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Effect of Aquaponically Grown Duckweed as a Sustainable Feed on Growth Indices, Water Quality, and Digestive Activities, for the Nile Tilapia Reared in Aquaponic Culture

Yousef Ahmed Alkhamis
Department of Animal and Fish Production, Agricultural and Food Sciences College, King Faisal University, P.O. Box 400, Al-Ahsa 31982, Saudi Arabia
Fish Resource Research Center, , King Faisal University, P.O. Box 400, Al-Ahsa 31982, Saudi Arabia

yalkhamis@kfu.edu.sa

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ABSTRACT

One of the challenges facing aquaculture is finding a high-value, sustainable, and low-cost alternative feedstuff. Duckweed, a small aquatic plant, has a high-quality protein value. This study was conducted to evaluate the impact of aquaponically produced duckweed *L. minor* as a substitute feedstuff and its effect of different levels (0, 10, 20, and 30%) on water quality, growth indices, digestive activities, and profitability of the Nile tilapia *Oreochromis niloticus* fingerlings (15.27± 0.19g) using aquaponics system for 8 weeks. With a completely randomized design, fish were stocked (6kg/ m3) in 12 experimental aquaponic units (250L). Results showed that duckweed inclusion had a significant impact on water quality measures (P< 0.05), leading to a drop in nitrogen and phosphorus levels in fish tanks. Duckweed improved growth indexes, digestive enzyme activities, and profitability (P< 0.05). At a substitution rate of 20% of duckweed in the diet, the feeding impact was at its peak, resulting in a weight gain rate of 63.2g in 56 days, which was 32.7% greater than the control group (P< 0.05). Compared to the control group, the fish showed significantly higher body weight, specific growth rate, feed conversion ratio, condition factor, and hepatosomatic index (P< 0.05). Duckweed stimulated the digestive system's amylase, protease, and lipase activity (P< 0.05). Based on the findings, it can be reported that, the Nile tilapia fingerlings reared in an aquaponics system can include duckweed at a rate of 20% in diet to show better growth indices and profitability.

INTRODUCTION

Nowadays, intensive aquaculture systems such as recirculating aquaculture systems (RAS) are more used in the aquaculture industry to save water consumption, especially fresh water in dry areas **(Sarkheil *et al.*, 2023)**. To maintain water quality, RAS systems rely on biological and mechanical filtration systems **(Chen *et al.* 1997)**. High productivity is made possible by intensive rearing in RAS, which also lessens the activity's detrimental effects on the environment and the quantity of water required per unit of production **(Blancheton 2000; Colt 2006)**. To maximize the benefit, a combination of the hydroponic system (soilless agriculture) was combined with the recycled aquaculture system (RAS) in what is known as the aquaponic system **(Maucieri *et al.*, 2018)**. In aquaponic systems, fish waste contains nutrients that are used to nourish the plants. Bacteria must be at their optimal functioning for fish waste to be transformed into nutrients for plants, which is why the system depends on them **(Wong *et al*., 2020)**. The maintenance of nutrients and microbes, hydroponics, and aquaculture are all required for an aquaponics system to function well.

Approximately 42% of the world's total aquaculture is derived from tilapia **(FAO, 2020)**. *O. niloticus* is a heavily cultured fish that may be found in more than 100 countries **(Makori *et al.* 2017)**. Moreover, this species is characterized by its adaptation to a wide range of environmental stresses, fast growth, resistance to diseases, and ease of breeding. In recent years, there has been a more than twofold increase in the demand for and expense of fish feed globally **(FAO, 2020)**. On average, the intensive Nile tilapia farming has a feed cost of around 50% of overall operating expenditures **(El-Sayid *et al.*, 2015)**. Since the Nile tilapia are herbivores, feeding costs can be minimized by using plant protein sources in their diet due to their tolerance. Ensuring sustainable Nile tilapia aquaculture practices is crucial for the industry's long-term success, and researchers have been exploring alternative and cost-effective feed options **(Kari *et al.*, 2022)**. One such alternative is duckweed *Lemna minor*.

