# Indoor Aquaculture Potential of Duckweed (*Lemna minor*) and the Need for Adoption in Kenya Open Access

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## Abstract

Duckweed (*Lemna minor*) culture has been explored as a possible macrophyte based ingredient for partial or full substitution of both soy bean or fish meal in fish feeds in China and India but has seldom been explored in Kenya to establish its growth conditions. To demonstrate the culture potential of *L. minor* in aquaculture, culture technique was homogenized in indoor plastic tanks using chicken manure at an optimum water depth of 30 cm. Temperature ranged from (25.84±0.19) to (28.24±0.08) °C. After 10 days *L. minor* attained 100% cover and was harvested three times in a month. The overall yield was (1.09±0.09) kg/m$^3$/month. The Relative Growth Rates was higher during the first harvest (0.38 g/day) and decreased in subsequently (0.12 and 0.10 g/day) for the second and third harvests respectively. Dissolved oxygen decreased with *L. minor* growth and ranged between (0.84±0.19) and (2.52±0.52) mg/L. pH. values ranged between (5.89±0.32) and (7.23±0.23) throughout the study period. The present study demonstrates that *L. minor* can be cultured using cheap and locally available organic manure in aquaculture sector. Therefore, there is need to embrace *L. minor* culture technologies by small scale farmers for sustainable aquaculture production in Kenya.

## Keywords

Aquaculture; Duckweed; Adoption; Technology

## Introduction

Duckweed (*Lemna minor*) is a free-floating freshwater macrophyte growing in either still or slow moving shallow water forming green mat (Sree et al., 2016; Bog et al., 2020; Tippery et al., 2021). It is a monocotyledonous macrophyte belonging to Lemnaceae family comprising of 37 different species globally. The different species of duckweed are extensively distributed in various tropical and subtropical areas in various aquatic ecosystems (Chakrabarti et al., 2018). Its morphology consists of 2~4 fronds (Fu et al., 2020). Its vegetative part comprises of tiny leaflets with inconspicuous stems and roots (Figure 1).

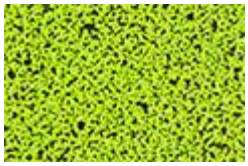

Figure 1 *Lemna minor* plant cultured in a circular plastic tank

Under optimum conditions, they grow freely thus occurs in diverse aquatic environments ranging from nutrient-rich stagnant water including irrigation canals, swamplands and wastewaters (Ziegler et al., 2015). *L. minor* is characterized by relatively high growth rates and rapidly reproduce vegetatively through budding both in natural and controlled laboratory or pond culture conditions, covering the entire water surface within 7~10 days (Ziegler et al., 2015; Ceschin et al., 2016). Ideal conditions including physico-chemical parameters as well as appropriate nutrient levels favors higher biomass within a short duration. For instance, water with fairly high concentrations of animal waste results in high biomass within a short period of time whereas natural underground water leads to slow growth rate, long roots and low protein content due to inadequate nitrogen (Xu et al., 2011). However, some ecological conditions such as pH, temperature and wind affects growth of *L. minor* (Sudiarto et al., 2019; Ceschin et al., 2020). Furthermore, plant nutrients like ammonium, calcium, magnesium, nitrogen and phosphorous also influence its biomass (Nafea, 2016; Chakrabarti et al., 2018).

*L. minor* is utilized as food in several parts of the world including China among other Asian countries where they are recognized to have sufficient amounts of protein, starch and fatty acids (Cheng and Stomp, 2009; Mwale and Gwaze, 2013; Ibrahim et al., 2017). Studies in the past reported higher harvested yields compared to average yield for major food crops hence possible indicators of the plant's potential to address food insecurity and nutrition (Mwale and Gwaze, 2013; Ibrahim et al., 2017). Unlike animal products, plant-based foodstuffs including *L. minor* are not associated with human health complications such as cardiac failure, cholesterols risks and diabetes (Tristan et al., 2017). Other than human food, *L. minor* are also used as livestock and poultry feeds and have been reported to be very nutritious providing phosphates and proteins (Negesse et al., 2009; Li et al., 2020; Zhao et al., 2021). The low fiber content is useful in digestibility and palatability of animal feeds (Chepkirui et al., 2022). Thus *L. minor* has great potential to address the growing demand against declining availability of animal feeds protein sources.

