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Research Article

Nutritional Potential and Apparent Digestibility of Organic Matter, Protein, and Energy of Two Duckweeds (*Lemna Minor* and *Lemna Eaquinoctialis*) In Juvenile Tilapia *Oreochromis Niloticus* (Brazil Strain)

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Abstract

The aquaculture products provided a relevant protein source in context of population growth and the mitigation of food insecurity in Sub-Sahara Africa. The objective of this study was to assess the nutritional potential and the digestibility of duckweeds (*Lemna minor and Lemna eaquinoctialis*) in juvenile *Oreochromis niloticus* (35.73 ± 0.08 g). Two experimental ponds were implemented for the culture of fresh duckweeds. The apparent digestibility was assessed using six experimental ponds connected to tanks running with an electric pump.

The results showed that total organic matter contents of *L. minor and L. eaquinoctialis* were 978.49 mg g^{-1} and 974.73 mg g^{-1}, respectively. Hydrolysable organic matter was 98.9% and 97.9% of the total organic matter content of duckweeds. Ash content was 2.42%, lipids (3.15%), proteins (38.80%), energy (19.12 kJ g^{-1}) and the protein/energy ratio (20.29 mg kJ^{-1}) of *L. minor* were statistically higher ($p<0.05$) than contents in *L.eaquinoctialis*. Variation of the digestibility of duckweeds in *O. niloticus* was observed with apparent digestibility coefficients (proteins, hydrolysable organic matter and energy) for *L. minor and L. eaquinoctialis*. The investigated duckweeds could be incorporated into the diet of *O. niloticus* as protein source for the partial substitution feed in aquaculture in Côte d'Ivoire.

Keywords: Apparent digestibility; Nutrients, Fresh duckweeds; Nutrition; Fish feed; *Oreochromis niloticus*; Côte d'Ivoire

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Introduction

The aquaculture practice represents an important alternative for increasing protein sources, and for reducing the food insecurity in Sub-Saharan African countries. Studies have reported the critical issue of fishery and aquaculture associated with nutrition improvement, food security, and economic growth in developing and emergent countries [1]. Other studies analysed this situation by showing that there are few knowledge on influences of climate change and demographic transition on aquaculture products. Indeed, aquaculture plays an increasing role in aquatic food security [2]. The World Food Organization reported that the consumption of fish has increased at 3.0% yearly, representing about twice the proportion of annual world population growth (1.6%), and the per capita consumption rate estimated at 20.2 kg [3]. The need of animal protein covered by fishing is increasing, with a fish production estimated at 178 million tons worldwide, where up to 88 million tons come from aquaculture [3]. Aquaculture is strongly dependent on food representing 60% of aquaculture production costs [4]. For the composition of farmed fish feed, the fishmeal and oils are main important ingredients because of their high contents on proteins and essential fatty acids, which are necessary for the growth of fishes [5]. These compounds are costly and limited access in developing countries. The use of fish from fisheries for the production of fishmeal and fish oil, for farmed fish feed in aquaculture reduced the availability of natural fish stocks that are already overexploited [6].

There is a need to investigate new sources of improved farmed fish feeds in aquaculture practices in Sub-Sahara Africa, particularly in Côte d'Ivoire. Moreover, some alternatives sources of feed have been experienced by many authors to increase in context-specific for ensuring food security and the sustainable management of natural resources. For example, recently a study showed that microalgal biomass has the potential to support the growth of fish and shrimp aquaculture [7]. Authors revealed that fish and microalgae have similar nutritional profiles and their use as highly digestible feed reduced production costs, feed waste generation, and the risk of eutrophication. Other investigations recommended the reduction of the feed production costs in aquaculture through the replacement of fishmeal and oil by additional protein sources [8]. Many plants have the potential to replace fishmeal in aquaculture [9]. Usually, food ingredients for aquaculture are cereals, oil cakes, oil seeds, and aquatic plants [4,10,11]. Duckweed is the aquatic plant used as food supplements, with the potential to produce a biomass for fish farming and animal husbandry [12]. Moreover, their protein, vitamin, micro and macronutrient content makes them suitable for fish feed [13]. Specifically, Tilapia fed by *Lemna valdiviana* and *Heterobranchus longifilis* by *Lemna pauciscostata* have shown high growth performance rates [14,15].

