To estimate the % hatchability, unhatched eggs which appeared pale yellow or white in colour were counted and subtracted from the total egg count to obtain number of hatched eggs. This was used to estimate % hatchability as:

TABLE 2 Levels of selected physico-chemical parameters of the fish culture environment.

Feed	Temperature (°C)	Dissolved oxygen (mg L^{-1})	pH	Salinity (ppm)
LM0	24.17 ± 0.28	8.00 ± 0.16	6.60	0.08
LM10	24.47 ± 0.27	7.91 ± 0.17	6.39	0.09
LM15	24.32 ± 0.30	7.84 ± 0.18	6.41	0.08
LM20	24.33 ± 0.26	7.98 ± 0.10	6.61	0.08
LM25	24.2 ± 0.24	7.88 ± 0.17	6.27	0.10

Note: Average values of temperature dissolved oxygen and salinity in each pond during the experimental period and presented as means ± SE.

$$\% \text{ Hatchability} = \frac{\text{hatched egg count}}{\text{total egg count}} \times 100 \quad (10)$$

To estimate % survivability, the total numbers of hatchlings and fertilized eggs were counted and then calculated as

$$\% \text{ Survivability} = \frac{\text{total hatchlings egg count}}{\text{totalfertilized egg count}} \times 100 \quad (11)$$

2.6 | Water Quality Monitoring

Water exchange in the box ponds was conducted once weekly to maintain water quality at recommended levels. Food remains and faecal waste were siphoned out daily after the last daily feeding at 5.00 p.m. Water quality parameters were measured weekly using a multi-parameter meter model H19828 (Hanna Instruments Ltd.).

3 | DATA ANALYSIS

Data analysis was conducted using the IBM statistical package SPSS 18.0 software (SPSS Inc.). Results were expressed as means ± standard deviation. Differences among treatments were examined using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The relationship between the predictor variables (% *L. minor* feed inclusion levels) and response variables, namely growth and reproductive performance parameters of *O. niloticus*, was tested using orthogonal polynomial contrasts-linear, quadratic and cubic models. *p*-Values of <0.05 were considered statistically significant.

4 | RESULTS

4.1 | Water Quality Parameters

The estimated means of water quality parameters measured during the experimental period are presented in Table 2. Temperature ranged between 24.17 and 24.47°C, dissolved oxygen oscillated between 7.84 and 8.00 mg L^{-1}, and pH varied from 6.27 to 6.60, whereas salinity ranged from 0.08 to 0.01 ppm. All water quality parameters were within acceptable levels for fish survival during the experimental period. There were no significant differences between parameters within each column.

4.2 | Growth Performance of *O. niloticus*

Table 3 presents results of ANOVA and orthogonal polynomial regression analyses (linear, quadratic and cubic models) which were used to find out if there were significant differences between mean values of growth performance parameters of *O. niloticus* subjected to various *L. minor* diets. Initial and final fish weights ranged from 18.00–18.93 to 33.46–40.17 g, respectively. Initial and final lengths ranged between 10.20–10.43 cm and 12.31–13.16 cm. ANOVA tests for final lengths and weights showed significant differences ($p < 0.05$). Orthogonal polynomial analysis for the final weights indicated that a cubic polynomial model gave the best fit ($p = 0.000$), suggesting an increase in weight of fish between 0% and 10% *L. minor* diets, respectively, followed by a decrease to the lowest at 15% *L. minor* diet and then marginal increases for diets 20% and 25%, respectively. On the other hand, there was a linear inverse significant ($p < 0.05$) relationship between final mean lengths and different *L. minor* diets – 0%–25% (Table 3, Table S2a and Figure S1a).

Weight gain ranged from 15.36 g at 15% *L. minor* diet to 21.36 g at 10%, respectively. ANOVA test indicated that there was a significant difference in the percentage weight gain among the *L. minor* diets treatment groups ($p < 0.05$). Further, Turkey's post hoc showed that fish fed on the 10% *L. minor* diet presented a significantly higher final weight gain than all the other *L. minor* treatments. Weight gain recorded significant linear, quadratic and cubic model fits (Table 3) with *L. minor* levels increasing from 0% to 25% ($p < 0.05$). However, the cubic model was a better fit suggesting an increase in weight gain of fish between 0% and 10% *L. minor* diets, respectively, followed by a decrease at 15% *L. minor* diet and then marginal increases (Table 3, Table S2a and Figure S1a).

