Egypt. J. Vet. Sci., pp. 1-11

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

The Impact of Partially Whole Diet Replacement by *Moringa oleifera* Leaves in Terms of The performance, Feed Utilization, and Various Blood Indices in Nile Tilapia (*Oreochromis niloticus*)

Abd El-Rhman A. E. Abo-Yehia[1]*, Abd El-Mageed E. Nasr[1], Mostafa I. Abdelglil[2], Mohammed A.E. Naiel[1], Adel M. A. Akr[3] and Sabry A. M. Shehata[1,2]

[1]*Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt.*

[2]*Al-Farahidi University, Baghdad, Iraq.*

[3]*Central Laboratory of Aquaculture Research, Al-Abbasa, Abo-Hamad, Sharkia, Egypt.*

Abstract

THIS TRIAL was conducted to examine the influence of replacing basal diet of Nile tilapia fingerlings at different levels by *Moringa oleifera* leaves (MOL). Five diets were formulated which contain MOL levels 0, 5, 10, 15 and 20% instead of fish basal diet. Using MOL instead of basal diet at 5% level indicated remarkable (p≤0.05) decline fish body weight, weight gain, specific growth rate and relative growth rate. The mortality rate was 10% for fish-fed diets 0, 10, 15 and 20% MOL in compared to 6.67 for fish-fed 5% MOL. The feed intake and feed conversion ratio were significantly (*P*≤0.05) decrease in fish- group fed diets 3, 4 and 5 in compared to fish-fed diets 1 and 2. The blood parameters results showed that the total protein and globulin increased (*P*≤0.05) in all levels of MOL on the other hand, the creatinine and uric acid were significantly decreased. The protease, lipase and amylase activities were significantly reduced (*P*≤0.05) by 10, 15 and 20% MOL. Meanwhile, the blood liver function enzymes (AST and ALT) activities decreased (*P*≤0.05) at 5% MOL level in comparison with control and other groups of MOL. The antioxidant parameters including glutathione, total antioxidant capacity and superoxide dismutase increased (*P*≤0.05) by all levels of MOL. In conclusion, The results obtained indicated that using MOL instead of the basal diet at 5% had no significant effect on the growth performance and health status of Nile tilapia fish.

Keywords: *Moringa oleifera*, fish, weight gain, feed utilization, blood parameters.

Introduction

Egypt is recognized as a leading country in aquaculture, producing an average of over 1.1 million tons annually [1]. Approximately 65% of this production is derived from *Oreochromis niloticus*, which is the main famous farmed fish species [2]. It is well known that Nile tilapia is the most profitable farmed fish species due to its rapid growth and higher resistance to various ecological conditions [3]. Therefore, Nile tilapia is regarded as a well-known and widely distributed food fish in Egypt and around the globe [4,5]. Fish feeds account for approximately 75 to 85% of daily costs in fish production. The use of antibiotics as feed promoters and the dependence on animal protein as the primary protein source raise health concerns and are becoming increasingly costly. To address these issues, alternative plant resources are essential as an alternative to antibiotics and as replacements for animal protein. This approach aims to achieve growth-promoting functions while reducing reliance on fishmeal, thereby helping to mitigate potential health and environmental risks [6,7].

In semi-arid and arid areas, *Moringa oleifera* (MOL) has been observed to grow naturally in North Sinai, Aswan, and El-Sharkyia [8,9]. Recently, extensive reports have been established to determine the efficiency of substituting plant protein sources for dietary fish meal (FM) in aquafeed. MOL possess medicinal properties, such as anti-inflammatory, antioxidant, hepatic protective and anti-bacterial effects [10-13]. *Moringa oleifera* leaves (MOL) have notable contents of minerals, vitamins, and amino acids [14,15]. The in-vivo and in-vitro studies suggested that the MOL could be considered a good protein resource for FM replacement. For instance, it has been previously indicated that enriched diets with MOL in *O. niloticus*, improved performance and

*Corresponding authors: Abd El-Rhman A. E. Abo-Yehia, Email: abdelrhmanaboyhia@gmail.com, Tel.: 01029279260
(Received 19 December 2024, accepted 02 March 2025)
DOI: 10.21608/EJVS.2025.345544.2569

general health status [16,17], and promoted consumed feed utilization measurements, whilst, boosting the digestibility coefficients of diet nutrients of catfish [18]. MOL could substitute 25-30% of the fish meal diet content of *O. niloticus* without inducing any negative consequences on the performance and consumed feed utilization measurements [14,19]. This study was carried out to study the effect of replacing the basal diet of Nile tilapia fish with MOL at levels 0, 5, 10, 15, and 20% instead of the basal diet. The effect of diets on weight gain, feed utilization, and blood parameters of Nile tilapia fish were studied.

Material and Methods

This trial was done at the wet lab belonging to the Animal Production Department, Faculty of Agriculture, Zagazig University, Egypt, during the summer of 2022. The experimental work included 5 diets. MOL was used instead of the basal diet at levels 0, 5, 10, 15, and 20%, respectively (diets 1-5). The experiment lasted 10 weeks.

Fish and aquaria

One hundred and fifty Nile tilapia fingerlings, each averaging 10.84 ± 0.12 g, were evenly distributed into five groups, with each group consisting of 30 fish (n=3). Fish were obtained from a hatchery located at Tal El-Kabir, Ismailia Governorate, Egypt. Each fish groups were housed in glass aquariums (70 × 50 × 30 cm). Fingerlings were preserved for 15 days to acclimate prior to the feeding trial period. Aquariums were filled with dechlorinated tap water from a storage tank, and air was supplied by aquarium air pumps. To manage fish waste, one-third of the water volume was drained daily using suction. The glass aquariums were cleaned every two weeks to prevent the accumulation of uneaten feed and excessive algal growth. Daily water samples were collected from the rearing water to assess water quality measurements. The average dissolved oxygen concentration ranged from 5.5 to 6.5 mg/L, with a pH value of 7.5. Water temperature, measured with a thermometer, averaged 26 ± 2 °C throughout the feeding trial period.