In Côte d'Ivoire, located in West Africa, the national fishery production is estimated at 116 028 tons/year. This quantity is still low for covering the fish demand for the population that is estimated 618 182 tons/year [16]. Overall, 549 233 tons are imported to fix the demand of fish requirements. In this context, many efforts are undertaken by the government in Côte d'Ivoire, through piloted projects and scientific research to increase fish production locally [16]. This challenge faced some difficulties, because there are few investigations of food supplements, particularly plant food ingredients in aquaculture for reducing the fish feed production cost. It is therefore, necessary to provide scientific evidence on aquaculture potential addressing the critical issue of nutritive characteristics, physico-chemical quality of fishes, and associated risk for improving decision making in Côte d'Ivoire. This applied research assessed the nutritional profile and digestibility potential of two duckweeds (*L. minor* and *L. eaquinoctialis*) in Tilapia juvenile (*O. niloticus,* Brazilian strain), in order to improve a feed-based ingredient for aquaculture practice in Côte d'Ivoire.

Materials and Methods

Study design

The overall methodology of this study used experimental designs comprising duckweed leaves production, and fish cultivated in six experimental ponds. The apparent digestibility coefficient of leaves by Tilapia *O. niloticus*, and physico-chemical characteristics of the aquatic environment were subsequently assessed.

Duckweeds production in experimental ponds

The culture of duckweeds (*L. minor and L. eaquinoctialis*) was carried out at the laboratory of the *Centre de Recherches Océanologiques*, in two experimental ponds (13.78 m^3 each) following the protocol developed by [17]. Indeed, each pond comprised a polyethylene liner filled with water (5000 L). On the first day of the experiment, 5 kg of pig manure from the piggery were added to two ponds. Then, 100 g of *L. minor* and *L. eaquinoctialis*, harvested from the natural environment, were used to seed the ponds. After one week of the cultivation process, a biomass of duckweeds had formed on the surface of the ponds. The sample of this biomass was harvested for chemical composition analyses.

Experimental design

The digestibility of duckweeds was assessed through the experimental design using six aquaria (45 L of water each). Practically, these aquaria integrated a closed-circuit system tank that were connected to an electric pump (5 L/min, 550 Watts). Every day, 30% of water in aquaria was renewed. Fishes (35.73±0.08 g) were then randomly distributed in aquaria with 10 fishes per aquarium. Fishes were adapted to the experimental conditions for one week by feeding with a commercial diet (35% of protein). Wastes in aquaria were cleaned by siphoning, and three batches were used for feed testing (*L. minor* and *L. eaquinoctialis*). Physico-chemical parameters analyses concerned *in situ* variables, such as temperature (T°), pH, and dissolved oxygen of the water of aquaria, daily assessed at 8:30. In each aquarium, the average values of temperature, pH and dissolved oxygen were 27.2°C; 7.3 and 4.7 mg/L, respectively. Every week, the aquarium water was sampled for laboratory analyses of phosphorus and ammoniacal nitrogen. The values of these parameters were ranged between 2.29 and 2.39 mg/L, and 0.41 and 0.43 mg/L, respectively.

Feeding and faeces collection

Duckweed was harvested with a dip net and rinsed with tap water. Fishes were fed with fresh duckweed manually and apparent satiety twice a day at 9:30 AM and 2:30 PM. Uneaten leaves were recovered 45 minutes after feeding. Fish faeces collection started three days after the beginning of the trial, by siphoning at one-hour intervals before the second feeding phase, where faeces from each triplicate were stored at -20°C until analysis. The digestibility test was performed over 15 days.

Analytical methods

Analytical methods were used for the physico-chemical characteristics of duckweeds and fish faeces. Indeed, the composition of duckweeds, particularly *L. minor*, *L. eaquinoctialis* and fish faeces were assessed in the laboratory to estimate dry matter after drying at 105°C for 24 hours. The content of total organic matter, hydrolysable organic matter, resistant organic matter to hydrolysis and minerals were determined according to the method described by [18], modified by [19]. The analysis of proteins, lipids and fibres were done by a standard method [20]. Indeed, Proteins were determined by the Kjeldahl method, lipids were with hot hexane soxhlet approach and fibres by acid and alkaline hydrolysis. The ash content was determined by incineration of samples of duckweeds and faeces in a muffle furnace at 550°C for 24 hours [21]. The non-nitrogenous extractives content were determined by the method used by [22] and the energy by [23]. The essential amino acid profile was determined by the method of the High-Performance Liquid Chromatography (HPLC,) (Waters Alliance, Model 2695, USA).

