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RESEARCH PAPER

Fishmeal partial replacement using duckweed (*Lemna minor*) enhances growth performance and body composition of Nile tilapia (*Oreochromis niloticus*) L.

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Abstract

Nile tilapia (*Oreochromis niloticus*) juveniles were fed experimental diets with duckweed (*Lemna minor*) supplementing fish meal at 0% (LM0), 5% (LM5), and 15% (LM15) and compared to a commercial diet as a positive control (COMM). Growth performance, feed utilization, and body composition were evaluated and compared with the control diet. The final weight and specific growth rate were significantly higher in the fish fed the commercial diet and in LM15 when compared to LM0 ($P < 0.05$). The fish could utilize the *L. minor*-based feed although the feed conversion ratio was significantly lower in fish fed the LM15 and COMM diets than the other diets ($P < 0.05$). Fish body composition was significantly affected by *L. minor*-based diets. Protein content was significantly higher in fish fed on control diet and diet LM10 ($P < 0.05$) compared with other diets. In contrast, lipid content was significantly higher in fish fed *L. minor* -based diets than in the control, with LM15 having the highest levels ($P < 0.05$). Partial replacement of fish meal with *L. minor* at 15% in the diet of *O. niloticus* is therefore recommended because it enhances growth performance, improves feed utilization, and increases the lipid content in *O. niloticus.*

Introduction

Fish feed is the most expensive input in fish farming, constituting more than 60% of the total production cost in an aquaculture enterprise (El-Sayed, 2008; Charo-Karisa et al., 2013). Feed ingredients, especially fish meal, have continuously experienced fluctuating prices and competition from other animal feed industries, thus affecting aquaculture feed production and, consequently, fish production (Shati et al., 2022). Plant-based protein sources from agricultural produce and by-products have been used as

alternatives to animal proteins (Abowei & Ekubo, 2011; Munguti et al., 2012; Montoya-Camacho et al., 2019). However, the sustainability of these plant protein sources is often threatened by unpredictable weather conditions due to climate change (Shati et al., 2022). Aquatic macrophytes are a sustainable source of protein for fish feed production because they can be grown in large quantities in nutrient-rich water lagoons that are not being used by communities and ponds in tropical and subtropical countries (Hassan & Edwards, 1992; Hasan & Chakrabarti, 2009; Chakrabarti *et al.*, 2018; Naseem et al., 2021).

Duckweed (*Lemna minor)*is considered a novel feed ingredient for the replacement of fish meal for omnivorous/herbivorous fish such as Nile tilapia (*Oreochromis niloticus*) (Hasan & Chakrabarti, 2009; Chakrabarti, 2018) and are considered beneficial for increasing the sustainability of small-scale aquaculture (Slembrouck et al., 2018). *Lemna minor* is a free-floating freshwater macrophyte belonging to the family Lemnacea and is found in freshwater ponds, lagoons, ditches, and streams in both tropical and subtropical climates (Culley et al., 1981; Hassan & Edwards, 1992; Young et al., 2006). They have multiple uses, including wastewater treatment, as food for humans, and as feed ingredients for fish and terrestrial animals (Culley et al., 1981; Chakrabarti et al., 2018; Nesan et al., 2020; Sosa et al., 2024). In aquaculture, *L. minor* is readily consumed as a raw macrophyte by *O. niloticus*, the Common carp (*Cyprinus carpio)* and other omnivorous fish (Hassan & Edwards, 1992; Yılmaz et al., 2004). It is reported to contain 35–45% CP, essential amino acid and mineral profiles of the plant dry weight (El-Sayed, 1999). It is also characterized by the availability of essential amino acids, vitamins A, B, and E, and carotenoids, which are required by the fish (El-Sayed, 1999; Cruz et al., 2011; Naseem et al., 2021).

Previous studies have documented the use of *L. minor* as a protein source for the larval stages of various fish species, *O. niloticus* (El-Shafai et al., 2004; Solomon & Okomoda, 2012; Uddin et al., 2014; Cipriani et al., 2021; Achoki et al., 2024), *C. carpio* (Yılmaz et al., 2004), Silver barbs (*Barbonymus gonionotus*) (Noor et al., 2000) and other omnivorous fish. However, the use of *L. minor* to replace fish meal in the diets of grow-out *O. niloticus* cultured in ponds has not been documented. This study aimed to determine the effects of *L. minor* as a replacement for fish meal on *O. niloticus* grow-out in ponds, focusing on growth performance and body composition.

