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Research Article
Effect of Four Treated Forms of *Lemna minor* on Zootechnical Balance and Digestive Performance of Juvenile Tilapia (*Oreochromis niloticus)* in Côte d'Ivoire (West Africa)

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Abstract
Background and Objective: The incorporation rate of fish meal in compound feeds of aquaculture is currently reduced due to the higher demand and price. This study was conducted to assess the nutritional value and digestive utilization of four treated forms of *Lemna minor* in the diet of tilapia. **Materials and Methods:** Four treatments (shade-drying, oven drying, pre-cooking-drying and fermentation-drying) were applied to reduce anti-nutritional factors (oxalate, phytates, tannins and saponins). Four experimental diets, each including 70% of reference diet and 30% of treated forms of *L. minor*, were formulated. After 8 weeks of feeding in hatchery of aquaria, faeces were sampled for analyses. **Results:** The results showed that the reduction of anti-nutritional factors by the three heat treatments was less than 30% for oxalate, while tannins, phytates and saponins were reduced from 50-90%. The best reduction rates (90%) were found with fermented *L. minor* for tannins, phytates and saponins and 30% for oxalate. The study revealed high apparent digestibility coefficients (ADC) in proteins (81.68%) and energy (78.47%) for fish fed with fermented *L. minor*. Low digestive coefficients in protein (65.56%) and energy (62.20%) was observed for fish fed with pre-cooked-dried *L. minor*, while high digestible protein content (33.16 mg g^{-1}) was observed for fermented leaves. Fermented leaves diet had the highest protein retention coefficient (47.56%) and the pre-cooked-dried leaves diet had the lowest value (14.30%). **Conclusion:** In Côte d'Ivoire, fermented *L. minor* could be used as a protein supplement in fish feed formulation.

Key words: *Lemna minor*, nutritional value, nutrient digestibility, zootechnical balance, *Oreochromis niloticus*, Côte d'Ivoire

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish production has remained stable with minimal fluctuations and it has the highest share that is around 66% of global aquaculture production[1], though aquaculture production has gradually increased over the years in African countries, the progress remains still slow[2], accounting for 17.9% of fish production[3]. Recent investigations revealed that since 2000, aquaculture production in sub-Saharan Africa has grown by 11% annually on average[4]. The contribution of fish production in food security was estimated at 57.5 million tons of fish worldwide in 2020[1]. The sustainability of the aquaculture practice depends on many factors, where the high cost of feed is one of the key issues to be addressed. The sustainability of the sector of aquaculture required capacity development, enabling environment, biosafety frameworks, public policy and research and applications of biotechnology[5]. Furthermore, the inclusion rate of fish meal and fish oil in compound feeds used in aquaculture has considerably decreased[6], due to the significant decrease in the supply of fishmeal, high demand and increase of costs. But fish meal is the important content of compound feed due to provision of essential nutrients [7]. In Sub-Saharan Africa, though freshwater fisheries contribute significantly to the livelihoods and food security of millions of people, these activities are experiencing multiple anthropogenic stressors, mainly overfishing, illegal fishing, pollution and climate change[8]. In most of these countries, many challenges have been reported concerning the low production and aquaculture practice including site and species selection, environmental impact, lack of awareness, inefficient fishing gears, poor transportation access, poor postharvest handling, low price at the landing site, improper marketplace and lack of infrastructure and technologies. There is an increased support of aquaculture development in many countries, as a potential to creating new jobs and improving food security among poor households in Ghana, Tanzania and Kenya[9]. The fishmeal represented 60% of overall operating cost for the implementation of aquaculture project. Fishmeal is a major ingredient in the formulation of farmed fish feed[6]. It contains 66-72% of proteins. It is also known to be rich in essential amino acids and is considered as a suitable ingredient for fish growth in aquaculture[10].

Therefore, the development of cheaper feed contributes to increase the growth of aquaculture[11]. To date, the major challenge for fish nutritionists and producers is the development of cost-effective commercial feeds using available, less expensive and non-conventional resources[10]. The use of various plant sources in the diet of tilapia have been investigated[12,13]. For the growth of freshwater fish, aquatic and terrestrial, macrophytes are suggested as good supplementary food. These plants are known to playing an important role in the extensive farming system as fish feed. Duckweed (*L. minor*) able to accumulate nutrients from wastewater, in addition to a high content of minerals, vitamins and protein (45% of dry weight), with a high growth rate[14]. They have potential to produce biomass as a feed supplement in fish farms and animal husbandry. Duckweed can be considered as a cheaper and sustainable alternative source of protein[15]. Their nutrient contents making them an excellent nutritional source for fish[16]. The presence of anti-nutritional factors in plants limit their use in animal feed. Anti-nutritional factors have been reported in duckweed species in the genus *spirodela* et *Lemna*[17]. The heat treatment can detoxify these anti nutritional factors of duckweed consumed by fish[18]. Furthermore, the fermentation process also reduces the toxicity of duckweed caused by anti nutritional factors[19]. *Bacillus* isolated from the digestive tract of tilapia *O. niloticus* has positive effects on the growth and health of tilapia[20-22], catfish *Pangasianodon hypophthalmus*[23] and common carp *Cyprinus carpio*[24]. Determination of feed digestibility would be an important tool for the formulation of balanced diets with low cost and low environmental impact. In Côte d'Ivoire, there are few studies on the critical issues of fish quality, availability, improvement technologies, health risk and feed supplement. In this country in 2017, it is estimated that 70% of the coastal fishery is considered as artisanal, when 2.38×10^5 t are produced from marine capture and 4900 t in aquaculture[25]. Furthermore, the commercial aquaculture could contribute to reducing the risk associated with artificial reservoirs of schistosomiasis in poor infrastructure of water management[26]. Particularly, freshwater duck weeds are available in most stagnant water in Côte d'Ivoire. They are able to provide abundant and necessary biomass as low-cost sources of protein for fish farming. Aquaculture activities reduce the food insecurity for vulnerable populations, it is therefore, required to develop new available, accessible and low-cost diets for fish farming in Côte d'Ivoire. The objective of this study was to assess the nutritional potential of four treatments of *L. minor* as a source of protein in diet of juvenile tilapia *O. niloticus*.