According to **Les *et al.* (2002)** Lemna, Spirodela, Landoltia, Wolffia, and Wolffiella are the five genera that comprise the family Lemnaceae, which includes duckweed. Lemna has 38 species in total. It is known for growing quickly, typically achieving growth rates of 7– 12mg NH4–N L-1 or more in a medium containing a high nitrogen content (ideally as ammonium) **(Leng *et al.*, 1995)**. The protein levels in duckweed are comparable to, or even exceed, those found in conventional feed ingredients like soybean meal and fishmeal up to 45.5% of crude protein **(Appenroth *et al.*, 2017)**. Additionally, duckweed exhibits well-balanced essential amino acids and minerals for the optimal growth and health of the Nile tilapia **(Stadtlander *et al.*, 2019)**. Countless strains of duckweed have previously been used as chicken feed **(Haustein *et al.*, 1990)**. Moreover, several commercial fish were used including silver barb **(Noor *et al.*, 2000)**, snakehead **(Raj *et al.*, 2001)**, rohu **(Bairagi *et al.*, 2002)**, striped catfish **(Da *et al*., 2013)**, common carp **(Sirakov & Velichkova, 2018)**, and rainbow trout **(Stadtlander *et al*., 2019)**. For the Nile tilapia, several studies have investigated the nutritional content of different duckweed species, emphasizing their potential to replace or complement the traditional feed sources up to 50% of a tilapia diet without affecting the weight gain of fingerlings **(Tavares *et al*., 2008; De Matos *et al.*, 2014; Cipriani *et al*., 2021; Han *et al*., 2022)**.

Consequently, the current experiment aimed to evaluate the impact of aquaponically produced duckweed *L. minor* as a substitute feedstuff and its effect of various levels on water quality, growth indices, and digestive activities for the aquaponic Nile tilapia cultures.

MATERIALS AND METHODS

1. Aquaponic system design and establishment

The experiment was conducted in the greenhouse at King Faisal University Research and Training Station in Saudi Arabia (26◦23018.5600 N, 50◦11016.0100 E). The greenhouse is a protective structure measuring 15 x 8m, covered with a plastic layer of polyethylene (PE), treated with UV rays, and a 50% shade net was utilized as the growing area. The environmental conditions during the present trial did not have a control system. Twelve aquaponic systems were installed in the greenhouse. A 250L mechanical filter, a 250L biofilter tank, a 250L circular fish tank with a 200L water volume, and a duckweed culture tank were included with every aquaponic recirculation system. The dimensions of the duckweed tanks were 1.5 x 0.50 x 0.30 meters. Each tilapia tank is equipped with a submersible water pump (flow rate: 1200L/ h), which delivers water to the mechanical filter ($2m^3$/ hour) and then to the biological filter. The water was changed three times each hour by adjusting the water flow. The dissolved oxygen (DO) level remained over 8mg/ L by, installing an air blower in each fish tank. In a hydroponic system, duckweed obtains nutrients dissolved in aquaculture waste, and the resulting cleansed water is subsequently pumped back into the Nile tilapia tank. Water was circulated rather than exchanged between the Nile tilapia tank and the duckweed culture unit during the testing period.

2. Duckweed meal (DWM) preparation and experimental diets

The duckweed *Lemna minor* was freshly collected from the aquaponic system. Every one to two days, one-third of the biomass was removed, depending on the weather and the rate of growth of the *L. minor*. It was drained, sun-dried for at least 6h per day and after that, it was dried in an electric dryer at 60°C for 6 hours. The duckweed was powdered and stored at 4ºC after sifting using a 100-µm mesh screen. The chemical analysis of duckweed was done according to **AOAC (1995**), and the crude protein content was 38.2%, moisture was 8.6%, ash was 8.6%, ether extract was 2.34%, and crude fiber was 1.41%. The following quantities of DWM powder were added to the basal diet to produce the experimental dietary treatments: 0g kg^{-1} (DWM0) as the control group, 10g kg^{-1} (DWM10), 20g kg^{-1} (DWM20), and 30g kg^{-1} (DWM30) (Table 1). The diets were isonitrogenous and isocaloric (32% crude protein and 3200kcal).