Experimental diets

The MOL was purchased from a local market in Zagazig, Egypt. The ingredients and formulation of the basal diet is shown in Table (1). The chemical composition of basal diet and MOL are shown in Table (2). The gross energy of basal diet and MOL (Table 2) was calculated according to NRC [20] The pelleted basal diet and MOL were milled and mixed to formulate the diets, then pelleted again. The MOL levels in experimental diets (diet 1-5) were 0, 5, 10, 15 and 20% instead of control diet as presented in Table (3). The calculated chemical analysis of the formulated diets are shown in Table (4). The proximate composition of MOL, basal diet and experimental diets were determined according to AOAC [21].

Feeding regime

Fingerlings were afforded-feed daily at two different times (09:00 and 15:00 hrs) at a rate of 3% of BW. Fish were weighed every two weeks. The diets were offered 6 days/week. the relative growth rate (RGR) and specific growth rate (SGR) were calculated. RGR= $[(BWn - BW0) / BW0] \times 100$, SGR = $[(\ln BWn - \ln BW0) / d] \times 100$. Where, BWn is the final body weight (g), BW0 is the initial body weight (g), and d number of days.

Feed utilization

The diet was provided at a rate of 3% of fish BW. The fish was weighted biweekly. The feed intake (FI) was corrected biweekly according to fish weight. The feed conversion ratio (FCR) was calculated as [feed intake (g) / weight gain (g)].

Mortality rate

Mortality rate is calculated per each fish group as follows: Mortality rate = [number of dead fish / initial fish number] × 100.

Chemical Composition of Tested Diets

The chemical composition of the basal diet and MOL were carried out according to AOAC (2006) [21].

Blood parameters

By the end of the feeding trial period, blood specimens were obtained from the tail vein of non-anesthetized fish (15 fish per group; 5 fish per replicate) using a sterile heparinized syringe. Plasma was obtained by centrifugation at 1006 g for 10 minutes. Plasma was collected in Eppendorf tubes and stored at 20°C until analysis. The blood specimens were analysed in a private external laboratory. Plasma total protein and albumin were analyzed, and plasma globulin value was calculated by subtracting the albumin value from the value of total protein. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activate, creatinine, and uric acid were determined. The immunity parameters [lysozyme, immunoglobulin (IGM and IGA), complement activity, ACH5O], antioxidants parameters [malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), total antioxidant capacity (TAC) and content of digestive enzymes (amylase, lipase, protease) were analyzed.

Statistical Procedure

The calculated and obtained data from the trial were examined statistically by employing the SAS program using a general linear model [22]. The data in the Excel sheets were examined by one-way ANOVA. The variation between experimental groups means were determined using Duncan's Multiple Range Test [23].

Results

Chemical composition of diets

The chemical composition of *Moringa oleifera* leaves (MOL) showed a decrease in organic matter (OM), crude protein (CP), and nitrogen free extract (NFE) in comparison with the control diet (Table 2). On the other hand, the crude fibre (CF), ether extracts (EE), and ash increased in MOL. The Table (3 and 4) content illustrates the experimental diets and their chemical composition, respectively. MOL showed a decrease in the values of OM, CP and NFE compared to the control diet. The decrease in these parameters was linearly by levels of MOL. On the other hand, the CF, EE and ash of the diets increased by MOL, while the coliform counts were 3.21 ± 0.16 $\log^{10}$ CFU/g, with counts in the range 2.71–3.48 $\log^{10}$ CFU/g in the thigh and 2.02 ± 0.11 $\log^{10}$ CFU/g in the breast, with range 1.60–2.31 $\log^{10}$ CFU/g (Table 2). The *Staphylococcus aureus* counts were 2.11 ± 0.09 $\log^{10}$ CFU/g, with counts in the range 1.71–2.23 $\log^{10}$ CFU/g in the thigh and 1.85 ±0.08 $\log^{10}$ CFU/g with a range of 1.50–2.02 $\log^{10}$ CFU/g in the breast (Table 3).

Effect experimental of diets on Nile tilapia fish performance

The results of Table 5 revealed a notable ($p \leq 0.05$) decrease in WG, FBW, SGR, and RGR of fish fed diets 3, 4, and 5. On the other hand, no significant effect was found between fish fed control diet (diet 1) and 5% MOL (diet 2). The mortality rate (Table 5) was 10% in fish fed diets 1, 3, 4, and 5 compared to 6.67% in (diet 2). Significant ($p \leq 0.05$) decrease in feed intake (Table 5) and the worst feed conversion was obtained in fish fed diets 3, 4, and 5 (10, 15, and 20% MOL). While no significant results were found between fish fed diet 1(control) and diet 2 (5% MOL).

Effect of diets on blood parameters

The blood parameters results (Table 6) exhibited significant improvement in total protein and globulin ($p \leq 0.05$) in all fish groups afforded feed containing any level of MOL in comparison to control. Conversely, all estimated liver function enzymes (AST and ALT) remarkably decreased ($p \leq 0.05$) in fish fed diet 2 (5% MOL) in comparison with the control. Also, the creatinine and uric acid values were decreased ($p \leq 0.05$) significantly by all levels of MOL compared to the control.

Effect of diets on digestive enzymes

The lipase, amylase, and protease activities decreased ($P \leq 0.05$) significantly in the fish groups that received 3, 4, and 5 diets (10, 15, and 20% MOL) (Table 7). The decline in the gastrointestinal enzyme levels was linear with rising MOL levels.

Effect of diets on antioxidants parameters

The redox status measurements including glutathione (GSH), total antioxidant capacity (TAC), and superoxide dismutase (SOD) increased ($p \leq 0.05$) significantly by all levels of MOL. On the other hand, the malondialdehyde (MDA) decreased ($p \leq 0.05$) linearly in fish fed diets 3, 4, and 5 (Table 8). No significant difference was found between fish fed diets 1 and 2 in MDA and TAC.

Effect of diets on immunity

The results of all immunity parameters (lysozyme, immunoglobulin A and M, complement activity, ACH50) (Table 9) increased ($p < 0.05$) significantly in fish fed all levels of MOL. All parameters linearly increased ($p < 0.05$) significantly with increased MOL level.

Discussion

The chemical composition of MOL agrees with Ayoola *et al.* [24] who reported MOL contains 25% CP, 13.06% CF, and 6.7% EE. Also, Richter *et al*. [8] found that MOL contains 25% CP and 8.4% ash. In addition, Wu *et al*. [25] found that MOL contains 23.0-30.3% CP and 12.0% ash. Moreover, Idowu *et al*. [26] reported MOL contains 26.94% CP, 4.38% CF, 3.36% ash and 51.08% NFE. Moyo *et al*. [27] found that MOL contains 30.3% CP. The difference in the chemical composition of MOL may be due to plant age, agriculture location, and soil fertility.