Average daily growth ranged from 0.19 g at 20% and 25% *L. minor* diets to 0.25 g at 10%, respectively (Table 3). ANOVA test indicated significant differences amongst the *L. minor* diets ($p = 0.000$) with Turkey post hoc showing that it was significantly higher in fish fed 10% and 15% *L. minor* diet than the rest of the treatments. SGR ranged from 0.75 in fish fed on a diet containing 15% *L. minor* to 0.90 in those fed at 10%, respectively. The diet formulated at 20% *L. minor* feed inclusion gave similar SGR results like that of the control (0%). ANOVA test

TABLE 3 Growth performance of *Oreochromis niloticus* fed on *Lemna minor*-based diets.

Parameter	% *L. minor* diets (0%–25%)					Overall *F* test	*p*-Value		
	LM0	LM10	LM15	LM20	LM25		Linear	Quadratic	Cubic
Initial weight (g)	18.06 ± 0.40	18.80 ± 0.41	18.10 ± 0.40	18.00 ± 0.44	18.93 ± 0.42	0.362	0.485	0.537	0.071
Initial length (cm)	10.20 ± 0.81	10.37 ± 0.89	10.27 ± 0.91	10.27 ± 1.06	10.43 ± 0.76	0.455	0.245	0.809	0.148
Final weight (g)	34.97 ± 1.02^{b}	40.17 ± 0.95^{a}	33.46 ± 0.59^{c}	34.32 ± 0.84^{b}	34.59 ± 0.74^{b}	0.000	0.015	0.470	0.000
Final length (cm)	13.16 ± 1.49^{a}	12.97 ± 1.21^{ab}	12.60 ± 00.85^{bc}	12.92 ± 1.40^{ab}	12.31 ± 1.02^{c}	0.000	0.000	0.789	0.059
Weight gain (g fish^{-1})	16.90 ± 0.64^{b}	21.36 ± 0.58^{a}	15.36 ± 0.20^{b}	16.32 ± 0.40^{b}	15.65 ± 0.41^{b}	0.000	0.000	0.005	0.000
Average daily growth (g day^{-1})	0.20 ± 0.00^{b}	0.25 ± 0.00^{a}	0.24 ± 0.00^{a}	0.19 ± 0.00^{c}	0.19 ± 0.00^{c}	0.000	0.000	0.000	0.000
%SGR (% day^{-1})	0.77 ± 0.10^{b}	0.90 ± 0.05^{a}	0.75 ± 0.11^{bc}	0.77 ± 0.06^{b}	0.77 ± 0.10^{b}	0.000	0.000	0.000	0.000
FCR	1.56 ± 0.35^{a}	1.04 ± 0.01^{d}	1.26 ± 0.02^{b}	1.20 ± 0.01^{a}	1.24 ± 0.02^{b}	0.238	0.339	0.171	0.200
% Survival	86.67%^{a}	86.67%^{a}	81.11%^{a}	78.89%^{a}	77.78%^{a}	0.356	0.046	0.941	0.601
CF/K (g cm^{-3})	1.51 ± 0.11^{c}	1.95 ± 0.74^{a}	1.66 ± 0.08^{b}	1.58 ± 1.61^{bc}	1.84 ± 0.13^{a}	0.000	0.011	0.256	0.000

Note: Mean = mean ± std. deviation. One-way ANOVA followed by Tukey post hoc. Means between a column followed by different superscript letters (a–e) are significantly different ($p < 0.05$) with respect to the type of growth performance parameters in respective *L. minor* diets. Linear, quadratic and cubic effects of *L. minor* levels were assessed using orthogonal polynomial contrasts; <0.05 was considered statistically significant.

showed significant differences amongst the *L. minor* diets ($p = 0.000$) with Turkey post hoc showing that it was significantly higher in fish fed on a diet containing 10% *L. minor* than those fed on the control and the rest of the treatments. Average daily growth and SGR displayed highly significant ($p < 0.05$) linear, quadratic and cubic model fits across the *L. minor* feeding levels (Table 3, Table S2a and Figure S1b).