3. Experimental design and management

All male-sex reverse *O. niloticus* fingerlings with an initial weight of 15.27± 0.19g were randomly stocked at 6kg/ m^3. The Nile tilapia fingerlings were randomly distributed into four feeding treatment groups: 0g kg^{-1} (DWM0) as the control group, 10g kg^{-1} (DWM10), 20g kg^{-1} (DWM20), and 30g kg^{-1} (DWM30). The study employed a completely randomized design with three replicates per treatment. The experiment lasted for 8 weeks. During the 2-week acclimation period, all aquaponic systems were initiated by adding a starter duckweed and the Nile tilapia fingerlings to the tanks with fish feed on a basal diet. During the trial, the fish were fed twice daily at 8.00 and 16.00hrs, at the rate of 4% of the fish's body weight per day. Fingerlings were weighed every two weeks to adjust the feeding rate based on body weight.

Table 1. The feed components and a chemical analysis of the experimental diet for the Nile tilapia

Component	**DWM0 (g)**	**DWM10 (g)**	**DWM20 (g)**	**DWM30 (g)**
Fish meal	207.5	182.5	157.5	132.5
Soybean meal	207.5	182.5	157.5	132.5
Corn meal	207.5	182.5	157.5	132.5
Rice bran	207.5	182.5	157.5	132.5
Duckweed meal	0	100	200	300
Soybean oil	50	50	50	50
Premix[1]	20	20	20	20
Binder	100	100	100	100
Total	1000	1000	1000	1000
Chemical analysis (%)				
Moisture	82.0	81.3	77.6	78.8
Crude protein	328.2	325.8	322.9	321.3
Crude fat	45.7	43.6	44.2	43.1
Fiber	65.2	67.9	72.1	71.1
Ash	107.0	96.3	96.8	95.3
Gross energy (cal/g)	3484	3371	3279	3256

[1] Premix (g/kg pemix): Vit. C, KCL, 90; KI, 0.04; $CaHPO.2H_2O$, 500; NaCl, 40; $CuSO_4.5H_2O$, 4; $ZnSO_4.7H_2O$, 4; CoO_4, 0.02; $FeSO_4.7H_20$, 20; $MnSO_4.H_2O$, 3: $CaCo_3$, 215; MgOH, 124; Na_2SeO_3, 0.03; NaF1.

4. Water quality indices

Throughout the experiment, the following water parameters were taken daily in fish tank: temperature and dissolved oxygen (YSI 556 multiparameter meter), pH (pH10A Pen Tester), and electric conductivity, and total dissolved solids (Hach test kits). While, water samples were taken weekly to analyze the following measurements: ammonia (NH_3), nitrite (NO_2), nitrate- (NO_3), orthophosphate (PO_4), and alkalinity ($CaCO_3$ mg L^{-1}), utilizing an optical photometer YSI 9500 in accordance with the manufacturer's suggested methodology (YSI Incorporated, Yellow Springs, OH, USA).

5. The Nile tilapia growth indices and body composition analysis

Each fish was individually weighed, and their body length was determined at the end of the experimental period. The growth index was computed as follows:

Weight gain rate = (Weight $_{final}$ – Weight $_{initial}$)/ Weight $_{initial}$ × 100

Specific growth rates = (ln $_{Weight\ final}$ − ln $_{Weight\ initial}$) /days × 100

Feed conversion ratio = Feed consumed / Weight gain

Condition factor = Weight $_{final}$ / Total length^3 × 100

Viscerosomatic index = Viscera weight / Final weight × 100

Hepatosomatic index = Liver weight / Final weight × 100

Survival rate = (Final number of fish - Initial number of fish) ×100

The whole body composition of the Nile tilapia (protein, lipids, moister and ash) was determined for six fish from each treatment by using the standard methods mentioned by **AOAC (1995)**.