Materials and methods

Experimental design

The study was conducted in cages installed in an 800 m^2 with a depth of 1.5 m pond at the Kenya Marine and Fisheries Research Institute (KMFRI), National Aquaculture Research Development & Training Center (NARDTC), Sagana. Twelve cages whose length, width and height/depth were 2 m × 2 m × 1.2 m respectively were installed in earthen ponds that were previously limed and treated with agricultural lime at 100 g m^{-2}. The cages were stocked with *O. niloticus* juveniles of an average weight of 30.5 g. *L. minor* previously harvested from ponds at KMFRI Sagana was processed by drying under a shed for feed formulation. The other feed ingredients (Table 1) were purchased from local agrovet shops and ground separately into finer particles using a hammer mill (Thomas-Wiley intermediate mill, 3348-L10 series, USA). Three isonitrogenous (approximately 30% crude protein) experimental diets were prepared by replacing levels of fishmeal with dry *L. minor* meal 0% (LM0) (control), 10% (LM10), and 15% (LM15), following *L. minor* inclusion levels by (Opiyo et al., 2022). A commercial diet (COMM) sourced from a local feed manufacturer was used as a positive control. During the production of diets (LM0, LM10, and LM15), the ingredients were mixed thoroughly with water to make a homogenous dough and pelletized using a 2-3 mm commercial pelletizing machine into floating pellets. The pellets were dried, packed, and stored in a clean, dry, and cool environment. The experimental fish were hand-fed twice a day (1000 and 1600 h) to apparent satiation for 84 days.

Water quality monitoring

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

Table 1. Ingredients, formulations, and proximate compositions of the experimental diets

	Diet			
Ingredients (%)	**LM0**	**LM10**	**LM15**	**COMM**
Wheat bran	27	23	20	-
Soybean meal	23	22	18	-
Maize bran	25	24	25	-
Fish meal	20	16	17	-
Lemna minor	0	10	15	-
Soybean oil	1	1	1	-
Monocalcium Phosphate (MCP)	3	3	3	-
Vitamin premix	1	1	1	-
Proximate composition (% of dry weight)				
Dry matter	93.8	94.2	94.1	94.1
Crude protein	30.2	30.1	30.3	30.2
Crude lipids	5.7	4.5	5.5	8.5
Ash	12	14.2	13.3	9
Carbohydrates	52.1	51.2	50.9	52.3

***** LM0 (0 % *L. minor*); LM10 (10% *L. minor*); LM15 (15% *L. minor*); COMM (commercial diet).

Fish sampling for growth parameters and feed utilization

Fish were monitored for growth and mortality were recorded daily. Fish sampling was performed every 21 days. Fish were fasted for 24 h before sampling to allow gut emptying. All the fish in the cage were sedated with clove oil at 20 g L^{-1} and individually weighed with a digital balance (model EHB-3000, China) to the nearest 0.01 g and total length with a measuring board to the nearest 0.1 cm according to Caspers, (1969). At the end of the experiment, the dead fish were subtracted from the number stocked, and the percent survival was calculated. Growth performance and feed utilization were assessed in terms of weight gain, average daily growth, specific growth rates (SGRs) and condition factors as follows:

$$\text{SGR (\%)} = \frac{100\,(\ln(W\text{t}) - \ln(W0)}{t} \quad (1)$$

Where;

$W0$ is the natural logarithm of initial weight (g), Wt is the natural logarithm of final weight (g), and t is the period in days.

$$\text{Daily Weight gain (WG)} = \text{final weight (g)} - \text{initial weight (g)} \quad (2)$$

$$\text{Weight gain (WG\%)} = [(\text{final weight (g)} - \text{initial weight (g)}] \times 100 \quad (3)$$

$$\text{Feed conversion ratio (FCR)} = \text{Average feed given (g)} / \text{weight gain (g)} \quad (4)$$

$$\text{Survival (\%)} = \frac{\text{number of fish at end of experiment}}{\text{number of fish stocked}} \quad (5)$$

At the end of the experimental period, a random sample of three fish was collected from each replicate cage (n=9) and euthanized by placing fish in a container with ice water before culling (Lambooij et al., 2008). The fish samples were pooled and homogenized to form one sample per cage (n=3 per diet treatment) for body composition analysis using standard methods.

Proximate composition analysis of feeds and fish

The proximate composition of the experimental diets and fish carcasses was analysed using standard methods by the Association of Official

Analytical Chemists (AOAC, 2023). Dry matter content was measured by gravimetry, and moisture content was determined by oven drying for 12 h at 105 °C to a constant weight. Protein content (N × 6.25) was determined using a micro-Kjeldahl apparatus (Labconco Corporation, Kansas City, USA), lipid content was measured using a Soxhlet extractor (VELP Scientifica, Milano, Italy), and ash content was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, IO, USA) at 550 °C for 12 h. Carbohydrates were determined by subtracting of crude protein, crude lipids, and ash from 100.