Feed conversion ratio (FCR) ranged from 1.04 ± 0.01 in fish fed on a diet containing 10% *L. minor* to 1.56 in those fed on the control diet. All the *L. minor* formulated diets gave slightly lower FCRs which were not statistically significant ($p > 0.05$) than the control feed (0%). This shows that the *L. minor* diets had more or less the same effect as the control. The orthogonal polynomial analysis was also not statistically significant ($p > 0.05$). Survival rates ranged from 77.78% in fish fed on a diet containing 25% *L. minor* to 86.67% in those fed at 10%, respectively. Fish fed on the control feed presented slightly similar survival rates to those fed on a diet containing 10% *L. minor*. ANOVA test established no significant differences in the percentage survival rates amongst the various *L. minor* diets ($p > 0.05$). However, the orthogonal polynomial analysis showed a significant linear decrease in the percentage survival rate with increasing *L. minor* inclusion levels from 0% to 25% ($p = 0.046$). All the *L. minor* diets presented good condition factors. ANOVA test showed significant differences across the *L. minor* diets with Turkey post hoc indicating that the 10% diet was the highest. The cubic polynomial orthogonal contrast gave the best fit (Table 3 and Figure S1b).

In general, fish that were fed on a diet containing 10% *L. minor* gave the highest final weight and weight gain, average daily growth, SGR and condition factor with the lowest FCR.

4.3 | Reproductive Performance Parameters in Female *O. niloticus*

Estimated reproductive performance parameters are presented in Table 4, Tables S1b and S3, Table S2b as well as Figure S2. Fish fed on 10% *L. minor* diet had a higher number of spawnings compared to the control. There were minor differences in the number of spawnings among the other feed trials (Tables S3).

One-way ANOVA test as well as polynomial orthogonal contrasts on all the reproductive performance parameters showed no significant differences between control and all the other *L. minor* diets (Table 4). However, there were slight notable variations within means of the parameters. The numbers of fertilized eggs, total egg count and hatchlings were highest in mouth brooders that were fed on the control diet and had a general declining trend with increasing *L. minor* feed inclusion levels of 0%–25% (Table 4, Table S3). Percentage fertilization and survivability were highest in fish-fed diets containing 10% and 15% *L. minor* feed inclusion levels (Table 4).

There were four reproductive performance parameters that indicated slightly better performances among the *L. minor* feed inclusion levels than the controls, namely %: fertilization at 10%, hatchability at 20% and survivability at 15% (Table 4).

TABLE 4 Reproductive performance parameters in female *Oreochromis niloticus*.

Parameter	LM0	LM10	LM15	LM20	LM25	*p*-Values			
						Overall *F* test	Linear	Quadratic	Cubic
Total eggs count	302 ± 8.75	242.33 ± 8.41	200.00 ± 49.10	206.50 ± 62.57	113.00 ± 33	0.398	0.065	0.974	0.447
% Fertilization	98.25 ± 0.79	99.05 ± 0.34	98.61 ± 0.32	98.08 ± 0.85	97.95 ± 2.05	0.597	0.463	0.464	0.317
% Hatchability	85.39 ± 1.97	90.60 ± 3.48	88.74 ± 1.83	94.18 ± 2.33	86.61 ± 0.89	0.141	0.546	0.075	0.581
% Survivability	84.83 ± 8.06	87.32 ± 5.10	92.39 ± 2.31	86.67 ± 5.06	81.55 ± 10.11	0.920	0.849	0.454	0.786

Note: Mean = mean ± std. deviation. ANOVA test followed by Tukey post hoc. Means = mean ± std. deviation. Linear, quadratic and cubic effects of *Lemna minor* levels were assessed using orthogonal polynomial contrasts ($p < 0.05$).

5 | DISCUSSION

5.1 | Proximate Analysis of *L. minor*

Proximate analysis of whole *L. minor* showed that the % crude protein and lipid concentrations in the current study were relatively higher than those found by Herawati et al. (2020) and Solomon and Okomoda (2012), Opiyo et al. (2018). On the contrary, ash contents from the three latter studies were much higher than those of this study. The % fibre content of *L. minor* in the current study was almost sixfold lower than that recorded by Herawati et al. (2020) and fourfold lower than that of Okomoda op cit. These differences may be due to nutritional analysis of *L. minor* from the wild (Opiyo op cit), different culture conditions (Herawati, op cit), processing methods and geographical location associated with soil type, local *L. minor* varieties and climatic conditions. Presence of high fibre content as reported by the authors indicates that their diets were less digestible than those used in this study due to the fact that *O. niloticus* are monogastric and do not have enzymes that effectively digest fibre. *L. minor* protein and lipid contents were within the range of *O. niloticus* protein requirements for adult fish (Morton et al., 2017).

The higher lipid and protein as well as lower fibre contents established in the whole *L. minor* used in this study have also been documented in earlier studies on its nutritional status (Appenroth et al., 2018; Asimi et al., 2018; Chakrabarti et al., 2018). The observation that *L. minor* has higher lipid content than those of past studies further demonstrates the significance of the macrophytes in fish feed formulation. This is because a feed with high lipids greatly improves fish growth rates and reproduction (Craig & Helfrich, 2017; Naseem et al., 2021).