Assessment of apparent digestibility coefficient

The Apparent Digestibility Coefficient (ADC) of fresh duckweeds leaves was assessed for four compounds (organic matter, proteins, lipids, energy) following the method of [24] quoted by [25], using minerals (Ash) as reference material. The apparent digestibility coefficient was determined as follows:

$Ni\ (\%)=[(N_{Ff}/Min_{Ff}-N_f/\ Min_f)/(N_{Ff}/Min_{Ff})]\times 100$ With

Ni: Nutrient content in duckweeds

N_{Ff}: Percentage (%) of nutrient content in duckweeds

N_f: Nutrient content in fish faeces (%)

Min_{Ff}: Mineral content in duckweeds (%)

Min_f: Mineral content in faeces (%)

Statistical analysis

Statistical analyses were performed using STATISTICA 7.1 software (Informer Technologies Inc, France). Practically, descriptive statistics were implemented for assessing physico-chemical characteristics of duckweeds. The inference for analyzing the significant difference between *L. minor* and *L. eaquinoctialis* the apparent digestibility coefficient were done using ANOVA statistics One-way Analysis of Variance. Additional multiple comparisons of means were conducted with Tukey test at 5%.

Results

Chemical composition of duckweeds *L. minor* and *L. eaquinoctialis*

Table 1 shows chemical composition of duckweeds *L. Minor* (LM) *and L. Eaquinoctialis* (LE) used as feed in the experimental ponds. Total Organic Matter (TOM) contents were ranged from 974.73 to 978.49 mg g^{-1}, Hydrolysable Organic Matter (HOM) from 954.84 to 967.74 mg g^{-1}, Hydrolysable Resistant Organic Matter (HROM) from 10.75 to 19.89 mg g^{-1} and minerals (Min) from 21.51 to 25.27 mg g^{-1}. Furthermore, the results showed that the proportion of hydrolysable organic matter (967.74 mg g^{-1}) in *L. minor* are higher ($p<0.05$) than those observed in *L. eaquinoctialis* (HOM: 954.84 mg g^{-1}). But, hydrolysable resistant organic matter (19.89 mg g^{-1}) and mineral (25.27 mg g^{-1}) contents in *L. eaquinoctialis* are higher ($p<0.05$) than those in *L. minor* (HROM: 10.75 mg g^{-1}; Min: 21.51 mg g^{-1}). Lipid (3.15%), ash (2.42%), and energy (19.12 kj g^{-1}) determined in *L. minor* are statistically higher ($p<0.05$) than those obtained in *L. eaquinoctialis* (1.49% lipid, 1.13% ash and 18.41 kj g^{-1} energy). Protein contents estimated at 38.80% in *L. minor* were also high, with essential amino acid concentration of leucine were 5.29 g/16gN, 4.22 g/16gN for arginine, valine (3.66 g/16gN), phenylalanine (3.65 g/16gN), tryptophan (3.64 g/16gN) and 3.18g/16gN for lysine. The lowest values are observed for methionine (0.99 g/16gN) and histidine (1.39 g/16gN) (Table 2). The protein/energy ratio of *L. minor* is estimated at 20.29 and 16.14 for *L. eaquinoctilias*.

Anti-nutritional contents in *L. minor* and *L. eaquinoctialis*

Table 3 shows the composition of oxalate, tannin, saponin, and phytate in *L. minor* and *L. eaquinoctialis*. The concentrations of the compounds varied for *L. minor* and *L. eaquinoctialis*. Indeed, the concentration of oxalate was estimated at 2.06 mg/100g and 0.13 mg/100g for saponin, and particularly higher in *L. minor*. High concentrations of tannins (1.40 mg/100g) and phytates (0.81 mg/100g) were observed in *L. eaquinoctialis*. Among the investigated anti-nutritional contents, oxalate had the highest concentration, and saponin with the lowest levels in both duckweeds species.

Heavy metal contents in *L. minor* and *L. eaquinoctialis*

Table 4 shows the concentration of metals in duckweeds collected from the experimental ponds. The concentration of lead was estimated at 1.215 mg kg^{-1} and 0.042 mg kg^{-1} for cadmium in *L. eaquinoctialis.* The lead was estimated at 0.009 mg kg^{-1} and 0.000 mg kg^{-1} for cadmium in *L. minor.* The concentration of mercury was 0.001 mg kg^{-1} for *L. eaquinoctialis* and 0.002 mg kg^{-1} for *L. minor*. The results indicated that a statistically significant difference ($p<0.05$) was observed between heavy metals contents of *L. minor* and *L. eaquinoctialis*.