Data analysis

The data were cleaned, and normality was determined using the Shapiro-Wilk test. Mean comparisons were performed using one-way analysis of variance (one-way ANOVA) followed by Tukey's HSD post-hoc test to determine the pairwise differences among the diets. Differences were considered statistically significant at $P < 0.05$. Percentage data were arcsine-transformed to normalize the data before analysis. All statistical analyses were performed using Statistical Package and Service Solutions (SPSS version 23).

Results

Water quality

The mean values of the pond water quality parameters were stable with minimal variations during the experimental period. The mean values were as follows: Temperature (26.41 ± 0.23 °C), dissolved oxygen (DO) (5.58 ± 0.08 mg L^{-1}), conductivity (102.46 ± 1.68 µS cm^{-1}), total dissolved solids (64.06 ± 1.09 mg L^{-1}), and pH (8.13 ± 0.02). Low nutrient values were recorded for phosphate (0.3 ± 0.01 µg L^{-1}), nitrite (0.1 ± 0.01 µg L^{-1}), nitrates (0.4 ± 0.03 µg L^{-1}), and ammonium (2.0 ± 0.1 µg L^{-1}). All parameters were within the recommended levels for *O. niloticus* growth and survival when cultured in ponds.

Growth performance and feed utilization

The growth performance parameters are listed in Table 2. Among the fish fed with *L. minor*-based diets, those fed with LM15 containing 15% *L. minor* had significantly higher final weight and specific growth rate (SGR) ($P < 0.05$) than the other diets. Fish fed a commercial diet presented the highest final weight, SGR, and the best FCR among all diets ($P < 0.05$). The final weight of the fish fed LM10 containing 10% *L. minor* was not significantly different from that of fish fed the LM0. The feed conversion ratio (FCR) was low in fish fed commercial feed and was not significantly different from fish fed diet LM15. Fish survival was the highest in fish fed the commercial diet, but no significant differences ($P = 0.543$) were observed among all the diets.

Table 2. Growth parameters of *O. niloticus* fed on *L. minor* diets for 84 days

Parameter	Diet					
	LM0	**LM10**	**LM15**	**COMM**	**P-value**	**F-value**
IL (cm)	11.96±0.07^{a}	11.86±0.08^{a}	11.84±0.07^{a}	11.74±0.07^{a}	$P < 0.005$	6.378
IW (g)	30.46±0.31^{a}	30.50±0.38^{a}	30.45±0.37^{a}	30.48±0.26^{a}	$P > 0.005$	5.230
FL (cm)	16.87±0.19^{a}	16.20±0.17^{a}	18.89±0.17^{b}	19.16 ±0.22^{b}	$P < 0.005$	50.545
FW (g)	100.51±1.99^{a}	94.07±0.02^{a}	121.05±3.10^{b}	148.64±4.50^{c}	$P < 0.005$	59.096
SGR (%)	1.10±0.02^{a}	1.03±0.02^{a}	1.32±0.03^{b}	1.51±0.04^{c}	$P < 0.005$	56.075
DWG	0.66±0.02^{a}	0.59±0.01^{a}	0.89±0.03^{b}	1.13±0.05^{c}	$P < 0.005$	61.635
WG (%)	225.35±8.18^{a}	202.45±6.53^{a}	281.02±11.77^{b}	412.81± 20.13^{c}	$P < 0.005$	54.92
FCR	1.72±0.05^{b}	1.94±0.05^{c}	1.30±0.05^{a}	1.28 ±0.05^{a}	P <0 .005	55.82
Survival (%)	97.33±3.53^{a}	98.67±1.33^{a}	97.83±4.81^{a}	98.67±1.34^{a}	$P = 0.543$	0.77

**Means within the same row with different superscript letters are significantly different at $P < 0.05$. LM0 (0% *L. minor*); LM10 (10% *L. minor*); LM15 (15% *L. minor*); COMM (commercial diet). IL-initial length, IW- Initial weight, FL-Final length, FW-Final weight, SGR-Specific growth rate, DWG-Daily weight gain, WG-Weight gain, FCR- Food conversion ratio.