Fishmeal and soybean are commonly used ingredients in animal feeds manufacturing industry including the aquaculture sector. However, the growing demand of soybean as both human and animal feedstuff has led to an increased cultivation thus elevated emission of a significant amount of Greenhouse Gases (GHG) into the ecosystem (Castanheira and Freire, 2013). The GHG coupled with an increasing competition of fishmeal from other feed manufacturing industries has resulted in limited soybean supply. Therefore, there is need to embrace novel sustainable plant-based ingredients in animal feed formulation. *L. minor* is considered as a novel ingredient in feed formulation industries since it can reduce the emission of GHG as well as the overall production cost. It can also help lessen eutrophication loads in aquatic ecosystem due to its phytoremediation properties. Therefore, this study explores the culture potential of *L. minor* under aquaculture conditions, possibility of its mass culture within selected agro-ecological zones and the need for adoption in Kenya.

## 1 Results and Discussions

### 1.1 Water quality

DO ranged between (0.84±0.19) and (2.52±0.52) mg/L and was higher (2.52 mg/L) at the beginning of experiment but gradually declined (0.84 mg/L) after manure application. Dissolved oxygen level decreased with *L. minor* growth covering the surface of the tanks. Under normal aquatic environment settings, sources of DO in an ecosystem include; diffusion from the atmosphere as well as photosynthetic by products from phytoplankton, algae and other aquatic organisms. In hatchery conditions for this experiment, it is presumed that atmospheric air was the major source of oxygen. However, aerobic decomposition of manure by biological action depletes DO with the aquatic environment. Microbes such as bacteria and fungi requires DO during decomposition of organic matter in the culture media leading to a decrease in DO levels in subsequent days of the growth trials period (Table 1).

Table 1 Water quality parameters and Relative Growth Rate throughout the culture period

*L. minor* is capable of tolerating wide range of pH levels although the optimum pH of 6.5~7.5 is recommended (Nafea, 2016; Kuznetsova et al., 2019). pH values ranged between (5.89±0.32) and (7.23±0.23) throughout the study period (Table 1). Upon application of manure, pH decreased from 6.01±0.12 to 5.89±0.32 then later increases. This corroborates with studies reported by Chakrabarti et al. (2018). Previous studies indicated that pH is critical in determining the levels of converting ammonia ($NH_3$) to ammonium ($NH_4^+$) in the culture units (Su et al., 2014). High pH values increases the free $NH_4^+$ which hinders passage of anions in the *L. minor* cell membrane hence affecting the overall growth. On the other hand, high ammonia $NH_3$ (g) at lower pH leads to water toxicity which in turn negatively affects the plants' growth (Nafea, 2016). It is therefore imperative that pH is maintained between 6.5 and 7.5 for optimum results (Su et al., 2014).

Light intensity is directly proportional to *L. minor* growth rate. For instance; low light intensity slows down the growth rate and vice versa (Chakrabarti et al., 2018). This is assumed that increased light intensity leads to stomata opening thus encourages both photosynthesis and respiration hence better growth. Earlier studies reported that, in case of excess light, *L. minor* accumulates antioxidants thus preventing its damage. Temperature ranged from (25.84±0.19) to (28.24±0.08) °C throughout the study period. This agrees with Chakrabarti et al. (2018) who cited an optimum temperature of 24 °C to 30 °C for *L. minor* growth. However, low temperatures decrease duckweed multiplication rate whereas high temperatures above 35 °C slows down the plant's growth rate too (Lepedus et al., 2020). This is because optimum temperature is crucial for maximum enzymatic action in most plants' physiological processes including germination, respiration, transpiration, photosynthesis and flowering. More studies have been conducted in the wild and have found *L. minor* grows at an optimum temperature of 20 °C to 30 °C (Nafea, 2016; Kuznetsova et al., 2019). According to Nafea (2016), during late summer, *L. minor* undergo morphological changes referred to as as turion as a result of low temperatures. In such cases, *L. minor* sinks and remains dormant while accumulating starch reserve for the next growth season in the presence of optimum conditions. During spring season, *L. minor* germinates again in the presence of adequate light and temperature (Wang and Messing, 2012; Kuehdorf et al., 2014).