Analytical composition	Duckweeds	
	L. minor	*L. eaquinoctialis*
Dry matter (%)	6.43 ± 0.59^a	6.5 ± 0.08^a
TOM (mg g^{-1} of DM)	978.49 ± 0.91^a	974.73 ± 2.93^a
HOM (mg g^{-1} of DM)	967.74 ± 0.00^b	954.84 ± 3.23^a
HROM (mg g^{-1} of DM)	10.75 ± 0.63^a	19.89 ± 1.06^b
Mineral (mg g^{-1} of DM)	21.51 ± 1.40^a	25.27 ± 2.93^a
Protein (% of DM)	38.80 ± 0.04^b	29.72 ± 0.08^a
Lipids (% DM)	3.15 ± 0,15^b	1.46 ± 0.06^a
Fibres (% DM)	1.65 ± 0.05^a	2,30 ± 0.58^b
Ash (% DM)	2.42 ± 0.02^b	1.13 ± 0.04^a
NNE (% DM)	53.98 ± 0.09^a	64.38 ± 0.54^b
Energy (kJ g^{-1})	19.12 ± 0.04^b	18.41 ± 0.11^a
Ratio Protein/Energy (mg kJ^{-1})	20.29 ± 0.06^b	16.14 ± 0.06^a

Table 1: Approximate composition of *L. minor and L. eaquinoctialis* collected from the experimental ponds.

In each line the values with different letters are significantly different (p<0,05).

DM: Dry matter;

TOM: Total Organic Matter;

HOM: Hydrolysable Organic Matter;

HROM: Hydrolysable Resistant Organic matter;

Min: Mineral (ash); NNE: Non-nitrogenous extractives

Essential amino acids (g/16gN)	Duckweeds	
	L. minor	*L. eaquinoctialis*
Valine	3.66 ± 0.02^b	2.01 ± 0.02^a
Leucine	5.29 ± 0.19^b	2.52 ± 0.17^a
Isoleucine	2.95 ± 0.05^b	1.68 ± 0.08^a
Arginine	4.22 ± 0.11^b	1.64 ± 0.14^a
Phenylalanine	3.65 ± 0.05^b	1.80 ± 0.12^a
Methionine	0.99 ± 0.02^b	0.61± 0.07^a
Lysine	3.18 ± 0.03^b	1.17 ± 0.03^a
Tryptophan	3.64 ± 0.07^b	0.55 ± 0.05^a
Threonine	2.81 ± 0.08^b	0.50 ± 0.15^a
Histidine	1.39 ± 0.02^b	0.60 ± 0.10^a

Table 2: Essential amino acids composition in proteins of cultivated *L. minor and L. eaquinoctialis*

On each line, values with letters indicate a statistically significant difference (p<0.05).

Duckweeds	Content (mg/100g)			
	Oxalate	Tanins	Phytates	Saponines
L. minor	2.06 ± 0.05^b	0.72 ± 0.01^a	0.52 ± 0.02^a	0.13 ± 0.01^b
L. eaquinoctialis	1.98 ± 0.11^a	1.40 ± 0.04^b	0.81 ± 0.03^b	0.02 ± 0.00^a

Table 3: Anti-nutritional substances in *L. minor* and *L. eaquinoctialis*.

On each column, values with different letters indicate a statistically significant difference (p<0.05).

Duckweeds	Content (mg/kg)		
	Plomb	Cadmium	Mercure
L. minor	0.009 ± 0,001[a]	0.000 ± 0.000[a]	0.002 ± 0.000[b]
L. eaquinoctialis	1.215 ± 0,008[b]	0.042 ± 0.001[b]	0.001 ± 0.001[a]

Table 4: Heavy metal contents of *L. minor* and *L. eaquinoctialis* from experimental ponds.

On each column, values with different letters indicate a statistically significant difference (p<0.05).

Assessment of apparent digestibility coefficients of duckweeds

Table 5 shows the Apparent Digestibility Coefficients (ADC) of duckweeds in *O. niloticus*. The apparent digestibility coefficient of *L. minor* was estimated at 39.08% for Total organic matter, Hydrolysable organic matter (45.93%), protein (56.30%) and energy (44.38%). Furthermore, *L. eaquinoctialis* was estimated at 31.74% for Total organic matter, Hydrolysable organic matter (41.68%), protein (46.20%) and energy (37.69%). The results showed a significant difference ($p<0.05$) between the apparent digestibility coefficients of *L. minor* and *L. eaquinoctialis* in *O. niloticus*.

