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Productive performance, body measurements, and meat composition in Nile tilapia juveniles supplemented with fresh duckweed

Desempenho produtivo, medidas corporais e composição da carne de juvenis de tilápia-do-Nilo suplementados com lentilha d'água fresca

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Abstract: *Lemna minor* (LM) or duckweed is a cheap and sustainable protein source, mainly for use by small fish farmers. In this study was determined the composition of the used fresh LM and evaluated its effect on performance, body measurements, and meat composition of Nile tilapia. One hundred sixty juveniles were used in a completely randomized design with four treatments and four replicates. Treatments comprised a control group (commercial ration) and three experimental groups (90%, 80%, and 70% of the daily ration amount in the control group + 10%, 20%, and 30% of fresh LM, respectively). The used LM showed 96.55% of moisture, 17.24% of ash, 5.56% of total lipids, and 29.78% of crude protein. The 30% supplementation with LM reduced the feed intake and, consequently, the protein intake ($P \leq 0.05$) compared to the control group, resulting in a better feed conversion rate (g ration/g weight gain), and lower cost of fish kilo. There was no effect ($P \geq 0.05$) of the supplementation with fresh LM on body measurements and the contents of moisture, protein, fat, and minerals in the juvenile meat. It was concluded that Nile tilapia juveniles can be reared with 70% commercial ration + 30% fresh LM, reducing the costs of fish kilo.

Key-words: alternative feedstuff; fish feeding; *Lemna minor.*

Resumo: A *Lemna minor* (LM) ou lentilha d'água é uma fonte de proteína acessível e sustentável, principalmente para uso por pequenos produtores. Neste estudo, foi determinada a composição da LM fresca utilizada e foi avaliado o seu efeito sobre desempenho, medidas corporais e composição da carne dos juvenis de tilápia-do-Nilo. Foram usados 160 juvenis em delineamento inteiramente casualizado com quatro tratamentos e quatro repetições. Os tratamentos consistiram em grupo controle (ração comercial) e três grupos experimentais (90, 80 e 70% da quantidade diária de ração no grupo controle + suplementação com 10, 20 e 30% da quantidade em LM fresca, respectivamente). A LM utilizada apresentava 96,55% de umidade, 17,24% de minerais, 5,56% de lipídios totais e 29,78% de proteína bruta. A suplementação com

30% de LM reduziu o consumo de ração (P≤0,05) comparado ao grupo controle, resultando em melhor conversão alimentar (g de ração/g de ganho de peso) e menor custo do quilo de peixe. Não houve efeito (P ≥ 0,05) da suplementação com LM fresca nas medidas corporais e nos teores de umidade, de proteína, de lipídios e de minerais da carne de juvenis. Concluiu-se que juvenis de tilápia do Nilo podem ser criados com 70% de ração comercial + 30%, em peso, de *Lemna minor* fresca, com redução nos do quilo de peixe.

Palavras-chave: alimentação de peixes; alimentos alternativos; *Lemna minor*.

1. Introduction

As a country develops, the demand for meat increases and the nutrition and health of people improve. Although fish constitute a small fraction of the global protein ingestion (6.7%), they are considered an important source of this nutrient, comprising approximately 17% of the meat consumed worldwide [1]. Global fish consumption in 2023 was estimated to be 165 million tons, followed by poultry consumption at 140 million tons [2].

Feed and electrical energy costs contribute significantly to the effective operational costs of fish farmers. Feed costs can make up as much as 80% of the effective operating cost [3], underscoring the low efficiency of feed utilization and the impact of high input prices. This emphasizes the need to improve feed conversion rates to maximize feed efficiency.