6. Digestive activities

Nine fish from each treatment were given a 50µl solution of clove oil as a mild anesthetic after they fasting for a day. The stomach, intestines and liver were then pulled out and cleaned with cold distilled water (4°C) for each fish individually. The specimens were milled in the cold extraction buffer (20mM Tris/ 10mM phosphate, pH 7.0), using a motor-driven homogenizer after being weighed. After centrifuging the homogenates for 10 minutes at 4°C at 10,000× g, the supernatants were used right away for the enzyme test. Using a pepsin assay kit (Nanjing, China) according to the manufacturer's procedure, the amount of proteolytic activity in the stomach and intestine was measured. Liver proteolytic activity, lipase and amylase were measured using trypsin assay kit, lipase assay kit and amylase assay kit (Nanjing, China), respectively. All measurements were carried out in accordance with the manufacturer's methodology.

7. Economical evaluation

Feed ingredient pricing and fish sales were fixed in line with the going rates in the Saudi Arabian local market in September 2023. The rate of one United States dollar ($) is 3.77 Saudi Arabia riyal (SR). According to **Allam *et al.* (2020)** and **Kishawy *et al.* (2022)**, the following equations were used to determine economic efficiency:

Feed cost for 1kg weight gain/ $ = FCR x Feed cost of 1kg

Net profit for 1kg weight gain, $ = Sale price of kg – Gross cost

Economic efficiency for 1 kg weight gain, % = Net Profit / Gross cost

Where, 1kg cost of basal diet (DWM0) = 1.24$/ kg, DWM10 = 1.11$/ kg, DWM20 = 1.00$/ kg and DWM = 0.92$/ kg; gross cost = 60% for feeding cost + 40% of the remaining cost, and wholesale cost = 3.00$/ kg

8. Statistical analysis

The statistical software SPSS v26.0 (SPSS Inc., Chicago, IL, USA) was used for the analyses. Shapiro-Wilk and Levene tests were performed on the data to confirm the homogeneity of variances and the normality of the data distribution, respectively. One-way ANOVA was employed to estimate the data, and then Duncan's multiple-range test was performed. When $P< 0.05$, significant differences were taken into account. The mean values and their standard errors were displayed for the results.

RESULTS

1. Water quality indices

The water quality's physio-chemical characteristics that were observed during the experiment are listed in Table (2). There were no statistical differences ($P> 0.05$) in the means of temperature ranging from 30.61 to 30.85ºC and pH value, which ranged from 7.31 to 7.44. The DO was significantly higher with increased duckweed inclusion ($P< 0.05$). Increased inclusion of duckweed in the diet resulted in significantly reduced levels of total ammonia nitrogen ($NH_3 + NH_4^+$), nitrite (NO_2), nitrate (NO_3), and phosphate.

2. Analysis of growth indices

When the duckweed inclusion was added to the diet, the Nile tilapia fingerlings outperformed the control group (DWM0) in terms of final weight (FW), weight gain (WG), and specific growth rate (SGR%) (Table 3). These traits were significantly different ($P< 0.05$). The DWM20 group had the greatest SGR (3.57± 0.05) and WG (63.2± 0.98) values. However, fish fed the DWM0 and DWM30 diets showed no discernible variations in these parameters ($P> 0.05$). When compared to other treatments, the Nile tilapia-fed DWM20 feed had the lowest FCR value *(P*< 0.05), and the DWM0 treatment had the highest FCR value *(P*< 0.05). In contrast, no differences were observed in the viscerosomatic index or survival rates between the duckweed feed groups and the control group *(P*> 0.05). There is a statistical difference in the condition factor, which ranged from 1.53± 0.01 (DWM0) to 1.69± 0.03 (DWM20). Duckweed inclusion in the tilapia diets significantly increased the hepaticsomatic index among the tested groups, with the highest treatment via the inclusion of the duckweed at 20%.

