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Evaluation of Moringa (*Moringa Oleifera*) leaves meal as a growth promoter and immune stimulant for Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

One hundred and eighty Nile Tilapia fish (*Oreochromis niloticus*) with initial body weight (IBW) 28 ± 0.33 grams were used in the present experiment. The experiment was conducted in glass aquariums. Fish were stocked at a rate of 15 fish in each aquarium. Fish were allocated into four experimental groups (Each group includes three replicates) and one of them was used as a control. The control group was fed on a basal diet free from moringa leaves meal (MLM), whereas 2nd, 3rd and the 4th group were fed on diets containing 3.0, 4.4 and 7.4 % MLM, in replacement of 10, 15 and 25% of soybean meal (SBM), respectively (according to the percentage of crude protein in each). The experimental period lasted for 6 weeks (42 days). Parameters of growth efficiency, blood haematology, immune response, antioxidant status and histopathological changes in internal organs of Nile tilapia were used as indices for the study. The obtained results showed that MLM have high nutritive value. Inclusion of MLM in the fish diet at level 3.0% or 4.4%, significantly ($P < 0.5$) enhanced the growth performance indices of fish, including final live body weight, body weight gain, feed conversion ratio, and survival rate (%) either after three or six weeks of feeding MLM diets compared with those fed the control diet. Fish fed 7.4% MLM diet recorded the lowest growth performance traits compared with the control and those fed 3.0% or 4.4 MLM diets. The average of feed conversion ratio after 42 days; recorded:1.84, 1.58, 1.50 and 2.13 g feed per g gain, for fish groups fed the control, 3.0, 4.4 and 7.4% MLM diets, respectively. Survival rate (%) was improved with inclusion of MLM in the diet at level of 3.0 or 4.4%, while it decreased with 7.4% MLM diet. Significant improvement of immunity and antioxidant capacity of fish occurred with feeding MLM in the diet, where levels of IgM, lysozymes, RBCs, GPx, platelets (PLT) and nitric oxide (NO) were significantly increased ($p < 0.05$ or 0.01), with exception that levels of NO and GPx were decreased in blood of fish fed 7.4% MLM. The levels of WBCs and SOD were not affected significantly by feeding the experimental diets. The histopathological findings revealed normal structure of the liver, kidneys, spleen and intestinal cells of fish fed 3.0 or 4.4% MLM diet, whereas the same organs showed with fish fed 7.4% MLM some lesions ranged between sever diffuse cytoplasmic vacuolation, necrosis, hyperplasia and cell infiltration. In conclusion, Moringa leaves meal have high nutritive value and could be included successfully from 3.0 to 4.4% in the diet of Nile tilapia as enhancer for growth, immunity and antioxidant capacity.

Introduction

Nile tilapia fish (*Oreochromis niloticus*) are surface-feeding omnivore fish belong to the family *Cichlidae*. They are the most popular species for aquaculture in Egypt due to their fast growing and highly tolerant to different environmental conditions. Fish consumption in Egypt exceeded the local production by slightly more than half million tons (Wally and Verdonk, 2016).

Egypt is now interested in expanding aquaculture in order to increase the fish production to cover the gap between domestic consumption and the local production. The major element of fish production cost is the fish feed. Soybean meal is widely used as a cost-effective feed ingredient for many aquacultures and other animal species, because it contains high percentages of protein and essential amino acids. As the shortage of soybean production in Egypt, the price of soybean meal is high and therefore, it is necessary to search about untraditional potential and cheap alternative plant protein source in fish diets.