Lemna minor (LM), or duckweed, belongs to a group of floating aquatic macrophytic monocotyledons that grow extremely rapidly [4] and are a food source for fish, small invertebrates [5], and even humans [6, 7]. These plants accumulate large quantities of vegetal protein and, according to Mohedano *et al.* [8], can accumulate high nitrogen concentrations, as reflected by the high concentration of crude protein. They are also rich in unsaturated fatty acids from the omega-3 series [9]. According to Chakrabarti *et al.* [10], LM is a source of essential amino acids (39.2%), including leucine, isoleucine, and valine, which constitute 48.67% of the essential amino acids for tilapia. Thus, LM is a potential candidate for use as an economically viable and sustainable protein source, particularly for small fish farmers.

LM contains approximately 16–45% crude protein, 2–10% crude fiber, 2–10% lipids, 4–15% minerals, 0.015–0.28% calcium, and 0.1–0.43% total phosphorus (6, 10-13), based on dry matter, and has antimicrobial, antioxidant, immunostimulant effects [11, 14, 15]. The inclusion of 20% and 40% fresh LM in the diets of Nile tilapia (Oreochromis niloticus) juveniles has resulted in a higher deposition of minerals and protein and lower deposition of fat in the meat, indicating an improvement in the nutritional profile of the fillet [16]. The use of LM meal in the diet of tilapia juveniles has increased muscle levels of long-chain omega-3 polyunsaturated fatty acids - specifically eicosapentaenoic and docosahexaenoic acids - because, despite its lower lipid content, LM is rich in these fatty acids [17].

Several studies have shown positive results for the use of LM meal for tilapia [12, 18, 19], carp [9, 20], and trout [4]. However, tests with fresh LM are scarce. Thus, this study was performed to determine the composition of fresh LM and evaluate its potential use in the supplementation of Nile tilapia juveniles and its effects on performance, body morphometry, and fillet composition.

2. Material and methods

The Ethics Committee of Animal Use at the University of Rio Verde approved the study protocol on April 17, 2023 (No. 01/2023). Fresh LM was cultivated in a 1 m deep 16 m2 masonry tank containing dechlorinated water next to the fish farming laboratory. After the seedlings were placed on the water surface, slow-release NPK (10-10-10) fertilizer was applied at 40 g/m2 every 45 d. The tank was covered with a white screen to avoid mosquito reproduction. Solar exposure occurred from the morning for approximately 14 h. LM was collected daily, washed, and drained, and after excess water was removed, it was weighed and provided to the tilapia juveniles.

LM samples were dried in a convection oven at 55 °C for 48 h and ground before being analyzed. The proximate moisture, protein, lipid, and mineral composition was determined following the methodology described by Silva and Queiroz [21].

The experimental design was completely randomized with 4 treatments and 4 replicates, totaling 16 experimental units (94 L polyethylene containers) of 10 fish each. Therefore, 160 Nile tilapia juveniles with an initial mean weight of 20.88 ± 0.81 g were used. The experimental period was 48 d. All fish were fed the same extruded commercial diet (Raguife Rações, Santa Fé do Sul, SP, Brazil) for two weeks prior to the beginning of the experimental period to allow adaptation to the recirculating aquaculture system. Throughout the rearing period, the animals were maintained in an environment with continuous aeration (air blower: 12 m^3/h). The water used originated from an artesian well with a flow rate of 12,000 L/h. The recirculating aquaculture system was equipped with a heater, thermostat, digital thermometer, water compressor pump, and mechanical and biological filters. Water from each tank was passed through mechanical filters to remove solid waste (feces) and then through a biological filter for denitrification, achieved through the activity of free-floating or substrate-attached bacteria. During the experimental period, chemical parameters of the water collected from the filter - dissolved oxygen, pH, and total ammonia nitrogen - were measured daily in the morning using Labcom Test kits (Alcom®). Water temperature was monitored daily using an Incoterm® digital thermometer.