The weight gain, SGR, and RGR decreased ($p \leq 0.05$) in fish fed diets 3, 4, and 5 (10, 15, and 20% MOL). On the other hand, these parameters did not decrease in fish fed diets 2 (5% MOL). The results of our study agree with Bbole *et al*. [28] who showed that SGR and mean weight gain did not differ significantly among fish fed diets containing 0.0, 5, 10, and 15% MOL. Also, Billah *et al*. [29] found that the addition of 10% MOL powder to a commercial diet improves the growth, survival rate, immune response, and microbial challenge of *Nile tilapia* fish. El-Kassas *et al*. [30] reported that using 5% MOL instead of yellow corn, fish meal (FM), middling and soybean meal increased ($p \leq 0.05$) growth parameters of *Nile tilapia* fish. Zhang *et al*. [31] found that replacing 20, 40, and 60% of FM by fermented MOL induced remarkable ($p \leq 0.05$) improvements in FBW, and SGR of fingerlings received diets containing 20 and 40% replacement levels. The differences in results may be due to species, age, initial weight of fish, feed-stuff replacement, replacement level, and experiment length. Generally, using higher concentrations of MOL leads to a decline in fish performance. This effect may be due to containing MOL from tannins, phytates, and oxalates [32], hemolytic saponins [33].

The mortality rate of fish was reduced in fish-fed diet 2 (5% MOL) which was 6.67% compared to 10% in fish-fed diet 1 (control) and fish fed diets 3, 4, and 5 (10, 15, and 20% MOL). The results of the mortality rate agree with Billah *et al*. [29] who found the diet containing 10% MOL resulted in an increased survival rate. Also, Zhang *et al.* [31] found that the mortality rate decreased in fish when 40 and 60% of fish meal was replaced by fermented MOL in compared with the control and fish fed diet supplemented with 20%MOL.

Feed intake (FI) and feed conversion ratio (FCR) did not differ between fish fed control diet and fish fed 5% MOL (diet 2). The diets containing high levels of MOL (10, 15, and 20%) led to a decrease in FI and FCR. The results of our study agree with Zhang *et al.* [30] who found that replacing 20, 40, and 60% of fish meal with fermented MOL led to significant ($p \leq 0.05$) improvements in FCR of fish at a replacement of 20 and 40%. Our results agree with El-Kassas *et al*. [30] who reported that dietary supplementation with 5% MOL powder appreciably enhanced the FCR of Nile tilapia fish. The bad effect of MOL on FCR may be due to its negative effect on fish weight gain and reduced activities of digestive enzymes.

The globulin and total protein values increased ($p \leq 0.05$) under the effects of all levels of MOL. Also, AST and ALT activities decreased ($p \leq 0.05$) by 5% MOL. The creatinine and uric acid concentrations decreased ($p \leq 0.05$) by all levels of MOL. The blood analysis results align with those of Monir et al. [34], who demonstrated that *O. niloticus* fed a diet supplemented with MOL showed higher levels of globulin and total protein, along with lower levels of urea, ALT, creatinine, AST, triglycerides, and cholesterol. In addition, diets containing MOL meal up to 1.5% for *O. niloticus* led to a remarkable decline in both serum ALT and AST levels compared to the control group [34]. On the other hand, Tekle and Sahu [36] discovered that the levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), acid phosphatase (ACP), and lactate dehydrogenase (LDH) were elevated in *O. niloticus* that were afforded feeds enriched with 0.25% MO flower ethanolic extract. The observed variances in hepatic renal function enzymes and hematobiochemical parameters might be due to numerous factors, including the fish species, physiological status, maturation stage, and size of the fish, as well as the type and duration of MOL administration [37].

The negative effect of MOL at high concentrations may be due to its containing anti-national factors including high total content of phenolic, nonhemolytic saponin, and phytic acid. As well as the high content of MOL of NDF and ADF [8,38,39].

The redox status parameters including GSH, SOD, and TAC increased ($p \leq 0.05$) by all levels of MOL. The results obtained were inconsistent with the findings of Abd El-Gawad *et al.* [40], which indicated that dietary MOL improves the redox status of *O. niloticus* by increasing levels of SOD, CAT, and GPX, while significantly reducing MDA levels in comparison to the fish group received un-supplemented diets. Additionally, Monir *et al.* [34] demonstrated higher hepatic content from the SOD and CAT in *O. niloticus* fish afforded feeds containing MOL extract for 30 days. Meanwhile, dietary administration of 0.5% MOL extract for 60 days notably elevated hemolymph CAT, inducible nitric oxide synthase and SOD activities in freshwater prawns [41]. Furthermore, Zhang *et al.* [42] demonstrated that fermented MOL-based diets significantly increased serum content, along with elevated SOD and GPX activities, while substantially reducing serum MDA and protein carbonyl levels in juvenile gibel carp. The enhancement of the redox status in fish resulting from MOL-supplemented diets might be due to the high content of various phenolic compounds in MOL, such as flavonoids, carotenoids, ascorbic acid, and tocopherols [12, 43, 44], as well as the protective potential of glucosinolates in improving scavenging free radical capacity [45].

The positive impact of MOL on oxidative status may be attributed to its phytochemical content, which includes flavonoids, phenolic acids, alkaloids, carotenoids, tannins, terpenoids and lectins. These phenol-based compounds demonstrate various beneficial features, including oxidative and hepatic protection, antimicrobial activity, and immune-stimulating functions [37]. Additionally, MOL is rich in ascorbic acid and carotenoids, as well as essential minerals such as calcium, potassium, and iron [46]. Furthermore, it exhibits antioxidant, antibacterial, immunomodulatory, and anti-carcinogenic properties [47,48].