MATERIALS AND METHODS

Study site and design: This study was carried out in the Department of aquaculture at the Centre de Recherches Océanologiques, (CRO), in Côte d'Ivoire. Four experimental diets were formulated using duckweed *L. minor* for juvenile tilapia (*O. niloticus*). Aquaria containing fishes were designed for evaluating the digestibility of duckweeds and zootechnical parameters.

Feed ingredients: *Lemna minor* used in the study was grown at the Department of Aquaculture of the Centre de Recherches Océanologiques in Côte d'Ivoire (CRO) following the protocol of Tavares *et al.*[27]. Table 1 shows the proximate composition of feed ingredients.

The collected duckweeds were cleaned three times with tap water. Then, duckweeds were divided into four parts, where the first part was dried in the oven at 50°C, the second part was dried under the shade in a room at 23°C, the third part was pre-cooked with steam for 5 min and dried in oven at 50°C and the last part was fermented according to the method used by Utomo *et al.*[28]. After dewatering and cooling of the pre-cooked leaves, *bacillus* was extracted from the gut of Tilapia *O. niloticus* and added at 0.5% of the total weight of the pre-cooked leaves. Pre-cooked leaves and *bacillus* extract were manually mixed in a container and incubated at 37°C for 48 hrs. The fermented leaves were dried in oven at 50°C.

Diets preparation: A reference diet was formulated following the requirements of tilapia *O. niloticus*)and added with chromium oxide (Cr_2O_3) at the concentration of 1% used as an inert marker. Four experimental diets (Table 2), each containing 70% of the reference diet and 30% of the test ingredients (SDM, DLM, PDM, FDM) were formulated and prepared as described by Cho *et al.*[29]. They were prepared using a pellet press with a 2 mm diameter.

Experimental design: There were 40 aquaria containing 50 L of water and running in a closed circuit. It also used an electric pump (5 L min^{-1}, 550 Watts), a drum (200 L) for water supply in aquaria with a stable flow rate (1.5 L min^{-1}), water circulation pipes and a water collection tank. Pipes in aquaria were regulated to collect the overflow of water to maintain the water level at the targeted quantity. Every day, 30% of water in aquarium were renewed and filtered by decantation.

Feeding and feaces collection: A total of 150 male juvenile Tilapia (*O. niloticus*) (25.23±0.11 g) were randomly distributed in each aquarium, with 10 fishes per aquarium. Prior to experimental treatment, fishes were left for one week to acclimate to experimental conditions, during which they were fed with a commercial diet (35% of proteins). After this the aquaria were cleaned by siphoning. Triplicate groups of 10 fishes were formed per test diets.

Before feeding, the temperature (28.8-28.7°C), pH (7.72-7.89) and the dissolved oxygen (5.30-6.86 mg L^{-1}) were measured daily at 8:30 AM with a multi-parameter (HANNA, HI 9828). Fish were fed *ad libitum* twice daily (at 9 AM and 4 PM). In order to measure the exact value of feed intake, uneaten feed was siphoned after 45 min. Prior to collecting fish feaces, aquaria were cleaned. The fish feaces were collected three days after feeding trial by siphoning[30-33], with one-hour intervals before the second feeding. Feaces were then grouped in triplicates and oven-dried at 37°C for 24 hrs, then stored at -20°C for chemical composition analyses. The digestibility test was performed over 15 days. Fishes in each aquarium were weighted weekly. Ten fishes per diet were collected for chemical composition analyses. The monitoring of growth parameters was lasted for 56 days.

Analytical methods: For the analytical assessment, the chemical composition of ingredients tested, experimental diets and fish faeces was found. The dry matter of samples was determined by oven drying at 105°C for 24 hrs. The protein, lipid and fibers was assessed using the standard method[34]. Indeed, crude protein (%N×6.25) was determined by the Kjeldahl method and lipids by hot hexane soxhlet, while crude fibers were determined by acid/alkali digestion. Ash were evaluated by incinerating samples in a muffle furnace at 550°C for 24 hrs[35] and the non-nitrogenous extractives by the protocol of Kerdchuen[36]. The chromium oxide was analysed according to the method of Bolin *et al.*[37] and total oxalates through the acidification by potassium permanganate in hot acid medium[34]. The phytate content was analyzed using the method developed by Brooks *et al.*[38], while saponins with the