The percentage ash content represents the mineral matter of the feed, such as calcium, phosphorus, potassium and magnesium. The normal range of ash content in fish feed is 7%–12% (Morton et al., 2017). The ash content of the whole *L. minor* for the current study was lower than that of Chepkirui et al. (2022), Herawati et al. (2020) and Solomon and Okomoda (2012) and was within the acceptable range.

5.2 | Growth Performance, FCR and Survival

Fish feeds constitute an important input in aquaculture contributing on average 60% of total production cost and directly affecting fish growth rates (Fauzi et al., 2017). The quality and quantity of a feed determine growth performance in cultured fish. Thus, a good fish feed should contain a balanced mixture of dietary nutrient requirements for the cultured species. The attempt of this study to use *L. minor* aimed at establishing whether formulating it with other ingredients could yield equally good growth performance, FCR and survival equivalent or better than the conventional commercial soybean or fish meal-formulated diets. Studies on the relationship between growth performance and feeding level involving *L. minor* have been carried out (Herawati et al., 2020; Opiyo et al., 2022; Solomon & Okomoda, 2012). Findings of Solomon and Okomoda (2012) yielded similar results to the current

study, that is, an inverse relationship between the level of feed inclusion and growth performance. Results from the rest of the mentioned authors demonstrated no relationship between the two parameters. This can be attributed to differences in the formulated diets especially feeding levels, source of *L. minor* used and the composition of the ingredients in the experimental feed.

Fish fed on the 10% *L. minor* presented better growth performance compared to those fed on control diet. This finding was different from that of Opiyo et al. (2022) who established optimum performance at 15% and Solomon and Okomoda (2012) at 5% on Nile tilapia, respectively. Other studies using *L. minor* feed inclusions in common carp (*C. carpio*) showed optimum growth at 15% *L. minor* feed (Asimi et al., 2018; Mohapatra & Patra, 2013).

Higher growth rates at lower % *L. minor* feed inclusion levels can be attributed to better palatability and enzymatic digestibility than in the control diet (Naseem et al., 2021). Fibre, which comprises the polymeric cellulose, forms complex inhibitors which not only affects palatability but also hinders enzymatic digestion, absorption and assimilation of nutrients into the fish' metabolism (Opiyo et al., 2022; Solomon & Okomoda, 2012). Growth performance of a fish is controlled by its metabolism. Therefore, factors which adversely affect metabolism slow down the growth rate of fish. Fish are monogastric with simple stomachs and have reduced capacity to digest the cellulose present in the *L. minor* biomass. Lower feed intake, reduced digestibility and nutrient utilization associated with increased dietary *L. minor* have been reported in *O. niloticus* fed on macrophytes-formulated diets (Asimi et al., 2018; Chepkirui et al., 2022; Opiyo et al., 2022). Reduced growth performance with increase in *L. minor* inclusion in the treatment diets can also be due to increasing anti-nutritional factors in the plant (Naseem et al., 2021; Solomon & Okomoda, 2012). Preliminary analysis of *L. minor* in this study established that it has tannins, flavonoids and alkaloids, which are well-known anti-nutritional factors.

According to Jisr et al. (2018) condition, factor (*K*) mirrors the physiological well-being of fish, and that *K* values of 1 and beyond show good health of the fish. *K* values in the current study indicated that all the fish were in good health throughout the study period and were in concurrence with earlier studies by Opiyo et al. (2022) and Mohapatra and Patra (2013). The significant variations in fish' condition factor between the *L. minor* diet treatments can be attributed to the fish feeding behaviour and spawning (Degsera et al., 2020). Fish survival was also relatively impacted by the formulated diets with slightly higher survival rates in fish fed on the control (0%) and 10% *L. minor* inclusion and was within the range of values reported by Asimi et al. (2018) and Opiyo et al. (2022).

FCR is the efficiency with which feed is converted to body mass by an animal. In aquaculture, fish feeds are among important factor affecting FCR, whereas others are diet composition, culture practices, fish health, genetics and feeding environment (Novriadi, 2017). The FCRs observed in this study were significantly different, with those formulated with *L. minor* being lower than the control. This suggests that the formulated diets can be used in place of that of the control feed to achieve a better FCR and hence the suitability of the *L. minor* diets for aquaculture. Low FCR values in *L. minor* diets in the present study suggest efficient feed utilization hence low cost of production compared to the control. Therefore, the foregoing discussion demonstrates that there is a lot of promise in the use of *L. minor in* the production of a fish feed for culture of *O. niloticus* as well as for other cultured species.