The literature indicated that *Moringa Oleifera*, a member of the family *Moringaceae*, is a fast- growing plant widely distributed in the tropics and subtropics, which is characterized with several important industrial and medicinal uses (Abd El-Rahim, 2014). Moringa leaves contain about 26-30% crude protein, 87% from it is true protein (Makkar and Becker, 1996; Abdulkarim *et al*., 2005; Sherif *et al*., 2014; Abdel-Nabey *et al*., 2015; Abd El-Rahim *et al*., 2019). A comparison between the amino acid composition of raw moringa leaves and that of soybean meal revealed an almost identical pattern of all the essential amino acids (Foidl *et al*., 2001; Abd El-Rahim, 2014). Moringa leaves contain high levels of minerals (Yaméogo *et al*., 2011; Abo El-Haded, 2017). They are also rich in a wide range of vitamins such as ß-carotene, ascorbic acid, vitamin B1, B6 and niacin (Gopalakrishnan *et al*., 2016). Moringa leaves contain phenolics and flavonoids, which have various biological activities, including antioxidants (Yang *et al*., 2006; Makita *et al*., 2016; Ibrahim *et al*., 2019), anticarcinogenic, immunomodulatory and hepatoprotective properties (Rapatsa and Moyo, 2014, Sherif *et al*., 2014, Brilhante *et al*., 2017; Kaleo *et al*., 2019).

The literature showed discrepancy in the results of researchers concerning the optimal level of Moringa leaves meal which should be included in the diet of Nile tilapia fish as a source of plant protein and enhancer for growth, immunity and antioxidant activity. For this reason, the present study was planned to evaluate the effect of dietary inclusion of different levels of *Moringa Oleifera* leaves meal as a partial substitute for the soybean meal on growth performance and health status of Nile tilapia fish. Parameters of growth efficiency, blood haematology, immune response, antioxidant activity and histopathological changes in internal organs of Nile tilapia were used as indices for the study.

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Materials and methods

Experimental design and fish management

The research activity was conducted from September to November 2019 for 42 days in the Fish Research Unit belongs to Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt. One hundred and eighty Nile Tilapia fish (*Oreochromis niloticus*) with initial body weight (IBW) 28.0±0.33 grams were used in the present experiment. The experiment was conducted in glass aquariums. Fish were stocked at a rate of 15 fish in each aquarium. Fish were allocated into four experimental groups (Each group includes three replicates) and one of them was used as a control. The control group was fed on a basal diet free from moringa leaves meal (MLM), whereas the 2nd, 3rd and 4th groups were fed on diets containing 3.0, 4.4 and 7.4% MLM, in replacement of 10, 15 and 25% of soybean meal, respectively (according to the percentage of crude protein in each). Fish were obtained from El-Abassa fish farms, Sharkiya Governorate, Egypt. Fresh tap water was used for replacing about one third of the total water volume in each aquarium daily after the removal of fecal wastes daily by siphoning. Water temperature was controlled thermostatically and measured two times daily using a thermometer.

Water quality parameters; dissolved oxygen and pH were monitored during the whole trial. The experiment lasted 7 weeks and the first week was taken as a preliminary period in order to adapt fish to living in aquariums, where fish were fed the control diet and left without weighing and were weekly weighed after there.

Feed formulation

The moringa leaves were separated from the stalks of the plant, and then air dried, then the dried moringa leaves were mashed using a blender, and the resulting powder was obtained. The moringa leaves were used in the diets to replace 10, 15 and 25% of soya bean meal, respectively (according to the percentage of crude protein in each). The experimental diets were formulated to be isonitrogenous and isocaloric with about 32% CP and were prepared to meet the nutrients requirements of Nile Tilapia fish according to NRC (2011) by using different feed ingredients. The experimental diets were formulated and pelleted in the Fish Research Unit located at Faculty of Veterinary Medicine, Zagazig University, Egypt. Fish were fed the experimental diets at rate of 5% of their body weight twice a day during the entire trial.

Aquaria

For the experimental study a total of 12 glass aquariums were used. The diameter of each aquarium was 80 x 40 x 30 cm (60-liter water capacity),

each was filled with dechlorinated tap water provided with aerators. Water quality parameters were monitored and maintained within the appropriate range in the aquaria (APHA, 1998) as follows: temperature 28 ± 2.0°C, pH 7.2- 8.2, dissolved oxygen 7.4±0.34 mg L^{-1}, ammonia 0.02±0.01 mg L^{-1}, nitrite 0.03±0.010 mg L^{-1}, conductivity (Ec) 621 ppm, Ca^{+2} 33±0.12 mg L^{-1}, Mg^{+2} 19±0.25 mg L^{-1}, Na^{+} 10.0±0.13 mg L^{-1} and K^{+} 2.7±0.002 mg L^{-1}.