Table 1: Proximate composition of ingredients

Ingredients (Dry weight%)	Protein	Lipid	Ash	Fiber	Carbohydrate	Gross energy (kJ g^{-1})
Fishmeal	58	9.66	20.03	-	8.67	18.75
Soybean meal	45.25	4.3	5.95	5.22	44.50	18.73
Wheat bran	18.81	3.89	5.86	10.16	72.96	16.18
Fermented *L. minor*	40.59	0.72	15.82	1.38	42.87	16.43
Shade/dried at 23°C *L. minor*	36.70	2.56	14.52	2.35	43.86	16.69
Dried at 50°C *L. minor*	35.85	2.40	14.78	1.96	45.01	16.63
Pre-cooked/dried at 50°C *L. minor*	35.65	0.07	13.35	1.45	50.93	16.45
Cassava starch	-	-	-	-	100.00	17.02

Table 2: Formulation and composition of the experimental diets (dry weight%)

Description	Reference diets	Experimental diets DSDM	DDLM	DPDM	DFDM
Ingredient (g/100 g)					
Fishmeal	29	20.3	20.3	20.3	20.3
Soybean meal	53	37.1	37.1	37.1	37.1
Wheat bran	6	4.2	4.2	4.2	4.2
Shade/dried at 23°C *L. minor*	-	30	-	-	-
Dried at 50°C *L. minor*	-	-	30	-	-
Pre-cooked/dried at 50°C *L. minor*			-	30	-
Fermented *L. minor*					30
Cassava starch	3	2.1	2.1	2.1	2.1
Palm oil	5	3.5	3.5	3.5	3.5
Premix(1)	3	2.1	2.1	2.1	2.1
Chromium oxide	1	0.7	0.7	0.7	0.7
Total	100	100	100	100	100
Proximate composition					
Dried matter	88.05	89.85	89.45	88.55	88.62
Proteins (DM%)	38.23	37.77	37.51	37.45	38.94
Lipids (DM%)	10.31	7.80	7.75	7.06	7.25
Total carbohydrate (DM%)	36.85	37.07	36.95	36.80	36.78
Ash (DM%)	9.31	10.94	11.02	10.59	11.33
Fibers (DM%)	3.38	3.07	2.95	2.80	2.78
Digestible energy (kJ g^{-1})(2)	14.86	13.88	13.82	13.54	13.89
Ratio protein/energy (mg kJ^{-1})	25.73	27.20	27.15	27.66	28.02

DM: Dry Matter, DSDM: Diet with shade-dried *L. minor*, DDLM: Diet with dried *L. minor* at 50°C, DPDM: Diet with pre-cooked/dried *L. minor*, DFDM: Diet with fermented *L. minor*, (1)Per kg premix; Vitamin A1: 760 000 IU, Vitamin D3: 880 000 IU, Vitamin E: 22 000 mg, Vitamin B1: 4 400 mg, Vitamin B2: 5 280 mg, Vitamin B6: 4 400 mg, Vitamin B1: 236 mg, Vitamin C: 151 000 mg, Vitamin K: 4 400 mg, Vitamin P 35: 200 mg, Folic acid: 880 mg, Choline chloride: 220 000 mg, Pantothenic acid: D 14 080 mg, Cobalt: 20 mg, Iron: 17 600 mg, Iodine: 2 000 mg, Copper: 1 600 mg, Zinc: 60 000 mg, Manganese: 10 000 mg, Selenium: 40 mg, (2)Digestible energy: 18,8×protein +37,7×lipid content+11,3×carbohydrate content[30,31]

hot methanol in soxhlet[39]. The determination of tannins was performed with the casein precipitation method as cited by Seigler *et al.*[40].

Zootechnical and feed utilization parameters: Apparent Digestibility coefficient (ADC) of each nutrient or energy in the experimental diets (ADC N_{diet} or E_{diet}) and ingredients (ADC $N_{ingredient}$ or $E_{ingredient}$) were determined using the following equations as described by Robaina *et al.*[41]:

$$\text{ADC DM}_{\text{diet}}\ (\%) = 100\text{-}\left[100\left(\frac{\text{Ii}}{\text{If}}\right)\right] \quad (1)$$

$$\text{ADC N}_{\text{diet}} \text{ or E}_{\text{diet}}\ (\%) = 100\text{-}\left[100\left(\frac{\text{Ii}}{\text{If}}\right)\times\left(\frac{\text{Nf ou Ef}}{\text{Ni ou Ni}}\right)\right] \quad (2)$$

In the ingredient according to Sugiura *et al.*[42]:

$$\text{ADC DM}_{\text{ingredient}}\ (\%) = \frac{\text{ADC DM}_{\text{diet}}\text{-}0.7\times\text{ADC DM}_{\text{reference diet}}}{0.3} \quad (3)$$

$$\text{ADC N}_{\text{ing.}} \text{ or E}_{\text{ing.}}\ (\%) = \frac{(\text{N}_{\text{diet}} \text{ or E}_{\text{diet}}\times\text{ADC N}_{\text{diet}} \text{ or E}_{\text{diet}})\text{-}(0.7\times\text{N}_{\text{ref.}} \text{ or E}_{\text{ref.}}\times\text{ADC N}_{\text{ref. diet}} \text{ or E}_{\text{ref. diet}})}{0.3\times\text{N}_{\text{ing.}} \text{ or E}_{\text{ing.}}} \quad (4)$$

Where:

DM = Dry matter
I = Indicator (%)
i = Ingested
f = Feaces
N = Nutrient (%)
E = Energy (%)