Table 2. Effect of duckweed feeding levels on the water indices of *O. niloticus* fingerlings raised in an aquaponic system

	Duckweed inclusion			
Parameter	**DWM0**	**DWM10**	**DWM20**	**DWM30**
T°C	30.63±0.04	30.67±0.05	30.61±0.07	30.85±0.11
pH	7.31±0.09	7.36±0.15	7.44±0.10	7.39±0.08
DO – $mg.l^{-1}$	$5.35±0.21^{b}$	$6.35±0.31^{ab}$	$6.52±0.24^{a}$	$6.61±0.26^{a}$
conductivity - $\mu S.cm^{-1}$	543.4 ± 5.78	532.60 ± 31.71	534.73 ± 28.77	525.23 ± 29.27
NH_4^+ – $mg.l^{-1}$	$0.205±0.05^{a}$	$0.139 ± 0.08^{b}$	$0.115 ± 0.06^{c}$	$0.081 ± 0.09^{d}$
NO_2 - $mg.l^{-1}$	$0.31 ± 0.004^{a}$	$0.24 ± 0.006^{b}$	$0.12 ± 0.005^{c}$	$0.09 ± 0.004^{d}$
NO_3 - $mg.l^{-1}$	$4.29±0.32^{a}$	$3.35±0.33^{b}$	$2.79±0.39^{c}$	$1.62±0.42^{d}$
PO_4 - $mg.l^{-1}$	$0.79 ± 0.05^{a}$	$0.62 ± 0.03^{b}$	$0.56 ± 0.03^{c}$	$0.53 ± 0.04^{c}$

Values sharing the same superscript in each row do not differ at *P*< 0.05.

The duckweed inclusion did not influence the Nile tilapia fingerling's moister content whole-body (Table 4). The whole-body crude lipid of fingerlings decreased (*P*< 0.05) with increasing the duckweed inclusion in feed. The whole-body protein content was at its peak (*P*< 0.05) in the duckweed 20% fish feed group compared to the other groups in the experiment. The whole-body ash of fish increased *(P*< 0.05) with increased duckweed inclusion in feed.

Table 3. Effect of duckweed feeding levels on the growth indices of *O. niloticus* cultured in an aquaponic system

	Duckweed inclusion			
Parameter	**DWM0**	**DWM10**	**DWM20**	**DWM30**
IW (g)	15.60±0.17	14.89±0.21	15.41±0.18	15.11±0.20
FW (g)	$63.20±2.58^{c}$	$72.35±3.28^{b}$	$78.61±2.11^{a}$	$65.23±2.81^{c}$
WG (g)	$47.6±2.31^{c}$	$57.46±2.73^{b}$	$63.2±0.98^{a}$	$50.12±1.45^{c}$
SGR (%)	$3.19±0.06^{c}$	$3.23±0.05^{b}$	$3.57±0.05^{a}$	$3.23±0.07^{c}$
FCR	$1.41±0.03^{a}$	$1.32±0.02^{b}$	$1.25±0.02^{c}$	$1.38±0.01^{a}$
SR (%)	97.15±1.65	96.45±1.39	98.10±1.30	96.58±1.11
Condition factor (%)	$1.53±0.01^{b}$	$1.61±0.03^{ab}$	$1.69±0.03^{a}$	$1.63±0.02^{ab}$
Viscerosomatic index (%)	13.45±0.34	14.11±0.58	13.76±0.44	13.59±0.51
Hepatosomatic index (%)	$0.38±0.07^{c}$	$0.61±0.13^{b}$	$1.07±1.03^{a}$	$0.97±1.08^{a}$

Values that share the same superscript in each row are not different at *P*< 0.05.