**1.2 Water level and fertilization**

In natural environment, *L. minor* grows in shallow nutrient rich water bodies and are protected from wind (Nafea, 2016; Chakrabarti et al., 2018). In this study, *L. minor* performed well in fertilized tanks with an optimum depth of 30 cm (Chakrabarti et al., 2018) which was maintained and any water lost through evaporation was replaced. In deeper waters *L. minor* lacks ability to fully utilize the available

nutrients in the culture units resulting in decreased multiplication rate and development of longer roots. Furthermore, in such cases, *L. minor* will appear whitish in color due to nutrients deficiency thus produces lower biomass (Appenroth et al., 2018). According to Nafea (2016) and Xu et al. (2011), *L. minor* grows rapidly in waste water but lack of essential nutrients namely nitrogen, potassium and phosphorous affects its growth significantly. The need for fertilization relies on the water source and nutrient richness. For instance; rain water needs balanced NPK for *L. minor* growth unlike waste water. Previous studies have reported a direct relationship between nutrients availability in the culture units and crude protein of *L. minor.* Earlier studies reported that unlike inorganic manure, organic manure from livestock and poultry is highly effective for mass culture of *L. minor* (Srivastava et al., 2006; Chakrabarti et al., 2018). Further, preliminary studies indicated that chicken manure has a much higher NPK content compared to livestock manure making it ideal for the present study. However, nutrients availability is dependant on animal's diet, housing and bedding, storage as well as handling. High doses of manure above the recommended amount will lead to production of excess ammonia which might affect both growth, quality and overall *L. minor* multiplication. Therefore, the nature of the culture medium in terms of nutrients availability influence the physiological well-being of the plant which in turn affects its production (Lees et al., 2002).

### 1.3 Relative growth rate and biomass production

*L. minor* growth is strongly influenced by nutrients available, light, temperature and crowding degree (Hassan and Chakrabarti, 2009). First harvest was done after 10 days of inoculation upon *L. minor* attaining 100% cover and was harvested three times in a month. Earlier studies reported that younger plants have better nutrient profile and high growth rates compared to older ones (Mwale and Gwaze, 2013). Therefore, regular harvesting once the tanks attained 100% cover was strongly recommended to maintain high nutrient profile and faster growth of the plant. Further, choice of harvesting procedure depends on design of the system, labor as well as equipment availability. In tanks, simple harvesting techniques such as manual skimming of *L. minor* is employed using net from the water surface.

Studies reported that *L. minor* has high protein content and lipids with a favorable ratio of n6/n3 which enhance the nutritional value (Yan et al., 2013; Chakrabarti et al., 2018). RGRs recorded in the study were 0.58, 0.35 and 0.24 g/day for the 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ harvests correspondingly. The RGR was higher during the first harvest and decreased in the subsequent harvests. This agrees with results presented by Chakrabarti et al. (2018). A yield of (1.09±0.09) kg m$^{-3}$ month$^{-1}$ of *L. Minor* production was recorded.

*L. minor* dense cover is beneficial as it serves as a barrier against odour development and mosquito larvae. Past studies reported that, dense mat hinders the larvaes from reaching the water surface for respiration. Other reports also documented that *L. minor* produces compounds that are toxic to plasmodium larvae (Ifie et al., 2021). This contributes to possibility of acceptance of *L. minor* in areas with high prevalence of human diseases such as malaria. Also, the gaseous product of anaerobic decomposition from water column contributes to fouling in the environment. Kamyab et al. (2017) reported that aerobic *L. minor* acts as both physical as well as chemical barrier against such odours thus playing a critical role in environmental pollution management.