The growth performance parameters were determined as described by Yeo *et al.*[12]:

$$\text{Survival rate (SR\%)} = \frac{\text{Nf}}{\text{Ni}} \quad (5)$$

$$\text{Daily weight gain (DWG, g/j)} = \frac{\text{Wf-Wi}}{\text{t}} \quad (6)$$

$$\text{Specific growth rate (SGR, \%/j)} = \frac{\text{LnWf}\times\text{LnWi}}{\text{t}\times 100} \quad (7)$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Dry weight gain (g)}} \quad (8)$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Protéins gain (g)}} \quad (9)$$

The nutrient retention parameters were determined using the following equations as described by Carvalho[43]:

$$\text{Protein retention coefficient (PRC\%)} = \frac{\text{Protéins retained}}{\text{Protéines ingested}} \times 100 \quad (10)$$

$$\text{Lipid retention coefficient (LRC\%)} = \frac{\text{Lipids retained}}{\text{Lipids ingested}} \times 100 \quad (11)$$

$$\text{Energy retention coefficient (ERC\%)} = \frac{\text{Energy retained}}{\text{Energy ingested}} \times 100 \quad (12)$$

Where:

Wi = Initial weight
Wf = Final body weight of fish
t = The duration of the experiment
Ni = Initial number
Nf = Final number of fish

Statistical analysis: Data were analyzed using one-way analysis of variance (ANOVA) using STATISTICA 7.1. Software (Informer Technologies Inc, France). Descriptive statistics were performed for the collected data (nutrient contents, zootechnical parameters, percentages of Apparent Digestibility Coefficient). Multiple comparisons of means were performed using HSD Tukey test at 5% level of significance.

Ethical considerations: All experimental procedures were approved by the institutional review board of the Centre de Recherches Océanologiques (CRO), with minor suggestions for the experimental ponds concerning the fish growth.

RESULTS

Reduction potential of treatments on anti-nutritional factors in *L. minor*: Figure 1 shows the effect of treatments on anti-nutritional factors of *L. minor*. The results showed a significant difference ($p<0.05$) between fresh and different treated duckweeds. Concentrations of the investigated anti-nutritional factors (oxalate: 2.06 mg 100 g^{-1}, Tannins: 0.72 mg 100 g^{-1}, phytate: 0.52 mg 100 g^{-1}, Saponins: 0.13 mg 100 g^{-1}) in fresh duckweeds were higher than those in treated duckweeds. Concentrations of anti-nutritional factor varied from 1.32-1.84 mg 100 g^{-1} for oxalate, 0.04-0.08 mg 100 g^{-1} for tannins, 0.01-0.25 mg 100 g^{-1} for phytate and ranged at 0.003-0.03 mg 100 g^{-1} for saponins in treated duckweeds. The lowest concentrations were observed for fermented duckweeds, representing more than 94% reduction of anti-nutritional factors (tannins, phytate and saponins). Oxalate was reduced to 35.92% with the fermentation treatment (Fig. 2). The highest reduction was found for pre-cooked and dried *L. minor* treatment, ranging from 79.68 and 90.46% for tannins, phytates and saponins, while only 28.19% for oxalate. Statistical analyses showed that concerning the reduction of tannins, phytate and saponins, there was no significant difference ($p>0.05$) between the treatment with dried *L. minor* in the shade at 23°C and dried at 50°C. The percentages varied from 52.01-89.08% for tannins, phytates and saponins and less than 20% for oxalate.

Nutritional value after the treatment of duckweed *L. minor*: Table 3 shows the analytical composition of the nutritional value after treating duckweed (*L. minor*) with thermal-treatment and fermented processes. The results showed that there was a significant difference ($p<0.05$), between the

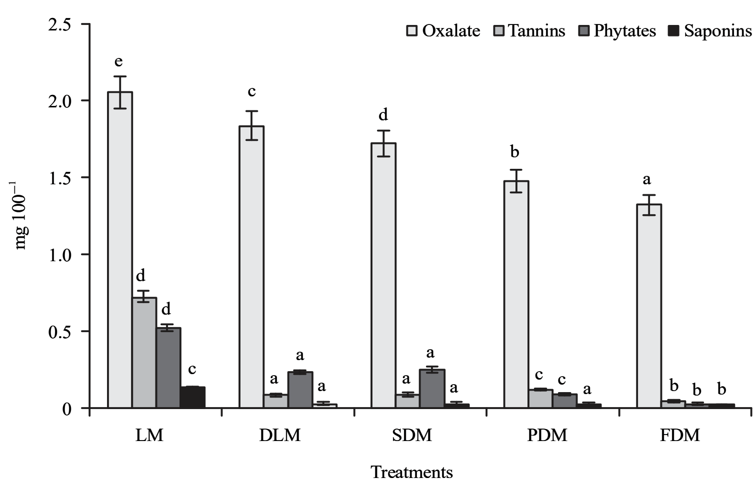

Fig. 1: The effect of applied treatments on anti-nutritional factors in *L. minor*

*a, b and d showed the statistical differences ($p<0.05$), LM: Fresh *L. minor*, DLM: Dried *L. minor* at 50°C, SDM: Shade-dried *L. minor*, PDM: Pre-cooked and dried *L. minor*, FDM: Fermented *L. minor*