Growth performance parameters

Growth performance was calculated according to the following equation: Average daily gain (ADG) = (FBW-IBW)/ T

Where, IBW and FBW were the initial and final body weight per gram, respectively and T is the number of days of the feeding experiment (Castell and Tiews, 1980). Feed conversion ratio (FCR) = Total feed intake (g)/ Total weight gain (g), where the total weight gain is the biomass of the fish at the end of the experiment - the biomass of fish at the start of experiment (Tacon, 1987). Survival rate (SR%) = No. of fish at end of experiment / No. of fish at the start of experiment ×100.

Blood and histopathological assay

At the end of the experimental period (42 days), nine fish per each group (three fish per each aquarium) were taken for the hematological and biochemical blood tests and histopathological findings. Blood samples were taken from tail vein by 1ml sterile syringa with EDTA anticoagulant (Lucky, 1977) for measurement of red blood cell (RBCs), white blood cell (WBCs) and platelets (PLT) counts which were counted by haemocytometer according to Stoskopf (1993). Other blood samples were collected without anticoagulant and left to clot at room temperature, then centrifuged at 3000 r.p.m. for 15 min to obtain blood serum which was stored in freezer at – 200°C until conducting the assays of determination of superoxide dismutase (SOD), glutathione peroxidase (GPx), lysozymes, nitric acid (NO) and immunoglobulin M (IgM) concentrations. Blood analysis was performed at one of the special accredited medical laboratories.

Estimation of immune indices

Immunoglobulin M (IgM, catalog no. CSB-E12045Fh) and complement 3 (C3, catalog no. MBS281020) was assayed using kits from Cusa bio Co. (Houston city, TX, USA) and My Bio Source Co. (San Diego, CA, USA), respectively, based on the instructions provided by the manufacturer. Lysozyme activity was evaluated in serum following Ghareghanipoor *et al*. (2014)based on the lysis of Micrococcus lysodeikticus (Sigma Co., MO, USA) with some modifications. The optical density was measured by Spectrophotometer (Model Spectro 22, S.N 221101, Manufacturer Labomed, Inc., USA) at wave length 540 nm. A calibration curve was constructed using dilutions of lyophilized chicken egg-white lysozyme (Sigma Co., MO, USA) to determine the serum lysozyme content.

Estimation of antioxidants capacity parameters

The activities of GPx, SOD and NO in the blood serum were evaluated spectrophotometrically using commercial kits (Bio diagnostics company, Cairo, Egypt). Blood serum glutathione peroxidase GPx (GP, catalog no. 2524), superoxide dismutase (SOD, catalog no. SD 2521) were measured according to Bryan and Grisham (2007). Serum nitric oxide (NO) was determined according to Montgomery and Dymock (2061).

Histopathological changes

The kidney, liver, spleen and intestine of each fish were removed and placed in bottles contain 10% formalin and left for three days, then the organs were treated with gradient levels of alcohol; 50, 70, 90 and 100%, respectively for 3 days to allow paraffin wax to penetrate the tissue during embedding. The organs were then embedded in malted wax. The tissue was sectioned into thin sections (5-7 mm) by a rotatory microtome and were dehydrated and stained with haematoxylin-eosin (H&E) stain (Bancroft and Cook 1994; Suvarna *et al*., 2018). The stained slide was observed under a light microscope at x100 magnification. Sections were examined and photographed using a stereo microscope.

Chemical analysis of experimental diets and water quality

Chemical analysis (%) of feed ingredients and the experimental diets was performed at Central Laboratory for Soil, Foods and feedstuffs (International accredited laboratory according to ISO 17025 / 2017 according to the International Standard Methods (ISO) as follow: Moisture content was determined according to ISO 6496: 1999, crude ash according to ISO 5984:2022, crude protein according to ISO 5983-1:2002, crude fat and

crude fiber contents were determined according to the method described by Official Journal of the European Union (EN) (2009). Mineral elements contents in diets and water with exception of sodium, potassium and phosphorus were determined by atomic absorption spectrophotometer (Perkin Elmar 2380, Serial No.13186, USA) using ISO 6869:2000. Sodium (Na) and potassium (K) elements contents were determined by flame photometer spectroscopy apparatus (CIBA Corning model 410, USA, Serial No., 4887). Phosphorus was determined by spectrophotometer (Manufacturer Labomed, Inc., USA, Model Spetro22, S.N 221101) according to ISO 6491:1998. Water pH was determined by using pH meter (Model Adwa 1030) according to ISO 10390:2021, EC by using conductivity meter (Model Jenway 4510) according to ISO 11265:1994. Potassium and sodium were determined according to Westerman (1990).