The increase in immune parameters such as lysozyme, IgM, IgA, and ACH50 at all levels of MOL is consistent with the findings of Mbokane and Moyo [49], who indicated that diets supplemented with MOL significantly enhanced immunological parameters (lysozyme activity), resistance to infections, and the increased the survival rate of challenged *O. mossambicus* with pathogenic bacterium (*Aeromonas hydrophila*). Additionally, Mansour *et al*. [50] observed a notable enhancement in IgM levels, as well as in phosphatase, lysozyme, and peroxidase activities in skin mucus, resulting from MOL dietary administration. Meanwhile, Bisht *et al*. [51] demonstrated that diets enriched with 15% MOL remarkably stimulated skin mucosal immune activities, including total immunoglobulin, lysozyme, and myeloperoxidase contents in guppies. The improvement in immune activities in fish-afforded MOL-supplemented diets might be due to the presence of biologically active phytochemicals, including polyphenols, volatile oils, tocopherols, and ascorbic acid [52,53]. Furthermore, MOL contains other beneficial compounds, for instance, phenolic molecules (e.g., chlorogenic, ferulic, ellagic, caffeic, salicylic, coumaric, and protocatechuic acids), flavonoid compounds (e.g., rutin, quercetin, kaempferol, catechin, apigenin, epicatechin, isorhamnetin, and myricetin), saponins, carotenoids, alkaloids, tannins, sterols, terpenoids, anthraquinones, anthocyanins, coumarins, and proanthocyanins [54,55,44]. Additionally, the presence of isothiocyanates and glycoside cyanides positively contributes to promoting immune function pathways [56].

Conclusion

The results obtained indicated that using MOL instead of the basal diet at 5% had no significant effect on the growth performance and health status of Nile tilapia fish.

Acknowledgments

Not applicable.

Funding statement

This research received no external funding

Declaration of Conflict of Interest

The authors declare no competing interests.

Ethical of approval

The animal study was reviewed and approved by the Zagazig University animal ethics committee.

TABLE 1. Ingredients and formulation of the basal diet.

Ingredients	%
Fish meal	20
Soybean meal	37.5
Yellow corn	20.5
Wheat flour	15
Fish oil	3
Vegetable oil	2
Premix [a]	2
Total	100

[a]; Minerals and vitamins mixture consisted of (mg/kg dry diet): 30000 Mg Copper, 250 Iodine, 300 Selenium, 50000 Manganese, 400 Cobalt, 60000 Zinc and 3000 Gm\ 30Kg CaCo3. retinol (VA) 10,000 international units (IU); cholecalciferol (VD) 1,500 IU; tocopherol (VE) 2500 IU; menadione (VK), 40; thiamin (VB1), 1; riboflavin (VB2), 9; pyridoxine (VB6),3; cyanocobalamin (VB12), 0.1 folic acid, 1.5; anti ascorbic acid (VC),60.

TABLE 2. Chemical composition of basal diet (control) and *Moringa oleifera* leaves.

Items (%)	Basal diet	*Moringa oleifera* leaves
Moisture	10.71	12.03
Dry matter	89.29	87.97
Organic matter	82.34	77.22
Crude protein	31.25	24.38
Crude fibre	4.69	14.78
Ether extract	3.00	7.23
Nitrogen free extract	43.40	30.83
Ash	6.95	10.75
GE (kcal/kg diet) *	398.90	332.47

*Gross energy calculation based on values of CP, EE and NFE, 5.64, 9.44 and 4.11 kcal/g respectively. (According to NRC 1993) [20].

TABLE 3. Design of experimental diets.

Items	Experimental diets				
	1	2	3	4	5
Basal diet (%)	100	95	90	85	80
Moringa oleivera leaves (MOL) (%)	0	5	10	15	20
Total	100	100	100	100	100

TABLE 4. Calculated chemical composition of diets.

Items (%)	*Moringa oleifera* leaves (MOL)				
	0%	5%	10%	15%	20%
Moisture	10.71	10.78	10.84	10.91	10.97
Dry matter (DM)	89.29	89.22	89.16	89.09	89.03
Organic matter (OM)	82.34	82.08	81.83	81.57	81.32
Crude protein (CP)	31.25	30.91	30.56	30.22	29.88
Crude fiber (CF)	4.69	5.19	5.70	6.20	6.71
Ether extract (EE)	3.00	3.21	3.42	3.63	3.85
Nitrogen free extract (NFE)	43.40	42.77	42.14	41.51	40.89
Ash	6.95	7.14	7.33	7.52	7.71

TABLE 5. Effect of diets on the performance of Nile tilapia fish

Items	*Moringa oleifera* leaves (MOL)					*P* value
	0%	5%	10%	15%	20%	
IW (g)	10.76 ± 0.16	10.87 ± 0.05	10.91 ± 0.01	10.97 ± 0.05	10.68 ± 0.08	<0.13
FW (g)	31.92 ± 0.7^{a}	29.29 ± 0.72^{a}	25.85 ± 0.58^{b}	25.3 ± 0.09^{c}	25.07 ± 2.17^{c}	<0.001
WG (g)	21.16 ± 0.86^{a}	18.42 ± 0.67^{a}	14.94 ± 0.57^{b}	14.33 ± 0.04^{c}	14.39 ± 2.25^{c}	<0.001
SGR (g d-1)	1.55 ± 0.06^{a}	1.41 ± 0.03^{a}	1.23 ± 0.03^{b}	1.20 ± 0.0^{c}	1.21 ± 0.14^{c}	<0.001
RGR (%)	196.65 ± 10.92^{a}	169.46 ± 5.39^{a}	136.94 ± 5.10^{b}	132.81 ± 0.11^{c}	134.74 ± 22.08^{c}	<0.001
TFI (g/fish)	31.06 ± 0.56^{a}	30.53 ± 0.51^{a}	28.26 ± 0.87^{b}	27.47 ± 0.54^{b}	27.43 ± 0.08^{b}	<0.001
FCR (g/g)	1.47 ± 0.09^{c}	1.66 ± 0.03^{bc}	1.89 ± 0.02^{ab}	1.92 ± 0.04^{a}	1.91 ± 0.3^{a}	<0.001
MR (%)	10	6.67	10	10	10	

$^{a, b, and c}$ means in the same row with different superscript differ significantly (P≤ 0.05). IW = initial weight; FW= final weight; TFI= total feed intake; WG= weight gain; FCR= feed conversion ratio; SGR= specific growth rate; RGR= relative growth rate; MR= mortality rate.

TABLE 6. Effect of diets on blood parameters of Nile tilapia fish.