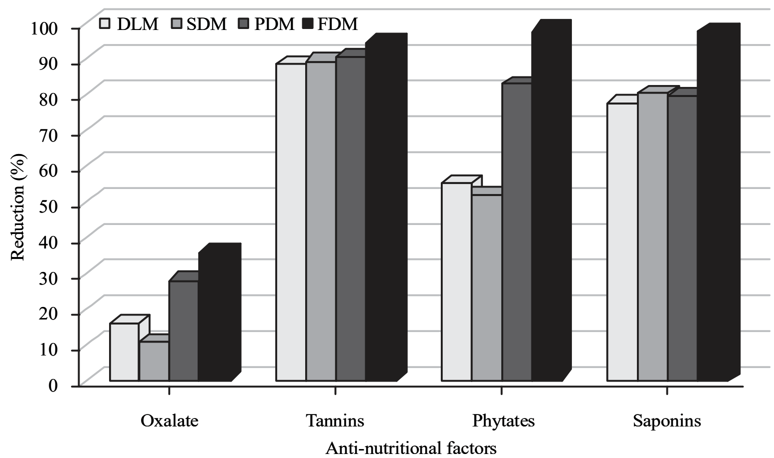

Fig. 2: Anti-nutritional factors reduction percentage after the treatments of *L. minor*
DLM: Dried *L. minor* at 50°C, SDM: Shade-dried *L. minor*, PDM: Pre-cooked-dried *L. minor*, FDM: Fermented *L. minor*

Table 3: Proximate composition of *L. minor* heat-treated and biologically

Analytical composition	Fresh *L. minor*	Treatments SDM	DLM	PDM	FDM
Dry matter (%)	6.43±0.59^{a}	88.00±1.00^{b}	96.45±0.32^{d}	93.85±0.11^{c}	93.55±0.50^{c}
Protein (%)	38.80±0.04^{c}	36.70±0.26^{b}	35.85±0.06^{a}	35.65±0.10^{a}	40.59±0.46^{d}
Lipids (%)	3.15±0.15^{d}	2.56±0.04^{c}	2.40±0.22^{c}	0.07±0.00^{a}	0.72±0.04^{b}
Carbohydrate (%)	55.63±0.14^{d}	46.21±1.00^{b}	46.96±0.34^{b}	50.93±0.39^{c}	42.87±1.94^{a}
Fiber (%)	1.65±0.05^{c}	2.35±0.06^{e}	1.96±0.01^{d}	1.45±0.10^{b}	1.38±0.02^{a}
Ash (%)	2.42±0,02^{a}	14.52±0.96^{c}	14.78±0.55^{c}	13.35±0.45^{b}	15.82±1.44^{d}
NFE (%)	53.98±0.09^{e}	43.86±1.00^{b}	45.01±0.34^{c}	49.48±0.50^{d}	41.49±1.92^{a}
GE (kJ g^{-1})	19.12±0.04^{b}	16.69±0.17^{a}	16.63±0.14^{a}	16.45±0.10^{a}	16.43±0.21^{a}
DE (kJ g^{-1})	14.58±0.04^{b}	12.82±0.12^{a}	12.73±0.12^{a}	12.32±0.07^{a}	12.59±0.12^{a}
P/E (mg kJ ED^{-1})	26.61±0.06^{a}	28.62±0.29^{b}	28.16±0.19^{b}	28.93±0.10^{b}	32.24±0.60^{c}

In each line the values with different letters are significantly different (p<0.05). SDM: Shade-dried *L. minor* DLM: Dried *L. minor* at 50°C, PDM: Pre-cooked/dried *L. minor*, FDM: fermented *L. minor*, NFE: Nitrogen-free extract, GE: Gross energy, DE: Digestible energy, P/E: Ratio protein/energy

contents of all treatments of leaves in the study. The percentages of dry matter were ranged from 6.43-96.45%. *L. minor* dried at 50°C had the highest value (96.45%) and fresh duckweed (*L. minor*) had the lowest value (6.43%). Protein contents were ranged from 35.65-40.59%. The highest value (40.59%) was observed for fermented *L. minor*. The thermal treatment of leaves showed lower contents (38.80%) compared to fresh *L. minor*. The study revealed low value of lipid in dried pre-cooked leaves (0.07%) and the highest value (3.15%) in fresh *L. minor*.

Furthermore, among the treated leaves, the carbohydrate content (50.93%) in precooked-dried leaves was the highest and lower (55.63%) than that of the fresh *L. minor*. The quantity of fibers and ashes were ranged from 1.38-2.45% and 2.42-15.82%, respectively. The lowest value of fiber was recorded in dried fermented leaves (1.38%) and the ash (2.42%) in fresh *L. minor*. The results showed that the gross energy, digestive energy and protein/energy ratio (P/E) of the treated leaves (thermal and fermented) were ranged from 16.43-16.69 kJ g^{-1}, 12.32-12.82 kJ g^{-1} and 28.62-32.24 mg kJ^{-1}, respectively. There was no significant difference (p>0.05) between treatments of the investigated nutrients. High values were noted for fresh leaves of *L. minor* in terms of gross energy (19.12 kJ g^{-1}) and digestive energy (14.58 kJ g^{-1}). The protein/energy ratio (32.24 mg kJ^{-1}) of fermented leaves was higher than that of the fresh *L. minor* (P/E of 26.61 mg kJ^{-1}).