Table 4. Effect of duckweed feeding levels on the whole-body composition (wet basis) of *O. niloticus* cultured in an aquaponic system

Parameter (%)	Duckweed inclusion			
	DWM0	**DWM10**	**DWM20**	**DWM30**
Moister	78.25±0.84	77.69±1.31	79.15±1.74	78.39±
Crude protein	16.42±0.24^{b}	16.81±0.16^{b}	17.36±0.19^{a}	16.37±0.28^{b}
Crude lipid	3.17±0.05^{a}	2.84±0.21^{b}	2.61±0.23^{b}	2.53±0.14^{b}
Ash	1.59±0.15^{b}	1.78±0.13^{b}	2.17±0.09^{a}	2.34±0.17^{a}

Values that share the same superscript in each row are not different at $P< 0.05$.

3. Duckweed and digestive enzyme activities

After 8 weeks of the feeding trial, duckweed inclusion led to higher ($P< 0.05$) digestive enzyme activities versus the control group for the Nile tilapia cultured in an aquaponic system (Table 5). The proteolytic, amylase, and lipase activities were higher for fish fed with 20% duckweed meal ($P< 0.05$) in gastric, enteric, and hepatopancreas compared with other treatments. When the addition rate of duckweed reached 30%, the activity of proteolytic and amylase declined in the gastric ($P< 0.05$). All digestive enzyme activities were at their lowest values for fish fed with DWM0 (control group) except lipase in gastric ($P< 0.05$). Proteolytic activity values ranged from 7.51 in the enteric with the control treatment to 23.26 in the gastric with the DWM20 treatment (Table 5). A significant improvement was recorded in amylase activity in the gastric and enteric with the start of duckweed inclusion.

Table 5. Effect of duckweed feeding levels on digestive activities of the Nile tilapia cultured in an aquaponic system

Digestive activities		Duckweed inclusion			
Enzyme ($U.mg^{-1}.prot$)	Organ	DWM0	DWM10	DWM20	DWM30
Proteolytic	Gastric	17.11±2.35^{bc}	14.21±1.05^{c}	23.26±1.91^{a}	13.54±1.27^{c}
	Enteric	7.51±1.33^{b}	15.11±1.67^{a}	15.64±1.43^{a}	16.98±1.62^{a}
	Hepatopancreas	14.26±1.57^{b}	14.88±1.09^{b}	18.34±1.53^{a}	17.09±1.21^{a}
Amylase	Gastric	0.71±0.2^{b}	1.2±0.06^{a}	1.9±0.8^{a}	0.79±0.9^{b}
	Enteric	0.12±0.03^{b}	0.18±0.09^{a}	0.21±0.09^{a}	0.17±0.05^{a}
	Hepatopancreas	0.51±0.04	0.46±0.07	0.54±0.03	0.52±0.03
Lipase	Gastric	19.86±1.67^{a}	16.37±1.17^{b}	20.34±1.16^{a}	19.55±1.02^{a}
	Enteric	2.52±0.28^{b}	6.11±0.39^{a}	6.52±0.34^{a}	6.34±0.23^{a}
	Hepatopancreas	7.35±1.81^{b}	7.97±1.64^{b}	12.17±1.41^{a}	8.24±1.40^{b}

Values sharing the same superscript in each row do not differ ($P< 0.05$).

4. Economic efficiency

The effect of duckweed feeding levels on the economic factors of *O. niloticus* fingerlings cultured in an aquaponic system based on feed cost, gross cost, net profit, and economic efficiency are shown in Table (6). The highest feed cost for 1kg weight gain was 1.75$ for the DWM0 group, followed by 1.46$ for DWM10, 1.26$ for DWM30, and 1.25$ for DWM20, respectively (P< 0.05). The gross cost of fish feed for both the DWM20 treatment and the DWM30 treatment was lower statistically than the other groups during the trial. The net profit of fingerlings fed on the DWM20 group and the DWM30 group is higher (P< 0.05) than the remaining groups. The highest value of economic efficiency was in the treatment of feeding 20% duckweed (71.42%), followed by feeding 30% duckweed (70.45%), without a significant difference, while the economic efficiency was the lowest (P< 0.05) in the control group (22.44%).