Items	*Moringa oleifera* leaves (MOL)					*p* value
	0%	5%	10%	15%	20%	
Total Protein (g/dl)	3.37 ± 0.21^{c}	4.07 ± 0.06^{b}	4.47 ± 0.31^{b}	4.37 ± 0.15^{b}	5.73 ± 0.32^{a}	<0.001
Albumin (g/dl)	1.47 ± 0.06	1.80 ± 0.26	1.77 ± 0.40	1.77 ± 0.40	1.73 ± 0.35	0.716
Globin (g/dl)	1.90 ± 0.20^{d}	2.27 ± 0.33^{c}	2.70 ± 0.10^{b}	2.60 ± 0.36^{b}	4.00 ± 0.10^{a}	<0.001
A/G Ratio	0.77 ± 0.09	0.79 ± 0.25	0.66 ± 0.18	0.68 ± 0.25	0.43 ± 0.10	0.182
AST (mg/dl)	26.14 ± 0.46^{a}	19.14 ± 0.43^{c}	25.08 ± 0.44^{a}	23.29 ± 0.38^{ab}	24.37 ± 0.43^{ab}	<0.001
ALT (mg/dl)	18.80 ± 0.21^{a}	16.32 ± 0.33^{c}	18.14 ± 0.32^{b}	18.83 ± 0.67^{a}	19.09 ± 0.12^{a}	<0.001
Creatinine (mg/dl)	0.64 ± 0.03^{a}	0.39 ± 0.02^{b}	0.38 ± 0.03^{b}	0.26 ± 0.01^{c}	0.19 ± 0.03^{c}	<0.001
Uric acid (mg/dl)	1.31 ± 0.02^{a}	1.10 ± 0.06^{b}	0.99 ± 0.02^{b}	0.87 ± 0.02^{b}	0.62 ± 0.05^{c}	<0.001

a, b, c and d: means in the same row with different superscript differ significantly ($P \leq 0.05$). A/G ratio, albumin-to-globulin ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

TABLE 7. Effect of diets on digestive enzymes of Nile tilapia fish.

Items	*Moringa oleifera* leaves (MOL)					*p-value*
	0%	5%	10%	15%	20%	
Amylase (u/l)	247.37 ± 5.31^{a}	228.70 ± 1.28^{a}	96.97 ± 4.20^{b}	81.37 ± 1.98^{c}	49.73 ± 5.39^{d}	<0.001
Lipase (u/l)	44.30 ± 5.98^{a}	37.90 ± 0.70^{a}	23.47 ± 0.43^{b}	16.44 ± 1.12^{c}	17.59 ± 0.77^{c}	<0.001
Protease (ng/mg)	463.67 ± 12.50^{a}	463.00 ± 23.90^{a}	265.33 ± 9.07^{b}	190.33 ± 4.93^{c}	124.00 ± 3.61^{d}	<0.001

a, b, c and d: means in the same row with different superscript differ significantly ($P \leq 0.05$).

TABLE 8. Effect of diets on antioxidant parameters of Nile tilapia fish.

Items	*Moringa oleifera* leaves (MOL)					*p* value
	0%	5%	10%	15%	20%	
GSH (mmd/ml)	25.33 ± 2.08^{e}	57.33 ± 1.53^{d}	63.00 ± 1.00^{c}	77.67 ± 1.53^{b}	90.00 ± 1.00^{a}	<0.001
SOD (pg/ml)	11.54 ± 0.51^{e}	22.97 ± 0.07^{d}	55.27 ± 1.10^{c}	63.50 ± 0.50^{b}	81.83 ± 1.77^{a}	<0.001
MDA (pg/ml)	4.81 ± 1.10^{a}	3.78 ± 0.98^{a}	1.10 ± 0.09^{b}	0.91 ± 0.15^{b}	0.64 ± 0.07^{b}	<0.001
TAC (mm/l)	25.87 ± 1.86^{d}	27.20 ± 1.22^{d}	42.97 ± 0.15^{c}	58.63 ± 0.60^{b}	63.63 ± 0.57^{a}	<0.001

a, b, c, d and e: means in the same row with different superscript differ significantly ($P \leq 0.05$). GSH, glutathione; SOD, superoxide Dismutase; MDA, malondialdehyde; TAC, total Antioxidant Capacity.

TABLE 9. Effect of diets on immunity parameters of Nile tilapia fish.

Items	*Moringa oleifera* leaves (MOL)					*p-value*
	0%	5%	10%	15%	20%	
LYZ (ng/ml)	1.06 ± 0.02^{d}	2.66 ± 0.25^{c}	3.86 ± 0.17^{b}	5.00 ± 0.20^{a}	5.10 ± 0.13^{a}	<0.001
IGM (ng/ml)	289.33 ± 80.35^{d}	329.33 ± 15.37^{d}	425.67 ± 34.20^{c}	537.00 ± 23.43^{b}	727.67 ± 48.81^{a}	<0.001
IGA (ng/ml)	203.67 ± 6.43^{e}	225.33 ± 4.16^{d}	269.33 ± 4.16^{c}	358.33 ± 16.07^{b}	409.00 ± 12.12^{a}	<0.001
ACH5O (mg/dl)	75.33 ± 3.06^{c}	90.67 ± 6.43^{b}	138.67 ± 8.33^{a}	141.67 ± 8.50^{a}	139.33 ± 4.93^{a}	<0.001

a, b, c, d and e: means in the same row with different superscript differ significantly ($P \leq 0.05$). LYZ, Lysozyme; IGM, immunoglobulin M; IGA, immunoglobulin A; ACH50, complement activity.

References

1. FAO, The State of World Fisheries and Aquaculture Contributing to Food Security and Nutrition for All (p. 200). Food and Agriculture Organization, Rome, Italy (2016).

2. Dickson, M., Nasr-Allah, A. and Kenawy, D., and Kruijssen, F., Increasing fish farm profitability through aquaculture best management practice training in Egypt. *Aquaculture*, **465**, 172–178 (2016).

3. Nandlal, S. and Pickering, T., Tilapia fish farming in Pacific Island countries, Volume 2: Tilapia grow-out in ponds, Secretariat of the Pacific Community, Noumea, *New Caledonia*, 49 (2004).