Apparent digestibility coefficient (ADC) of experimental diets of tilapia *O. niloticus*: Apparent digestibility coefficient of dry matter, protein, carbohydrate and energy in the experimental diets of tilapia (*O. niloticus*) are presented in Table 4. The statistical analyses revealed a significant difference (p<0.05) between the ADC of fishes fed the control and test diets. Indeed, ADC were ranged from 83-90% for dry matter and 87-96% for protein and 85-89% for carbohydrate and 89-94% for energy. The results showed that the fishes fed

Table 4: Apparent digestibility coefficients of dry matter, protein, carbohydrate and energy in experimental diets in Tilapia juvenile *O. niloticus*

		ADC of experimental diets (%)			
Parameters	ADC of control diet (%)	DFDM	DSDM	DDLM	DPDM
MS	89.92±0.21[e]	86.30±0.07[d]	85.32±0.75[c]	84.58±0.30[b]	82.97±0.05[a]
Proteins	96.01±0.11[e]	91.52±0.30[d]	89.94±0.26[c]	88.95±0.22[b]	87.33±0.17[a]
Carbohydrates	86,45±0.10[b]	85.21±0.03[a]	89.13±0.13[d]	85.60±0.33[a]	88.00±0.01[c]
Energy	93.62±0.04[e]	91.71±0.20[c]	92.03±0.05[d]	90.08±0.09[b]	89.17±0.10[a]

In each line the values with different letters are significantly different (p<0.05). Control diet: Diet without *L. minor*, DFDM: Diet with fermented *L. minor*, DSDM: Diet with shade-dried *L. minor*, DDLM: Diet with dried *L. minor* at 50°C, DPDM: Diet with pre-cooked/dried *L. minor*

Table 5: Apparent digestibility coefficient of dry matter, protein, carbohydrates and energy in test diets

	ADC of test ingredients (%)			
Parameters	FDM	SDM	DLM	PDM
Dry matter (%)	77.86±0.10[d]	74.60±0.09[c]	72.13±0.03[b]	66.75±0.13[a]
Protein (%)	81.68±0.30[d]	75.19±0.10[c]	71.35±0.04[b]	65.56±0.19[a]
Carbohydrates (%)	70.29±0.10[b]	77.48±0.2[c]	66.24±0.14[a]	66.01±0.1[a]
Energy (%)	78.47±0.02[d]	77.83±0.17[c]	69.88±0.95[b]	62.20±0.09[a]
Digestible Protein (mg g^{-1})	33.16±0.19[d]	27.60±0.2[c]	25.58±0.26[b]	23.38±0.1[a]
Digestible carbohydrates (mg g^{-1})	30.13±0.13[a]	35.81±0.10[d]	31.11±0.05[b]	33.62±0.26[c]
Digestible Energy (kJ g^{-1})	12.89±0.17[c]	12.99±0.21[c]	11.62±0.30[b]	10.23±0.23[a]

In each line the values with different letters are significantly different (p<0.05). FDM: Fermented *L. minor*, SDM: Shade-dried *L. minor*, DLM: Dried *L. minor* at 50°C, PDM: Pre-cooked/dried *L. minor*

Table 6: Growth performance and feed utilization by Tilapia juvenile *O. niloticus*

		Test diets			
Parameters	Control diet	DFDM	DSDM	DDLM	DPDM
IBW (g)	25.21±0.45[a]	25.33±0.24[a]	25.10±0.16[a]	25.12±0.61[a]	25.39±0.24[a]
FBW (g)	62.29±0.58[e]	58.83±1.37[d]	54.94±0.14[c]	52.01±0.25[b]	48.68±0.77[a]
SR (%)	83.33±5.77[a]	83.33±5.77[a]	83.67±8.05[a]	83.33±3.22[a]	83.33±4.55[a]
DWG (g/j)	0.66±0.06[e]	0.60±0.09[d]	0.53±0.06[c]	0.48±0.01[b]	0.42±0.02[a]
SGR (%/j)	1.61±0.63[e]	1.51±0.32[d]	1.40±0.08[c]	1.30±0.07[b]	1.16±0.2[a]
FCR	1.53±0.15[b]	1.77±0.08[c]	2.17±0.13[d]	2.39±0.03[e]	2.84±0.04[f]
PER	1.78±0.35[e]	1.60±0.04[d]	1.35±0.08[c]	1.27±0.25[b]	1.03±0.02[a]
PRC (%)	69.82±0.47[e]	47.56±1.14[d]	31.76±1.44[c]	22.11±0.77[b]	14.30±0.44[a]
LRC (%)	30.30±0.61[d]	23.25±0.73[c]	21.24±0.24[a]	21.07±0.02[a]	19.24±0.47[b]
ERC (%)	52.18±1.00[e]	30.36±0.42[d]	26.70±0.17[c]	23.19±0.30[b]	15.83±0.36[a]

In each line the values with different letters are significantly different (p<0.05). Control diet: Diet without *L. minor*, DFDM: Diet with fermented *L. minor*, DSDM: Diet with Shade-dried *L. minor*, DDLM: Diet with Dried *L. minor*, at 50°C, DPDM: Diet with Pre-cooked/dried *L. minor*, IBW: Initial body weight, FBW: Final body weight, SR: Survival rate, DWG: Daly weight gain, SGR: Specific growth rate, FCR: Feed conversion ratio, PER: Protein efficiency ratio, PRC: Protein retention coefficient, LRC: Lipid retention coefficient, ERC: Energy retention coefficient

with the control diet had the highest ADC values (89.92% for dry matter, 96.01% for protein, 86.45% for carbohydrate and 93.62% for energy). Among the tested diets, ADC of dry matter and protein was 86.30 and 92.52%, respectively, for fish fed fermented *L. minor*. The fishes fed with shade-dried leaf diet had the highest ADC of carbohydrate (89%) and energy (92%), followed by the fermented diet leaves, with 85.21 and 91.71%, respectively. The diet made with pre-cooked-dried *L. minor* had shown the lowest ADC.