## 2 Materials and Methods

### 2.1 Experimental set up

A 30-day culture experiment was conducted at Kenya Marine and Fisheries Research Institute (KMFRI), Kegati Aquaculture Centre's hatchery located at latitude 00°42"S; 034°47"E and an altitude of 1 700 m

above sea level. Circular plastic tanks measuring 3 $m^3$ were cleaned, sterilized and filled with clean water whose levels were maintained at 30 cm throughout the culture period (Chakrabarti et al., 2018). Dry chicken manure weighing 5 kg was mixed with water thoroughly and allowed to decompose in 50 liters buckets for five days. Fertilization of the tanks was done at a rate of 1.052 kg/$m^3$ followed by subsequent re-fertilization at a rate of 0.263 kg/$m^3$ after every seven. *L. minor* inoculants sourced from Ahero irrigation canals were sorted, cleaned and disinfected using potassium permanganate. One 5 mL teaspoon of potassium permanganate was allowed to dissolve in 10 L of water followed by dipping and removal of *L. minor* seed after 30 minutes. This was done to avoid the introduction of any parasites and diseases from wild into the aquaculture system. The sterilized *L. minor* weighing 2 kg wet biomass was introduced into the tanks immediately after fertilization (Figure 2).

Figure 2 *L. minor* culture experimental set up in circular plastic tanks

In-situ physico-chemical parameters namely conductivity, pH, dissolved oxygen (DO), temperature, Total Dissolve Solids and salinity were monitored daily using YSI multi-parameter probe (Yellow Springs OH 45387 USA). Water samples for nutrients analysis were collected weekly, preserved in cooler boxes with ice blocks prior to laboratory transportation for Total Nitrogen, Total Phosphorous and Total ammonium analysis according to APHA (2005). Harvesting was done after every 10 days and weights taken using a weighing balance (Model TX 4202 L, Shimadzu - Corporation, Philippines), with readability of 0.01 g. Partial harvesting (50%) was done using a scoop net with 100 µm mesh size to allow continuous propagation of *L. minor* in the tanks after harvesting. Washing of the garnered plant was done to remove any foreign materials and put in perforated bucket to drain excess water. After weighing the wet biomass, air drying on a shed and oven desiccation was done at 60 °C until a final constant weight was achieved for grinding into fine powder to be used in fish feed formulation.

**2.2 Relative growth rate (RGR)**

RGR of *L. minor* expressed as g/day was calculated as follows:

$$In(\frac{Wt}{W0})/t$$

RGR=

Where, $W_t$ is fresh weight of *L. Minor* during harvesting (t); $W_0$ is *L. Minor* weight during inoculation; T represent the interval of time in days

**2.3 Statistical analysis**

Collected data was cleaned, coded and presented as mean ± SE. Data were analyzed using SPSS version 2022 to calculate means and standard errors of physico-chemical parameters and RGR.

**3 Conclusions and Recommendations**

Literature indicates that *L. minor* farming in Kenya is scarce. Therefore, social acceptance and uptake of *L. minor* farming by small scale farmers will help in rural development through increased income, food security and nutrition especially in Arid and Semi-Arid areas. The plant has great potential of sequestering nutrients from water and lowering fouling, a major environmental concern in the fast growing world. In the tropics, it has great ability to minimize anopheles mosquito multiplication and thus minimize malaria infection. The present study demonstrates that *L. minor* can be cultured using cheap and locally available organic manure in aquaculture sector. Despite aquaculture and livestock farming being a major source of protein, its escalation exudes a lot of nitrogen and phosphorous contributing to eutrophication of ecosystems as well as increased carbon emissions (FAO, 2022).

Although there are no cases on public health concerns, more studies need to be done to inform on the possibility of the transfer of pathogens, diseases and heavy metals from animal waste used during propagation in aquaculture. There is also need to promote uptake of *L. minor* culture technologies in aquaculture by small scale farmers to help reduce the overall production cost as it can be used as animal food supplement for cattle and poultry thus promoting sustainable farming in Kenya.

**Authors' contributions**

CM is responsible for idea conceptualization, study design, data analysis, manuscript drafting and revision; OPS did idea conceptualization, study design, data analysis, manuscript drafting and revision; AJK is responsible for study design, data collection, analysis, manuscript drafting and revision; OT did study design, data collection, analysis, manuscript drafting and revision; KV is responsible for study design, data collection, analysis, manuscript drafting and revision; JR contributed in study design, data collection, analysis, manuscript drafting and revision; OJ is responsible for study design, data collection, analysis, manuscript drafting and revision. All authors read and approved the final manuscript.

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