Other major micronutrients present in *L. minor* may have contributed to the fish growth performance, food conversion ratio and survival (Chakrabarti et al., 2018). This calls for further research to identify and quantify the dietary micronutrients present in cultured and wild *L. minor* and their effects on fish growth and survival.

5.3 | Reproductive Performance Parameters in F.emale *O. niloticus*

The higher number of spawnings observed in fish given 10% and 20% feed with *L. minor* inclusions is beneficial as it is an indicator of higher fingerling production. Although there were a fewer number of spawnings in the control diet than in the 10% *L. minor* feed inclusion level, the fish in the control had a higher number of fertilized eggs but with lower survivorship.

Reproductive performance parameters, namely number of fertilized eggs, total egg count and hatchlings, declined with increase in *L. minor* feed inclusion level. This is attributed to increase in content of anti-nutritional factors as the % *L. minor* feed inclusion level increases (Opiyo et al., 2022). This may have interfered with the fish's nutrition hence retarding growth and reproductive performance.

It is known that when *O. niloticus* thrives in confined space, it does develop stunted growth (Massou et al., 2004). All fish used in this experiment were raised in confined space in raised wooden ponds hence the low weight gain observed. Further studies need to be carried out on the effect of *L. minor* diets in a free aquaculture environment on the reproductive parameters.

6 | CONCLUSIONS

L. minor included in *O. niloticus* feed formulation is readily accepted by the fish with 10% inclusion giving optimum growth and reproductive performance results. The study has shown that *L. minor* has great potential as an ingredient for inclusion in fish feed formulation and production. Their affordability, ease of culture and mass production over limited space and water are qualities that distinguish the plant as a novel fish feeds ingredient with potential to accelerate aquaculture commercialization.

The study recommends the inclusion of *L. minor* in fish feeds at 10%–20% levels for optimum growth and reproductive performance. However, criteria for selection of % inclusion level will always rest on the desired goals. For example, if one wishes to improve egg production, fertilization and hatching, 10% would be the most appropriate. However, if one wants to obtain the highest percentage of surviving larvae, then 15% will work, whereas 20% will give the highest hatching rates. Nevertheless, these suggestions are subject to further research.

Further trials need to be done with other fresh water fish species to establish the optimum growth and reproductive performance. More studies are required to explore possibilities for improving palatability, bioavailability of nutrients, harmonizing micronutrients and lessening effects of anti-nutritional factors and feeding approaches for effective fish growth using *L. minor*.

Generally, it is concluded that *L. minor* inclusion in the feed yielded slightly better reproductive performance than the control feed with the percentage: fertilization at 10%, hatchability at 20% and survivability at 15% than the control feed commercially sold to farmers. It is concluded that including *L. minor* in fish diets at 10%–20% can improve growth and reproductive performance of *O. niloticus*

AUTHOR CONTRIBUTIONS

Judith Kemunto Achoki: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; resources; validation; visualization; writing—original draft; writing—review and editing. **Catherine Kaluwa Kaingu**: Conceptualization; formal analysis; methodology; supervision; validation; visualization writing—original draft; writing—review and editing. **Jemimah Achieng' Oduma**: Conceptualization; methodology; supervision; validation; visualization; writing—original draft; writing—review and editing. **Paul Sagwe Orina**: Conceptualization; investigation; methodology; project administration; resources; supervision; validation; visualization; writing—original draft; writing—review and editing. **Robert Nyakwama Ondiba**: Formal analysis; investigation; methodology; resources; validation; visualization; writing—original draft; writing—review and editing. **Robert Nyamao Nyabwanga**: Data curation; formal analysis; writing—review and editing. **Albert Mochache Getabu**: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest or personal associations that could have affected the work reported herein.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the Supporting Information of this research article.

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ETHICS STATEMENT

The experiment was carried out in compliance with the requirements of the National Society for the Prevention of Cruelty to Animals, 1962 Act, CAP 360 (Reviewed 2012) of the laws of Kenya. The study was approved by the University of Nairobi Biosafety and Animal Use Ethical Committee (BAUEC). Handling of fish in the entire research duration was done as per recommendations in ethical justification for use and treatment of fishes used in research (Metcalfe & Craig 2011). All scientific ethics relating to accuracy, dependability, validity, systematic approach to investigation and due observance of bias were sustained in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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