4. Modadugu, V. G. and Belen, O., Review of global tilapia farming practices. *Aquaculture Asia,* **IX**(1), 1–16 (2004).

5. Mugwanya, M., Dawood, M. A., Kimera, F., & Sewilam, H., Updating the role of probiotics, prebiotics, and synbiotics for tilapia aquaculture as leading candidates for food sustainability: A review. *Probiotics and Antimicrobial Proteins,* 1–28 (2021).

6. Shourbela, R. M., El-Hawarry, W. N., Elfadadny, M. R. and Dawood, M. A, Oregano essential oil enhanced the growth performance, immunity, and antioxidative status of Nile tilapia (*Oreochromis niloticus*) reared under intensive systems. *Aquaculture,* **542**, Article 736868 (2021).

7. Kumar, J., Priyadharshini, M., Madhavi, M., Begum, S. S., Ali, A. J., Musthafa, M. S. and Faggio, C., Impact of Hygrophila auriculata supplementary diets on the growth, survival, biochemical and haematological parameters in fingerlings of freshwater fish Cirrhinus mrigala (Hamilton, 1822). Comparative Biochemistry and Physiology Part A: *Molecular & Integrative Physiology*, **263**, 111097 (2022).

8. Richter, N., Siddhuraju, P. and Becker, K., Evaluation of nutritional quality of Moringa (Moringa oleifera Lam.) leaves as an alternative protein source for Nile tilapia (Oreochromis niloticus L.). *Aquaculture,* **217**, 599–611 (2003).

9. Falowo, A. B., Mukumbo, F. E., Idamokoro, E. M., Lorenzo, J. M., Afolayan, A. J. and Muchenje, V., Multi-functional application of (Moringa oleifera Lam.) in nutrition and animal food products: a review. *Food Research International*, **106**, 317–334 (2018).

10. Cheenpracha, S., Park, E.-J., Yoshida, W. Y., Barit, C., Wall, M., Pezzuto, J. M. and Chang, L. C., Potential anti-inflammatory phenolic glycosides from the medicinal plant Moringa oleifera fruits. *Bioorganic & Medicinal Chemistry*, **18**(18), 6598–6602(2010). https://doi.org/10.1016/j.bmc.2010.07.057.

11. Abd Rani, N. Z., Husain, K. and Kumolosasi, E., Moringa genus: A review of phytochemistry and pharmacology. *Frontiers in Pharmacology*, **9**. (2018) https://doi.org/10.3389/fphar.2018.00108

12. Toppo, R., Roy, B. K., Gora, R. H., Baxla, S. L. and Kumar, P., Hepatoprotective activity of Moringa oleifera against cadmium toxicity in rats. *Veterinary World*, **8**, 537–540 (2015).

13. Napolean, P., Anitha, J. and Emilin, R. R., Isolation, analysis, and identification of phytochemicals with antimicrobial activity from (Moringa oleifera Lam.) *Current Biotechnology*, **3**(1), 33–37 (2009).

14. Afuang, W., Siddhuraju, P. and Becker, K, Comparative nutritional evaluation of raw, methanol extracted residues and methanol extracts of Moringa (Moringa oleifera Lam.) leaves on growth performance and feed utilization in Nile tilapia (Oreochromis niloticus L.). *Aquaculture Research*, **34**(13), 1147-1159 (2003). https://doi.org/10.1046/j.1365-2109.2003.00920.x

15. Murro, J. K., Muhikambele, V. R. M. and Sarwatt, S. V., Moringa oleifera leaf meal can replace cottonseed cake in the concentrate mix fed with Rhodes grass (Chloris gayana) hay for growing sheep. *Livestock Research for Rural Development*, **15** (11) (2003).

16. Elabd, H., Soror, E., El-Asely, A., El-Gawad, E. A. and Abbass, A., Dietary supplementation of Moringa leaf meal for Nile tilapia (*Oreochromis niloticus*): effect on growth and stress indices. *Egyptian Journal of Aquatic Research,* **45**, 265–271 (2019.

17. Hussain, M., Hussain, S. M., Iqbal, R., Javid, A., Shahzad, M. M. and Arsalan, M. Z., Haematological indices, nutrient digestibility and growth performance of Catla catla fingerlings fed citric acid supplemented Moringa oleifera leaf meal based diet. *Pakistan Journal of Zoology*, **50**, 2307–2315 (2018[a]).

18. Puycha, K., Yuangsoi, B., Charoenwattanasak, S., Wongmaneeprateep, S., Niamphithak, P. and Wiriyapattanasub, P., Effect of Moringa (Moringa oleifera) leaf supplementation on growth performance and feed utilization of Bocourti's catfish (Pangasius bocourti). *Agriculture and Natural Resources,* **51** (4), 286–291 (2017).

19. Hlophe, S. and Moyo, N., A comparative study on the use of Pennisetum clandestinum and Moringa oleifera as protein sources in the diet of the herbivorous Tilapia rendalli. *Aquaculture International,* **22**, 1245–1262 (2014).

20. National Research Council (NRC), Nutrient requirements of fish. National Acadamy Press, Washington, DC 114 pp (1993).

21. AOAC., Official Methods of Analysis 15th Edn. Association of Official Analytical Chemists (2006).

22. SAS Institute Inc. (1996). User's guide: Statistics 6th Edn. SAS Institute Inc.

23. Duncan, D. B., Multiple range and multiple F test. Biometrics, 11, 1–42 (1955).

24. Ayoola, S., Kuton, M. and Shokefun, O., Evaluation of nutritional quality and haematological parameters of Moringa (Moringa oleifera Lam.) leaves in the diet of African catfish (Clarias gariepinus). *Agrosearch,* **13**, 1–15 (2013).

25. Wu, D., Cai, Z., Wei, Y., Zhang, C., Liang, G. and Guo, Q., Research advances in Moringa as a new plant protein feed. *Chinese Journal of Animal Nutrition,* **25**, 503–511 (2013).

26. Idowu, E., Adewumi, A., Oso, J., Edward, J. and Obaronbi, G., Effects of varying levels of Moringa oleifera on growth performance and nutrient utilization of Clarias gariepinus post-fingerlings. *American Scientific Research Journal for Engineering, Technology, and Sciences,* **32**, 79–95 (2017).