There was a non-linear relationship between the replacement level of fish meal with *L. minor* and the growth performance of *O. niloticus*. In general, fish fed *L. minor* had a lower SGR and final weight than fish fed a commercial diet. Likewise, there was a higher FCR in fish fed the *L. minor* diet than in fish fed the commercial diet. The FCR in fish fed 15% *L. minor* (LM15) was

statistically the same to fish fed on the commercial diet ($P > 0.05$). LM15 represents the suitable level for the replacement of fishmeal with *L. minor* in *O. niloticus* diets for better feed efficiency and growth performance.

Fish body composition

The proximate composition of *O. niloticus* fed *L. minor* diets is presented in Table 3. The experimental fish had moisture content ranging between 40.23 to 41.47% and was not significantly different ($P > 0.05$). Total lipid content was significantly higher in fish fed LM15 and COMM ($P < 0.05$) compared to LM0 and LM10. The total protein content was significantly higher in fish fed the LM0 and LM10 diets ($P < 0.05$) than the fish fed LM15 and COMM. The ash content did not differ significantly among the treatments ($P > 0.05$). Carbohydrates were also not significantly different ($P > 0.05$) among the four treatments.

Table 3. Whole body composition of *O. niloticus* fed on *L. minor* diets for 84 days

Parameter (% wet weight)	Diet			
	LM0	**LM10**	**LM15**	**COMM**
Moisture	41.46±0.08[a]	41.47±0.06[a]	40.3±0.08[a]	40.23±0.08[a]
Protein	34.01±0.08[a]	33.22±0.04[a]	30.24±0.07[b]	30.46 ± 0.03[b]
Lipid	19.34±0.90[a]	20.32±0.98[a]	24.44±0.20[b]	24.2 ± 0.21[b]
Ash	4.04±0.09[a]	4.03±0.10[a]	4.02±0.13[a]	4.03 ± 0.09[a]
Carbohydrate	0.35±0.07[a]	0.35±0.02[a]	0.35±0.03[a]	0.35±0.04[a]

**Means within the same row with different superscript letters are significantly different at $P < 0.05$. LM0 (0% *L. minor*); LM10 (10% *L. minor*); LM15 (15% *L. minor*); COMM (commercial diet).

Discussion

This study has established that *O. niloticus* fed on *L. minor* at 15% (LM15) had a better growth performance than LM0 and LM10. This indicates that the inclusion of duckweed did not compromise the overall growth potential of the fish, as demonstrated by final body weights and growth rates that were higher than LM0, indicating that *L. minor* can replace 15% of fish meal in *O. niloticus* diets. These results align with previous research demonstrating that *O. niloticus* exhibits promising growth performance when fed diets containing 15% *L. minor* (Yen et al. 2015; Opiyo et al. 2022). This suggests that duckweed can effectively serve as a viable alternative protein source in tilapia diets, without negative effects on growth performance. A study by Uddin et al. (2014) reported significantly high SGR and final weight in Nile tilapia fed *L. minor* as supplementary feed. However, other studies reported higher levels of up to 20% of *L. minor* to replace fish meal for Indian major carp (*Gibelion catla*) (Shafi et al., 2024) and *C. carpio* (Yılmaz et al., 2004) to grow to juvenile size. Contrary to our study, better growth and feed utilization were reported in tilapia (*Sarotherodon galilaeus*) fed on a 33% CP diet containing duckweed as a partial replacement for fish meal (Mbagwu et al., 1990). Low feed utilization have been reported in *O. niloticus* fed high levels of dry or fresh *L. minor* with a 20% replacement level being utilized better than a 40% replacement level with fish meal (El-Shafai et al., 2004). The SGR and FCR ranged between 1.1 - 1.5% and 1.28 - 1.94% respectively. The SGR are within the range reported by (El-Shafai et al., 2004) in Nile tilapia while the FCR in the present study were higher compared to 0.9-1.1% in a related study by El-Shafai et al. (2004). The combination of both fishmeal and *L. minor* has been reported to lead to better FCR when compared to other plant sources (El-Shafai et al., 2004). The FCR in the LM15 were comparable to COMM, indicating that the fish had the same utilization of the feed as the commercial feed. A similar scenario was reported in Nile tilapia fingerlings, where the 15% *L. minor* inclusion had the same FCR to the control diet formulated to mirror the commercial feed (Opiyo et al., 2022). Goswami et al. (2020) reported improved SGR and FCR when fishmeal was partially replaced with duckweed in the diets of *Labeo rohita* fingerlings. Improved final weight, SGR, and FCR were reported in grow-out rainbow trout fed on *Spirodela polyrrhiza* at 12% (Stadtlander et al., 2023).