Table 6. Effect of duckweed feeding levels on the economic efficiency of the Nile tilapia cultured in an aquaponic system

Parameter	Duckweed inclusion			
	DWM0	**DWM10**	**DWM20**	**DWM30**
Feed cost for 1kg weight gain, US $	1.75±0.05[a]	1.46±0.07[b]	1.25±0.04[c]	1.26±0.03[c]
Gross cost for 1kg weight gain, US $	2.45±0.07[a]	2.05±0.03[b]	1.75±0.05[c]	1.76±0.07[c]
Net profit for 1kg weight gain, US $	0.55±0.08[c]	0.95±0.06[b]	1.25±0.05[a]	1.24±0.04[a]
Economic efficiency for 1kg weight gain, %	22.44± 1.02[c]	46.34±1.97[b]	71.42±2.13[a]	70.45±1.13[a]

Values with the same superscript in each row are not different at P< 0.05.

DISCUSSION

Aquaculture of freshwater fish in areas where water is scarce requires the use of unconventional systems such as aquaponics and biofloc **(Craig, 2019)**. It is noteworthy that the availability and quality of water and optimal, sustainable and cheap nutritional compositions are the main factors affecting the sustainability of aquaculture **(Harikrishnan *et al*., 2021; Rashidian *et al.*, 2021; Sinha *et al*., 2021)**. The current study focused on the water quality, growth indexes, and digestive activities while examining the viability of employing aquaponically produced duckweed, *L. minor*, as a substitute feed in the Nile tilapia diets raised in aquaponics systems.

1. Water quality indices

The experiment's water quality remained within the acceptable parameters for raising the Nile tilapia *(P*< 0.05), indicating that the culture system's water quality control was sufficient. The pH range of 7.31 to 7.44, which is typically regarded as an acceptable range for the aquaponic system, was not significantly affected by the variation in duckweed levels in the Nile tilapia diet during the experiment. Fish need a pH range of 6.5 to 9.0, while plants are guaranteed to have access to nutrients in the 5.8 to 6.5 pH range **(Tyson *et al*., 2008)**. Numerous researchers have concluded that the ideal electrical conductivity (EC) range for aquaponic solutions is between 300 and 1100s/ cm **(Lennard & Goddek, 2019)**. There are significant differences between the experimental treatments of duckweed inclusions and the level of DO *(P*< 0.05). The tilapia tank had dissolved oxygen (DO) levels that varied from 5.35 to 6.61mg/ L. There is a positive relationship between the level of duckweed and the level of DO. Tilapia tolerates dissolved oxygen levels as low as 2- 3mg/ L **(Somerville *et al*., 2014)**. In the aquaponics system of the current study, it was shown that, the more duckweed was included in the feed for Nile tilapia, the lower the concentration of total nitrogenous compounds in the fish tanks (*P*< 0.05). The addition of duckweed in the DWM30 group led to a significant decrease in ammonium concentration by 60.5% compared to the control group. This is consistent with the results of **Velichkova and Sirakov (2018)** when using *L. minor* as a biofilter; it reduces the ammonia concentration in the RAS by 19.6% compared to the control group. **Zhang *et al*. (2014)** found that duckweed prefers ammonia, with a maximum ammonium absorption rate of 0.082mg/ g fresh weight.h^{-1}. Similarly, the DWM30 group significantly lowered phosphorus levels compared to the control group (*P*< 0.05) **(Velichkova & Sirakov 2013)**. This finding is also consistent with that of **Boyd and Queiroz (1997)**, who found that aquatic plants in biofilter systems could dispose 97% of the phosphorus element in fish ponds.