27. Moyo, B., Masika, P. J., Hugo, A. and Muchenje, V., Nutritional characterization of Moringa (Moringa oleifera Lam.) leaves. *African Journal of Biotechnology,* **10**(60), 12925–12933 (2011).

28. Bbole, I., Mumba, C., Mupenda, N. and Kefi, A. S., Analysis of growth performance and haematological parameters of (Oreochromis niloticus) fed on a varying diet of (Moringa oleifera Lam.) leaf meal as an additive protein source. *International Journal of Fisheries and Aquaculture*, **8**, 105–111 (2016).

29. Billah, M. B., Haque, M. E., Sarkar, S., Hossain, M. M. and Dey, S. K., Growth performance, haematological disorder and bacterial challenge on Nile tilapia (Oreochromis niloticus) using Moringa oleifera plant leaf as feed supplement. *Bangladesh Journal of Zoology*, **48**(1), 151–166 (2020). https://doi.org/10.3329/bjz.v48i1.47884.

30. El-Kassas, S., Abdo, S. E., Abosheashaa, W., Mohamed, R., Moustafa, E. M., Helal, M. A. and El-Naggar, K., Growth performance, serum lipid profile, intestinal morphometry, and growth and lipid indicator gene expression analysis of mono-sex Nile tilapia fed Moringa oleifera leaf powder. *Aquaculture Reports,* **18**, 100422 (2020).

31. Zhang, X., Sun, Z., Cai, J., Wang, J., Wang, G., Zhu, Z. and Cao, F., Effects of dietary fish meal replacement by fermented Moringa (Moringa oleifera Lam.) leaves on growth performance, nonspecific immunity, and disease resistance against Aeromonas hydrophila in juvenile gibel carp (Carassius auratus gibelio var. CAS III). *Fish & Shellfish Immunology,* **102**,430–439 (2020[b]).

32. Shih, M.-C., Chang, C.-M., Kang, S.-M. and Tsai, M.-L., Effect of different parts (leaf, stem, and stalk) and seasons (summer and winter) on the chemical compositions and antioxidant activity of Moringa oleifera. *International Journal of Molecular Sciences,* **12**, 6077–6088 (2011).

33. Stevens, C., Ugese, F., Otitoju, G. and Baiyeri, K., Proximate and anti-nutritional composition of leaves and seeds of Moringa oleifera in Nigeria: A comparative study. *Agro-Science,* **14**, 9–17 (2015).

34. Monir, W., Abdel-Rahman, M. A., El-Din Hassan, S., Mansour, E. S. and Awad, S. M., Pomegranate peel and moringa-based diets enhanced biochemical and immune parameters of Nile tilapia against bacterial infection by Aeromonas hydrophila. *Microbial Pathogenesis*, **145**, 104202 (2020).

35. Salama, F., Labib, L., Al-Sheikh, H., Tonsy, H. and Zaki, M., Effect of Moringa (Moringa oleifera) leaves supplementation as a growth promoter on growth performance, body composition, and physiological profile of Nile tilapia (Oreochromis niloticus L.) fry. *Journal of the Egyptian Academic Society for Environmental Development*, **17**, 49–61 (2016).

36. Tekle, E. W. and Sahu, N., Growth and immunomodulatory response of Nile tilapia (Oreochromis niloticus) fingerlings to ethanolic extract of Moringa oleifera flower. In Aquaculture Europe 2015. European Aquaculture Society, Rotterdam, Netherlands (2015).

37. Abdel-Latif, H. M. R., Abdel-Daim, M. M., Shukry, M., Nowosad, J. and Kucharczyk, D., Benefits and applications of Moringa oleifera as a plant protein source in Aquafeed: A review. *Aquaculture,* **547**, 737369 (2022) . https://doi.org/10.1016/j.aquaculture.2021.737369.

38. Dongmeza, E., Siddhuraju, P., Francis, G. and Becker, K., Effects of dehydrated methanol extracts of Moringa (Moringa oleifera Lam.) leaves and three of its fractions on growth performance and feed nutrient assimilation in Nile tilapia (Oreochromis niloticus (L.)). *Aquaculture,* **261**, 407–422 (2006).

39. Francis, G., Makkar, H. P. S., & Becker, K., Anti-nutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture,* **199**, 197–227 (2001).

40. Abd El-Gawad, E. A., El Asely, A. M., Soror, E. I., Abbass, A. A. and Austin, B., Effect of dietary Moringa oleifera leaf on the immune response and control of Aeromonas hydrophila infection in Nile tilapia (Oreochromis niloticus) fry. *Aquaculture International,* **28**, 389–402 (2020). https://doi.org/10.1007/s10499-019-00472-9.

41. Kaleo, I. V., Gao, Q., Liu, B., Sun, C., Zhou, Q., Zhang, H., Shan, F., Xiong, Z., Bo, L. and Song, C., Effects of Moringa oleifera leaf extract on growth performance, physiological and immune response, and related immune gene expression of Macrobrachium rosenbergii with Vibrio anguillarum

and ammonia stress. *Fish & Shellfish Immunology*, **89**, 603–613 (2019).

42. Zhang, X. H., Sun, Z. Y., Cai, J. F., Wang, G. B., Wang, J. H., Zhu, Z. L. and Cao, F. L., Dietary supplementation with fermented Moringa oleifera leaves inhibits the lipogenesis in the liver of meat ducks. *Animal Feed Science and Technology*, **260**, 114336 (2020[a]).

43. Pakade, V., Cukrowska, E. and Chimuka, L., Comparison of antioxidant activity of Moringa oleifera and selected vegetables in South Africa. *South African Journal of Science*, **109**, 01–05 (2013).

44. Ahmadifar, E., Yousefi, M., Karimi, M., Fadaei Raieni, R., Dadar, M., Yilmaz, S., Dawood, M. A. O. and Abdel-Latif, H. M. R., Benefits of dietary polyphenols and polyphenol-rich additives to aquatic animal health: An overview. *Reviews in Fisheries Science & Aquaculture*, 1-34 (2020). https://doi.org/10.1080/23308249.2020.1795361.

45. Bennett, R. N., Mellon, F. A., Foidl, N., Pratt, J. H., Dupont, M. S., Perkins, L. and Kroon, P. A., Profiling Glucosinolates and Phenolics in Vegetative and Reproductive Tissues of the Multi-Purpose Trees (Moringa oleifera Lam.) (Horseradish Tree) and (Moringa stenopetala Lam.) *Journal of Agricultural and Food Chemistry*, **51**, 3546–3553 (2003).