The survival of the experimental fish was not affected by the replacement of fishmeal in any

diet. This indicated that the nutrients in the feeds supported fish well-being equally, as the survival of the fish was more than 90% in all the treatments. This could also be attributed to the overall experimental management and good health of the fish. The nutritional composition of cultured *L. minor* is sufficient to meet the nutritional requirements of cultured Nile tilapia because it contains essential fatty acids, especially polyunsaturated fatty acids (PUFAs), which are important for fish well-being and performance (Mukherjee et al., 2010; Kumar et al., 2022; Opiyo et al., 2023). The high survival rate and production of *O. nioloticus* fed *L. minor* as a supplementary feed in fertilized ponds was documented by Uddin et al. (2014). Similarly, a study replacing fish meal with fermented *L. minor* at 0, 2.5, 5, and 7.5% reported high survival with no significant differences among the treatments (Herawati et al., 2020). High percent survival has been recorded in Nile tilapia fed diets replacing fish meal with duckweed *Spirodela polyrrhiza* at 5% and 10%, and *C. carpio* fed on *L. minor* at 5% and 10% (Fasakin et al., 1999; Yılmaz et al., 2004) which is contrary to the present study, which had high survival at all the treatments.

The moisture content of the fish was not significantly different. The protein content in the fish body decreased with an increase in *L. minor* in the diets with the fish fed diet LM15, which had the lowest protein content. This is in agreement with studies of Hassan & Edwards (1992), who recorded low protein levels in Nile tilapia fed with whole *Lemna perpusilla* and a study by Opiyo et al. (2022), where *L. minor* inclusion levels ranged from 5-20% in plant based feeds. Lipid levels increased with increasing levels of *L. minor* in the fish diet. This could be a result of the size of fish, which increased with increasing levels of *L. minor* with diet LM15 having significantly bigger fish but were not significantly different from the fish fed on the commercial diet. The high lipid content in diets LM15 and COMM could also indicate that the diets were more energy dense, leading to lipid deposition (Hassan & Edwards, 1992). The ash content was not significantly affected by the diet. A similar trend was reported in silver barbs (*Barbonymus gonionotus*) fed on diets with *L. minor* partially replacing fishmeal at 10, 20, 30 and 35% (Noor et al., 2000). In contrast, El-Shafai et al. (2004) reported a high ash content in tilapia fed on duckweed diets. A reduction in ash content was reported when *O. niloticus* were fed on *S. polyrhiza* (Fasakin et al., 1999) and *L. minor* from 5 to 20% (Solomon & Okomoda, 2012). This study indicates that there were no significant differences in the body composition of the fish fed on *L. minor* at 15% and the commercial diet.

Conclusion

Replacement of fish meal with duckweed (*Lemna minor*) at 15% gave the best growth performance and could be used to replace fish meal in Nile tilapia grow-out diets to obtain similar outcomes to those of commercial feed for growth performance, feed utilization, and fish body composition. The fact that there was no significant effect on the survival of the fish indicated that the fish were in good condition. The use of higher levels of *L. minor* than those in this study requires further processing to improve digestibility due to the high fiber content and antinutritional factors that could be present in the macrophytes. The use of duckweed as a protein source in tilapia diets has important implications for sustainable aquaculture. Duckweed is a fast-growing aquatic plant that requires minimal input and can be cultivated using nutrient-rich water from fish culture systems, making it an environmentally friendly, climate-smart, and sustainable substitute for fishmeal. The collection of duckweed from unknown sources is not recommended because of possible contamination which may be present in the water. Only duckweed cultured with known manure and nutrient-rich water from an aquaculture facility is recommended for use in fish feed. More studies are recommended for high levels of *L. minor* to replace fish meal in tilapia growth after analysis of antinutritional factors which may pose challenges to utilization of *L. minor* by *O. niloticus*.

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Credit author statement

Mary A. Opiyo and Kevin Mbogo: funding acquisition, conceptualization, methodology, data collection, data analysis, writing of the original

draft, and revision of the manuscript. Jacob Abwao, Domitila Kyule, Charles Amahwa, Betty M. Nyonje, and Jonathan Munguti: conceptualization, writing of the original draft, and revision of the manuscript.

Ethical statement

The experiment was conducted following the standard operating procedures (SOPs) of the Kenya Marine and Fisheries Research Institute (KMFRI) guidelines for handling animals after ethical review and approval by KMFRI Ethical Review Committee registered with the National Commission for Science, Technology and Innovation (NACOSTI) registration number NACOSTI/2016/05/001. The SOPs comply with the Prevention of Cruelty to Animals Act 1962, CAP 360 (Revised 2012) of the laws of Kenya, and EU regulation (EC Directive 86/609/EEC).

Competing interests

The authors state that there is no conflict of interest to declare.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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