The treatments consisted of a control group (fed extruded commercial feed) and three experimental groups that received 90%, 80%, and 70% of the daily feed amount given to the control group, supplemented with 10%, 20%, and 30% fresh LM, respectively. The amount of feed provided was 4% of the biomass in each tank divided into two daily feedings. Juveniles were weighed weekly to adjust the feed and LM supply. Commercial feed was offered twice daily at 8:30 a.m. and 4:30 p.m. LM was provided daily at 5:30 p.m. Forty-five minutes after commercial feed distribution, uneaten feed was collected using a hand net, dried, and weighed to determine feed intake. The next morning, unconsumed LM was collected, drained of excess water, and weighed to assess plant intake.

The performance parameters evaluated included final weight, weight gain, feed intake, feed conversion ratio (g feed/g weight gain and g feed + LM/g weight gain), and specific growth rate. Economic viability was determined based on the amount of feed consumed (R$ 7.50/kg) × feed conversion ratio (g feed/g weight gain) × 1000, resulting in the cost per kilogram of fish for each treatment.

At the end of the experimental period, three randomly selected fish from each replicate were euthanized for body measurements, and 50 g of dorsal muscle was collected. Clove oil (eugenol) was used as a general anesthetic at a dose of 185 mg/L in water, followed by euthanasia following CONCEA (the Brazilian National Council for the Control of Animal Experimentation) [(22)]. The carcasses were measured and processed only after this procedure.

The morphometric measurements obtained included total length (from the anterior-most tip to the end of the caudal fin), standard length (from the anterior-most tip to the base of the caudal fin rays), head length (from the anterior-most tip to the edge of the operculum), and body depth (from the base of the dorsal fin to the base of the ventral side). Measurements were performed using a Digimess digital caliper with a 0.01 mm precision.

The fish were dissected by making a dorsal incision, followed by two lateral cuts on each side of the body. A 50 g sample of skinless dorsal muscle was collected, minced, homogenized, and analyzed for dry matter, crude protein, total lipids, and ash content, as described by Silva and Queiroz [(21)].

All results were subjected to analysis of variance using the SISVAR software [(23)]. When the F test was significant, the Scott–Knott test at a 5% probability level was used for mean comparisons.

3. Results and discussion

Water quality parameters during the experimental period are listed in Table 1. The mean total ammonia, pH, dissolved oxygen, total hardness, and temperature were within the reference ranges reported by Queiroz *et al.* [(24)].

Table 1. Mean water quality parameters for Nile tilapia juveniles fed commercial feed supplemented with fresh Lemna minor.

Parameters	Mean value	Queiroz *et al.* [(24)]
Total ammonia (mg/L)	1.54 ± 0.94	0.6–2.0
Non-ionized ammonia (mg/L)[1]	0.014 ± 0.012	< 0.6
pH	6.9 ± 0.23	6–9
Dissolved oxygen (mg/L)	5.72 ± 1.37	5–6
Total hardness (mg/L)[2]	± 8.66	> 20
Temperature (°C)	28.7 ± 1.35	26–28

[1]Determined according to the kit manufacturer's instructions.
[2]One to three drops correspond to 50 to 150 mg/L of calcium carbonate, with the water being classified as soft, according to the kit manufacturer (Labcon Test Toxic Ammonia for Freshwater, Alcon, Camboriú, SC, Brazil).

The water quality parameters were within the recommended ranges for Nile tilapia, except for ammonia levels, which were close to the upper limit. However, due to their interactions with other variables, these levels did not negatively affect fish performance.

On average, the LM used in this study contained 96.55% moisture, 17.24% minerals, 5.56% lipids, and 29.78% crude protein on a dry matter basis. These values are similar to those reported by Chakrabarti *et al.* [(10)], Herawati *et al.* [(18)], and Sońta *et al.* [(25)], who found ranges of 91.9–93.3% for moisture, 23.47–49.38% for protein, 1.15–7.81% for total lipids, and 8.64–23.6% for total minerals, all on a dry matter basis. Variations in these results may be due to the nutritional status of the cultivation water, which influences the LM protein content. Protein levels may vary from 9–20% under low or suboptimal nutrient conditions to 24–41% under optimal nutrient conditions.