46. Aborhyem, S., Ismail, H., Agamy, N. and Tayel, D., Effect of Moringa oleifera on lipid profile in rats. *Journal of the High Institute of Public Health*, **46**, 8–14 (2016).

47. Chumark, P., Khunawat, P., Sanvarinda, Y., Phornchirasilp, S., Morales, N. P., Phivthong-ngam, L., Ratanachamnong, P., Srisawat, S. and Klai-Upsorn, S. P., The in vitro and ex vivo anti-oxidant properties, hypolipidaemic and anti-atherosclerotic activities of water extract of (Moringa oleifera Lam.) leaves. *Journal of Ethnopharmacology*, **116**(3), 439–446(2008). https://doi.org/10.1016/j.jep.2007.12.010

48. Rapatsa, M. and Moyo, N., Effect of Moringa oleifera on the histology, haematology, and growth of Oreochromis mossambicus in aquadams® fertilized with chicken manure in South Africa. *African Journal of Aquatic Science*, **39**, 295–303 (2014).

49. Mbokane, E. M., & Moyo, N. A. G., Alterations of haemato-biochemical parameters pre and post-challenge with Aeromonas hydrophila and survival of Oreochromis mossambicus fed Moringa oleifera-based diets. Fish & Shellfish Immunology, **83**, 213–222 (2018).

50. Mansour, A. T., Espinosa, C., García-Beltrán, J. M., Miao, L., Francisco, D. C. C., Alsaqufi, A. S. and Esteban, M. A., Dietary supplementation of drumstick tree, Moringa oleifera, improves mucosal immune response in skin and gills of seabream, Sparus aurata, and attenuates the effect of hydrogen peroxide exposure. *Fish Physiology and Biochemistry*, **46**, 981–996 (2020).

51. Bisht, M., Kumar, A. and Shah, T. K., Effect of Moringa oleifera leaf powder on skin mucosal immune responses and growth performance of guppy, Poecilia reticulata (Peter, 1860). *Aquaculture Research*, **51**, 4984–4990 (2020).

52. Makkar, H., Francis, G. and Becker, K., Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. *Animal*, **1**, 1371–1391 (2007).

53. Anwar, F., Latif, S., Ashraf, M. and Gilani, A. H., Moringa oleifera: A food plant with multiple medicinal uses. *Phytotherapy Research*, **21**(1), 17-25 (2007). https://doi.org/10.1002/ptr.2023

54. Kou, X., Li, B., Olayanju, J. B., Drake, J. M. and Chen, N., Nutraceutical or pharmacological potential of (Moringa oleifera Lam.) *Nutrients*, **10**, 343 (2018).

55. Wang, L., Chen, X. and Wu, A., Mini review on antimicrobial activity and bioactive compounds of Moringa oleifera. *Medicinal Chemistry*, **6**, 578–582 (2016).

56. Sudha, P., Asdaq, S., Dhamingi, S. S. and Chandrakala, G. K., Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in animals. *Indian Journal of Physiology and Pharmacology*, **54**, 133–140 (2010).

تأثير استبدال الغذاء الكامل جزئيا بأوراق المورينجا أوليفيرا على الأداء والاستفادة من العلف ومؤشرات الدم المختلفة في أسماك البلطي النيلي

عبدالرحمن على السيد ابو يحيى[1]، عبدالمجيد السيد نصر[1]، مصطفى ابراهيم عبدالجليل[2] ، محمد عبدالهادى نايل[1]، محمد عبدالحميد عكر[3] وصبرى عبدالحافظ محمد شحاته[1،2]

[1] قسم الانتاج الحيوانى، كلية الزراعة، جامعة الزقازيق، الزقازيق 44519، مصر.
[2] جامعة الفراهيدى، بغداد، العراق.
[3] المعمل المركزى لبحوث الثروة السمكية، العباسة، ابو حماد، الشرقية، مصر.

الملخص

أجرى هذا العمل لدراسة تأثير استبدال مستويات مختلفة من العليقة الاساسية لاسماك البلطى النيلى باوراق المورينجا. (MOL) تم عمل 5 علائق استبدل فيها 5 ، 10 ، 15 و 20% من العليقة الاساسية لاسماك البلطى النيلى بأوراق المورينجا.

استخدام MOL بمستوى أعلى من 5% من العليقة الاساسية خفض معنويا وزن جسم السمك، معدل النمو، معدل النمو النوعى معدل النمو النسبى. كان معدل النفوق 10% للاسماك المغذاه على العلائق 1 المحتوية صفر، 10، 15 و 20% MOL بالمقارنة 6.67% للاسماك المغذاه على عليقة محتوية 5% MOL. انخفض معنويا الغذاء الموضوع ، ومعدل التحويل الغذائى فى الاسماك التى تغذت على العلائق 3، 4 و 5 بالمفارنة بالاسماك التى تغذت على العلائق 1، 2. أظهرت قياسات الدم زيادة معنوية فى البروتين الكلى والجلوبيولين بكل مستويات MOL، وعلى الجانب الاخر انخفض معنويا الكرياتينين. وحامض اليوريك. انخفض نشاط الانزيمات الهضمية (الاميليز، الليبيز، البروتييز) بمستويات المورينجا 10، 15 و 20%. (الاسماك المغذاه على العلائق 3، 4 و 5). انخفض نشاط انزيمات الكبد (الاسبرتيت أمينو ترانزفيريز و الالانين أمينوترانزفيريز) فى الاسماك المغذاه عليقة بها 5% MOL بالمقارنة بالكنترول وباقى المعاملات. ارتفع معنويا قياسات مضادات الاكسدة (glutathione, superoxide dismutase and total antioxidant capacity) بكل مستويات MOL.

التوصية: تشير معظم النتائج الى ان استخدام MOL بمعدل 5% بدلا من العليقة الاساسية ليس له اى تأثيرات معنوية على اداء اسماك البلطى النيلى كما يحسن الحالة الصحية لها.

الكلمات الدالة: أوراق المورينجا ، السمك، معدل النمو، الاستفادة بالغذاء، قياسات الدم.