Table 5 presents the ADC of the treated leaves of *L. minor*. The results showed that there was a significant difference (p<0.05) between ADC of the treated leaves. Apparent digestibility of dry matter was ranged from 66-78%, of protein from 66-82%, carbohydrates from 66-77 and energy from 62-79%. The results revealed that fermented *L. minor* had the highest ADC values for dry matter (77.86%), protein (81.68%) and energy (78.47%), followed by shade-dried *L. minor* (74.6, 75.19 and 77.83%), oven dried *L. minor* (72.13, 71.35 and 69.88%) and pre-cooked *L. minor* (66.75, 65.56 and 62.20%). This increase in ADC of digestible proteins was observed in treated *L. minor* where values were 33.16% for fermented leaves, 27.60% for shade-dried leaves, 25.58% for dried leaves at 50°C and 23.38% for pre-cooked leaves. For digestible energy, ADC was ranged from 10-13 kJ g^{-1}. The fermented and shade-dried *L. minor* had the highest value, 12.89 and 12.99 kJ g^{-1}, respectively.

Assessment of fish growth performance and use of nutrients: Table 6 shows the growth performance and the nutrient retention in juvenile (*O. niloticus*). Survival rates of fishes were ranged from 83-84%. The statistical analyses showed that there was no significant difference ($p>0.05$) between fishes fed with experimental diets. The final mean weights of fish were ranged from 49-63 g, with the highest value (62.29 g) for fish fed with the control diet. The lowest values were noticed for fish fed with the pre-cooked and dried *L. minor* diet (48.68 g). The specific growth rates (SGR) were ranged from 1.16-1.61% day^{-1}. Fishes fed with the control diet had the highest value (1.61% day^{-1}), followed by fermented leaves (1.51% day^{-1}). About the feed utilization by Tilapia juvenile (*O. niloticus*), the statistical analyses showed that there was a significant difference ($p<0.05$) between fish fed with the experimental diets. The Feed conversion ratio (FCR) varied from 1.5-2.8 and the protein efficiency ratio (PER) was ranged from 1.0-1. 8. The control diet had the lowest FCR (1.53) and the highest (1.78) value of PER. Furthermore, fishes fed with the test diets and fermented *L. minor* diet showed the lowest FCR (1.77) and the highest PER (1.6), followed by the shade-dried leaves diet with 2.17 FCR and 1.35 PER. Concerning the nutrient retention aspect, the results showed that protein retention coefficients (PRC) varied from 14 to 70%, while the energy retention coefficients (ERC) were ranged from 16-52%. The highest values of the nutrient coefficients were observed for the control diet (PRC = 69.82% and ERC = 52.18%), followed by the fermented leaves diet (PRC = 47.56% and ERC = 30.36%) where values were significantly higher ($p<0.05$) than those of the other tested diets. The results of this study showed that the fish diet based on fermented *L. minor* provided a better utilization coefficient than those of the other tested diets.

DISCUSSION

In this study, four treatments (shade-drying, oven drying, pre-cooking-drying and fermentation-drying) were applied to *L. minor* for improving the diet of juvenile Tilapia (*O. niloticus*). Then, the zootechnical parameters and apparent digestibility coefficient regarding four dietary treatments of *L. minor* were assessed in juvenile Tilapia (*O. niloticus*).

Overall, these treatments decreased the anti-nutritional factors of *L. minor*. Particularly, the fermentation of *L. minor* provided more reduction of anti-nutritional factors than those of the other three treatment forms. Adebayo *et al.*[44] reported that fermentation method was the best for reducing anti-nutritional factors in plants. In India, a similar result was observed by Bairagi *et al.*[45] who used *Lemna polyrhiza* and fermented it with a strain of *bacillus* extracted from the intestine of carp. These authors revealed a decrease in tannin content (1%-0.02%), phytates (1.23%-0.09%) and fiber (11%-7.5%).

Results of the present study showed that the pre-cooked-drying process had better reduction percentage than drying at 50°C and shade-drying. This result could be explained by the combined action of cooking and drying the leaves. In Nigeria, Okpara *et al.*[18] conducted an experiment to reduce the anti-nutritional factors in the leaves of *Gmelina arborea* and found similar results.

The crude protein content in pre-cooked-dried *L. minor* (35.65%) and dried *L. minor* at 50°C (35.85%) was different from shade-dried *L. minor* (36.70%) and fresh *L. minor* (38.80%). This variation is due to the effect of heat on the nutrients. Heat treatment process to reduce anti-nutritional substances, can reduce the availability of some amino acids particularly lysine[46].