Parameters	ADC (%)	
	Duckweeds	
	L. minor	*L. eaquinoctialis*
TOM	39.08 ± 0.85[b]	31.74 ± 1.52[a]
HOM	45.93 ± 1.53[b]	41.68 ± 1.13[a]
Proteins	56.30 ± 1.43[b]	46.20 ± 1.20[a]
Energy	44.38 ± 1.74[b]	37.69 ± 0.68[a]

Table 5: Apparent Digestibility Coefficient (ADC), organic matter, proteins, and gross energy of duckweeds.

In each line the values with different letters are significantly different (p<0,05).

Discussion

This study assessed the nutritional potential and the apparent digestibility of *L. minor* and *L. eaquinoctialis* on juvenile *O. niloticus*. It was observed that *L. minor* and *L. eaquinoctialis* had high nutritive potentials as fish feeds for aquaculture in Côte d'Ivoire.

This study revealed that investigated duckweeds had a significant biomass content of organic matter, with important quantity particularly of hydrolyzable organic matter (967.74 mg g^{-1} for *L. minor* and 954.84 mg g^{-1} for *L. eaquinoctialis*). This result is close to the study reported in periphyton, a preferred natural food source of phytophagous fishes, such as tilapia [26,27]. Additionally, the assessment of the analytical composition showed high concentration of lipids, proteins, and amino acids in *L. minor* leaves. Total lipid contents remained higher in *L. minor*, with high values close to those recorded (3 to 7% DM) in duckweeds grown in nutrient rich medium [28,29]. Revealed that a nutrient poor medium induced low lipid content in duckweeds. The results of this study show that *L. minor* grown under the same conditions as *L. eaquinoctialis*, would be less demanding of nutrient resources. Protein contents obtained in *L. minor* in the present study are similar to the finding reported by other author in similar contexts [30,13]. Protein contents and Protein/Energy ratio evaluated are close to values reported by [31] who showed that dietary protein contents (35 - 40% with P/E ratio of 20 mg kJ^{-1}) allow a good growth of tilapia *O. niloticus*. Among anti-nutritional substances (oxalates, tannins, phytates, saponins) in the investigated duckweeds, the highest value of oxalate was observed in both duckweeds species. These results corroborated the observations made by [32] in the duckweed of *Lemna species*. Overall, the concentrations of anti-nutritional substances in duckweeds remained low than those reported in aquatic plants used as fish feeds in aquaculture, where concentrations varied from 0.6% - 3.5% for calcium oxalate, tannin (0.25% - 0.93%) and phytate (0.004% - 0.005%) [33]. The investigated duckweeds in the experimental ponds, could not be considered as risk for fish health, particularly concerning heavy metals such as lead, cadmium, and mercury. These chemical concentrations in this study were in line with European community standards for heavy metals in the raw material of animal feeds, with thresholds fixed at 10 mg kg^{-1} for lead, cadmium 1 to 2 mg kg^{-1} and mercury 0.1 mg kg^{-1} [34].

Furthermore, the apparent digestibility coefficient of total organic matter, hydrolyzable matter of *L. minor* and *L. eaquinoctialis* show middle digestion of duckweeds organic matter in *O. niloticus*. These results are consistent with those obtained in *O. niloticus* reared in extensive fish ponds with a diet based primarily on natural nutrient resources [27]. The digestibility performance of the organic matter appeared to be consubstantial with plants, which in the natural state exhibit low nutrient availability associated with low digestibility [9]. However, the digestibility of the protein fraction and energy remained higher in fish fed with *L. minor* compared to *L. eaquinoctialis.* Overall, the apparent digestibility values observed for the duckweed-fed fish in this study remained low compared to those obtained when duckweeds are incorporated into the compound diet. Studies have already shown the relevance of protein apparent digestibility coefficient of 77% in *O. niloticus* fed with 20% by fresh *L. minor* [35].

The lower values of apparent digestibility coefficients in this study could be explained by the presence of anti-nutrients that could interfere with nutrient utilization [36], and consequently could reduce their digestibility by *O. niloticus*. Some substances such as tannins have the property of binding or precipitating proteins and various organic compounds, including amino acids and alkaloids [37]. Tannins are considered as inhibitors of the enzymatic activity of some enzymes, mainly lipase, proteolytic enzymes, alpha-galactosidase, cellulase, pectinase and amylase [38]. Therefore, the presence in duckweeds of anti-nutritional substances, suggest the application of adapted treatments for their efficient use in fish diets [39].

Conclusion

This study showed that *L. minor* and *L. eaquinoctialis* have important hydrolyzable organic matter. Among the two tested fresh duckweeds species, *L. minor* showed the best protein and energy utilization coefficient. However, adequate treatment of fresh duckweeds would improve the ingestion of these leaves by the fish as a source of protein.

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