2. Growth indices of duckweed-fed Nile tilapia

During an experimental study to test the nutritional efficiency of duckweed grown in an aquaponic system and its inclusion in the feed of the Nile tilapia fish that were raised using an aquaponic system for 56 days, growth measurements (FW, WG, SGR, FCR, CF, and hepatic index) were significantly and positively affected by an increase in the level of duckweed. The Nile tilapia growth indexes declined significantly when the inclusion rate of duckweed reached 30% as opposed to 20% inclusion, presumably since the duckweed contains anti-nutrition components such as phytic acid and tannin **(Bairagi *et al*., 2002)**. In comparison to most other plants, duckweed leaves have a lower amount of fiber and high protein **(Chaturvedi *et al*., 2003)**, and the duckweed's protein contains a well-balanced amino acid composition **(Gwaze & Mwale, 2015)**. Finally, the fingerlings' growth indices were significantly enhanced by adding the duckweed to the Nile tilapia diet. The strongest feeding impact was demonstrated by the 20% inclusion, which also increased the FW, WG, SGR, FCR, and CF (Table 3).

3. Digestive enzyme activities

Based on these findings, adding duckweed to the diets of the Nile tilapia stimulates the release of digestive enzymes, particularly at inclusion rates of 20%. One crucial indicator of a feed formula's nutritional worth is apparent digestibility. Following fish consumption, feed constituents function as digestive enzyme substrates, influencing the production and functionality of digestive enzymes **(Pervin *et al*., 2020; Santos *et al*., 2020)**. The stomach has the highest level of digestive enzyme activity among the three organs that house tilapia's digestive enzymes: the stomach, intestine, and hepatopancreas **(Einarsson *et al*., 1996)**. In their study, **Zheng *et al*. (2017)** postulated that, amylase, protease, and lipase are crucial digestive enzymes found in tilapia.

After being ingested by fish, flavonoids are mostly retained in the stomach, intestine, and hepatopancreas. Antioxidants called flavonoids can enhance the biological functioning and shield organs from biotic and abiotic stressors **(Liu 2012)**. For instance, feeding tilapia diets high in anthocyanins increased the fish's survival rate when exposed to ammonia stress, as well as its innate immunological characteristics and gene expression responses **(Yilmaz 2019)**. Flavonoids are abundant in duckweed **(Appenroth *et al*., 2018)**, perhaps stands as one of the explanations for why the addition of duckweed to the fish's diets increased the activity of digestive enzymes and improved the growth indices of the *O. niloticus* fingerlings in this investigation.

4. Profitability of feeding on duckweed

In light of the high costs of feeding in aquaculture, as well as the cost of operating an aquaponic system, this study aimed to answer one of the main concerns: Did the Nile tilapia achieve the optimal growth and profitability with the inclusion of duckweed in its diet? The price of Nile tilapia feed varies significantly ($P < 0.05$), according to the data, and it gets less expensive the more the level of duckweed is in the feed (Table 6). These results indicate that the inclusion of duckweed at a level of 20% in the Nile tilapia feed was the most profitable in terms of economic point of view. These results are consistent with **Eid *et al*. (2017)**, who demonstrated that substituting duckweed for soybean meal is a workable and practical way to reduce production costs and increase the profitability of *O. niloticus* culture.

CONCLUSION

Duckweed , rich in protein, can be cultivated in aquaponic systems and be included in the feed of the Nile tilapia. When the Nile tilapia fingerlings are fed up to 30% duckweed *L. minor*, the amount of ammonium, nitrite, nitrate, and phosphate is reduced by 60.48, 70.96, 62.23, and 32.91%, respectively, in the water tank, in comparison to the control group. When fed inclusion of duckweed at 20%, *O. niloticus* exhibited improved fish WG, SGR, FCR, and CF growth in comparison to the control group. Duckweed stimulated the activity of the digestive system's lipase, protease, and amylase enzymes. These results show that the *L. minor* can be used as a substitute for feedstuff for the Nile tilapia up to 20%. Therefore, the duckweed presents a cheap and easily accessible ingredient for feeding the Nile tilapia. Furthermore, treating wastewater in recirculating aquaculture systems in this manner, can improve the sustainability of those systems.

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