The LM treatments did not significantly affect ($P \geq 0.05$) final weight, weight gain, total feed conversion (feed + LM), survival rate, or specific growth rate. However, fish supplemented with 30%

fresh LM showed a 33% reduction in feed intake ($P < 0.05$) compared with the control group, resulting in an improved feed conversion ratio and a 23% lower cost per kilogram of fish (Table 2). Supplementation with 10–30% LM led to reduced feed conversion values when considering feed intake alone, which translated into lower production costs per kilogram of fish.

The reduction in feed intake observed with 30% LM supplementation may be attributed to the crude fiber content of the plant, which ranges from 11.51% to 29.7% [12, 26–31]. *Lemna minor* has protein levels comparable to, or even higher than, most grains and contains significantly more fiber than typical cereals [29]. Although fresh LM has lower levels of fiber and anti-nutritional factors such as tannins and phytic acid than dried LM, it may have contributed to an increased sensation of satiety in the fish, leading to reduced feed consumption [13, 26, 31].

Using L. minor as a protein source in tilapia feed can improve productive performance. However, there are limited studies on the use of fresh L. minor. Cipriani *et al*. [32] observed the same effect in juvenile tilapia that consumed fresh LM, with a subsequent reduction in feed intake and no change in productive performance.

Table 2. Performance of Nile tilapia juveniles fed commercial feed and supplemented with fresh *Lemna minor.*

Parameters	*Lemna minor* (%)				SEM	P value
	0	10	20	30		
Final weight (g)	74.45	78.22	73.92	68.92	3.83	0.43
Weight gain (g)	53.77	57.17	53.35	47.87	3.72	0.39
Feed intake (g)	77.61^{a}	67.62^{a}	64.79^{a}	51.55^{b}	3.96	0.005
L. minor consumption (g)	0.00^{d}	5.91^{c}	10.07^{b}	12.79^{a}	0.38	0.001
Feed: gain ratio[1]	1.45^{a}	1.18^{b}	1.21^{b}	1.12^{b}	0.09	0.05
Feed: gain ratio[2]	1.45	1.28	1.40	1.39	0.08	0.60
SGR	2.67	2.73	2.66	2.45	0.01	0.28
Survival rate (%)	94.94	96.61	88.96	98.80	2.84	0.14
Cost of fish kilo (R$)	10.87^{a}	8.85^{b}	9.07^{b}	8.40^{b}	1.40	0.05

[1]feed conversion (g feed/g weight gain).
[2]total feed conversion (g feed + L. minor/g weight gain)
[ab] Means followed by different letters differ significantly according to the Scott–Knott test ($P < 0.05$).
SGR, specific growth rate; SEM, standard error of the mean.

Other authors, such as El-Shafai *et al.* [16], have investigated the effect of fresh *Lemna* on the performance of tilapia, by offering 20–40% of the diet by weight as dry or fresh LM to juvenile tilapia. The inclusion of 20% fresh LM promoted a specific growth rate and feed conversion similar to those obtained in the control group and better than those obtained in the other treatments. According to Chowdhury *et al.* [33], improved specific growth rate and weight gain resulted in a 182.51% increase in tilapia fry production in tanks supplemented with fresh LM (60% of the total body weight of the fish per day).

Despite lower feed intake, weight gain was similar across all treatments, possibly because supplementation with fresh LM contributed, even in small amounts, to meeting the amino acid requirements of fish in the groups fed relatively lower feed quantities. LM has a good proportion of essential amino acids (g/100 g protein) that are important for optimal tilapia development, such as methionine (1.6–1.9%), threonine (3.6–4.0%), lysine (5%), arginine (4.8%), valine (4.6%), isoleucine (3.7–4.03%), leucine (6.5–7.3%), phenylalanine (4.4–4.6%), arginine (4.8–6.4%), tryptophan (1.11%), and histidine (1.5–2.3%) [6, 7, 13]. These values are similar to those found in soybean meal and fishmeal protein, which are standard components of fish feed [34].