The study showed that the protein content in fermented *L. minor* was higher than that of the fresh leaves. Firstly, the improvement in the protein content was associated with the increase of the overall free amino acids due to bacterial degradation of food proteins. Secondly, this rate of increase was linked to the production of amino acids by extracellular protease-producing bacteria during the fermentation process[47]. Additionally, results of the present study agree with a previous study conducted by Saha and Ray[48] who found increase in protein content (13.37-16.8%) of fermented water hyacinth with *Bacillus megaterium* and *Bacillus subtilis.* The crude protein content and the Protein/Energy (P/E) ratio of the treated *L. minor* are close to the values (protein: 40% and P/E: 27.75 mg kJ^{-1}) reported by Jauncey and Ross[49] for tilapia. However, our study revealed that fermented leaves had a better reduction rate of anti-nutritional factors and offer nutritive value that is required for fishes, particularly Tilapia, (*O. niloticus*)

The Apparent Digestibility Coefficient of nutrient and energy assessed for treated *L. minor* were ranged from 67-78% for dry matter, 66-82% for protein and 62-79% for energy. Among the four treated forms of *L. minor* in this study, the fermented *L. minor* had the highest Apparent Digestibility Coefficient for protein (81.68%) and energy (78.47%). The digestible protein concentration (33.16 mg g^{-1}) of the fermented *L. minor* was higher than that of the heat-treated *L. minor*. This result showed a better digestion of the fermented leaves by juvenile Tilapia (*O. niloticus*) compared to three other implemented treatments. Protein utilization of the fermented *L. minor* by tilapia could be related to the improvement of its nutritional value. The digestion of the fermented *L. minor* was also associated with the protein concentration in duckweed and the strong reduction of anti-nutritional factors by this treatment method. Recently, it has been reported by some authors[28,50] that the fermented plant ingredients can be used in fish diet to improve the micronutrient and essential amino acid content. The activity of digestive enzymes could be promoted by the bacillus used for fermentation[51], because bacillus are known for their probiotic potential[52]. Ray and Das[53] reported that fermented aquatic macrophytes had higher protein digestibility compared to thermally dried macrophytes. Similar results were reported for the digestibility potential of sun-dried and fermented aquatic macrophytes in the diet of Cachama (*Piaractus brachypomus*). Cruz-Velásquez *et al.*[54] reported that the Apparent Digestibility Coefficient of proteins for *Spirodela polyrhiza* (85%), *Lemna minor* (78%) and *Azolla filiculoides* (75%) leaves fermented by lactic acid bacteria were higher compared to those treated by sun-dried.

For the heat-treated *L. minor*, the apparent digestibility coefficient of protein (75.19%) and energy (77.83%) from shade-dried leaves were higher than those of the other implemented treatments (dried at 50°C and pre-cooked/dried). This low digestibility of protein and energy (dried at 50°C and pre-cooked/dried *L. minor*) compared to shade-dried *L. minor* could be attributed to a possible loss of nutrients, especially amino acids by heat[46,55]. Previous studies have addressed the issue of the Apparent Digestibility Coefficient of protein, energy for several plants and agricultural by-products used in the diet of *O. niloticus*. These products used in aquaculture concerned essentially maize (70-77%), millet (68-77%) and sorghum (52-77%), sunflower meal (90-72%), rapeseed meal (88-75%) and rice bran (84-75%)[12,13]. In this context, fermented leaves and shade-dried leaves could be introduced as proteins supplements in the formulation of Tilapia feeds in Côte d'Ivoire for improving the food security. For animals, an ingredient is considered a protein supplement if it contains at least 20% crude protein[56].

A significant difference ($p<0.05$) was observed between the apparent digestibility coefficient of dry matter for tested and the control diets. This difference could be due to the nature and the biochemical composition of the ingredients which influenced the digestibility because the tested diets were iso-protein and iso-lipid. The digestion, utilization and bioavailability of nutrients also depend on the forms and nature of food components[11]. The same digestive trend in the treated *L. minor* was also observed with the tested diets. Thus, the diet with fermented *L. minor* was the best digested by juvenile tilapia (*O. niloticus*) after the control diet. The probiotic action of the bacillus improves feed utilization by fish[57]. This result showed that the digestibility of the diet depended on the nature of the studied ingredients[12].

Result showed that the fish survival rate was not significantly different ($p>0.05$) between different tested diets. In terms of growth and nutrient utilization, these parameters were observed following the same trend as apparent digestibility coefficients of the dietary nutrients. The best zootechnical parameter values were obtained with control diet. Due to higher digestibility of nutrients in tilapia, diet with fermented *L. minor* provided a better growth performance and feed utilization compared to shade-dried, dried at 50°C and pre-cooked-dried *L. minor* diets. This would be explained by the incorporation of bacillus-fermented and heat-dried *L. minor* in the diet. Tran *et al.*[58] reported that the presence of heat-killed bacilli can improve feed utilization and growth performance of aquatic animals. Furthermore, an improvement in growth performance was observed after adding bacillus into the diet of carp koi (*C. carpio* var. koi)[59].

CONCLUSION

This study assessed the nutritional potential of four treated forms of *L. minor* as a protein source in the diet of juvenile Tilapia (*Oreochromis niloticus*). Among the heat treatments, pre-cooking-drying method is found to be the most effective to reduce the anti-nutritional factors. Therefore, the fermentation process provided the best reductions in anti-nutritional factors and improved the nutritional value, particularly increase the protein content. The diet containing fermented *L. minor* showed the best growth performance, protein utilization and digestion among all the tested diets. We recommend that in aquaculture practices in Côte d'Ivoire, fermented *L. minor* could be used as an ingredient for protein supplement in the fish feed formulation.

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