Supplementation with fresh LM had no significant effect (P ≥ 0.05) on the body measurements of juvenile tilapia after 48 days of study (Table 3). Different fish species may have varying productive aptitudes because of differences in muscle mass in specific body regions during growth, resulting in diverse body shapes and sizes [35].

Table 3. Mean body measurements (mm) of Nile tilapia juveniles fed commercial feed and supplemented with fresh *Lemna minor.*

Proportion of *L. minor* (%)	SL	TL	BD	BW	HL
0	144.6	172	53.5	24.7	49.6
10	144.8	175	54.2	24.6	46.7
20	142.7	175	52.0	23.8	45.7
30	140.3	171	54.2	23.8	49.0
SEM	2.5	3.0	1.2	0.8	1.3
P value	0.56	0.76	0.57	0.80	0.15

SL, standard body length; TL, total body length; BD, body depth; BW, body width; HL, head length; SEM, standard error of the mean.

Tilapia body measurements positively correlate with carcass and fillet weights, especially body width, depth, and length (36). Both width and standard length can be used as selection criteria for weight and fillet yield in Nile tilapia [37].

Dietary supplementation with LM did not affect (P > 0.05) the moisture, protein, lipid, or mineral contents of the juvenile tilapia (Table 4), indicating that sufficient nutrient absorption occurred during deposition, despite the reduction in feed intake due to supplementation with 30% LM. Studies involving fresh LM are scarce, but Solomon and Okomoda [19] evaluated the inclusion of dry LM in tilapia diets at 5, 10, 15, 20, and 25% and reported that protein levels in fish meat decreased as the LM inclusion level in the diet increased from 5%. Opiyo *et al.* [17] and Hassan and Edwards (38) observed a reduction in dry matter, lipid, and protein content in fish that consumed diets containing 2.5–15% LM meal inclusion.

Table 4. Mean composition (%) of fillets from Nile tilapia juveniles fed commercial feed and supplemented with fresh *Lemna minor* (based on natural matter).

Levels of L. minor (%)	Moisture	Protein	Lipids	Minerals
0	79,00	18,87	2,40	1,34
10	78,75	19,60	2,36	1,37
20	79,00	18,54	2,38	1,26
30	79,25	18,84	2,80	1,22
SEM	0,33	0,36	0,06	0,10
P value	0,78	0,25	0,59	0,74

SEM, standard error of the mean.

Notably, the cited studies used dry LM, which increases the concentration of nutrients, including crude fiber. High levels of crude fiber may reduce nutrient absorption, contributing to reduced nutrient deposition in fish meat [39].

Fasakin *et al.* [40] reported similar results in that they found no observable differences in tilapia meat with the use of LM meal (5, 10, 20, 30, and 100% replacement of fishmeal). However, lipid (4.5%) and mineral (2.9–3.6%) levels were higher, and protein levels (13.1–14.6%) were lower than those found in this study. Fiodelmondo *et al.* [4] reported that LM meal (10, 20, and 28%) did not affect moisture, fat, protein, or mineral levels in trout, and the values obtained were similar to those observed in the present study.

4. Conclusion

Nile tilapia juveniles can be raised on a diet composed of 70% commercial feed and 30%, by weight, of fresh LM, with a reduction in the cost per kilogram of fish produced.

Conflict of interest statements

The authors declare that there is no conflict of interest.

Data availability statements

The data will be made available upon request to the corresponding author.

Author contributionsr

Conceptualization and formal analysis: M. C. de Oliveira and Y. A. Attia. Data curation, project administration, and writing (original draft, review, and editing): M. C. de Oliveira. Investigation and visualization: T. F. F. Santos and J. L. F. Gomes. Resources and supervision: P. F. Santos.

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