Statistical analysis

Statistical analysis was performed using the analysis of variance (ANOVA) as a completely randomized design according to Snedecor and Cochran (1982). Duncan's Multiple Range Duncan (1955) was used to determine differences among treatments mean at significance level of $p \leq 0.05$. All statistics were run on the computer using the linear model program of SPSS (2014).

Results and Discussion

Chemical composition of moringa leaves meal (MLM)

Data in Table 1 showed the chemical composition of MLM versus soybean meal (SBM). The obtained results indicated that MLM has high nutritive value. Moringa leaves meal contained; 27.40 % crude protein, 8.42% crude fat, 33.52% soluble carbohydrates, 11.15% crude ash and 9.69 % crude fibre. Moringa leaves meal rich in macro elements (g/100g): 1.74 Ca, 0.36 P, 0.06 Na, 1.59 K and 0.46 Mg and micro elements (mg / kg), 321 Fe, 32 Zn, 65 Mn and 13 Cu. Table 1 showed that MLM are higher in content of EE, Ash, Ca, Na, Mg, Fe and Mn than SBM and lower content in CP, P, K and Zn than SBM. The obtained high nutritive value of moringa leaves was reported by many researchers (Batal *et al*., 2010; Abd El-Rahim, 2014; Abdel-Nabey *et al*., 2015; Gopalakrishnan *et al*., 2016; Oyeyinka and Oyeyinka, 2016; Magouz *et al*., 2016; Abo El-Haded, 2017; Brilhante *et al*., 2017; Chatepa and Mbewe, 2018).

Chemical analysis of the experimental diets

Chemical composition of different experimental diets is presented in Table 2. The experimental diets were isonitrogenous, and contained nearly similar DM, CP, EE, CF, Ash, NFE and GE content.

Growth performance of Nile Tilapia fish

Table 3 shows growth performance parameters of Nile Tilapia fish fed for three and six weeks on diets contained different levels of MLM as a partial replacement for SBM.

The obtained results revealed that inclusion of MLM in the fish diet at level 3.0% or 4.4%, significantly ($P < 0.5$) enhanced the growth performance indices of fish, including final live body weight, body weight gain, feed conversion ratio, and survival rate (%) either after three or six weeks of feeding MLM diets compared with those fed the control diet (without MLM). Fish fed 7.4% MLM diet recorded the lowest growth performance traits compared with the control and those fed 3.0% or 4.4 MLM diets. The average of feed conversion ratio through the experiment; recorded:1.84, 1.58, 1.50 and 2.13 g feed per g gain, for fish groups fed the control, 3.0, 4.4 and 7.4% MLM diets, respectively. Survival rate (%) was improved with inclusion of MLM in the diet at level of 3.0 or 4.4 %, while it decreased with 7.4% MLM diet, where it recorded at the end of experiment; 75.5, 82.2, 83.5 and 68.9% for fish groups fed the control, 3.0%, 4.4% and 7.4% MLM diets, respectively.

Table 1. Chemical composition of MLM versus SBM as fed basis (%).

Parameter	Ingredient	
	MLM	SBM
Proximate composition (%)		
DM	90.18	91.15
Moisture	9.82	8.85
CP	27.4	42.79
EE	8.42	2.08
CF	9.69	7.27
Ash	11.15	6.63
NFE	33.52	32.38
Total	100	100
Macro elements (g/ 100g)		
Ca	1.74	0.31
P	0.36	0.67
Na	0.06	0.01
K	1.59	2.05
Mg	0.46	0.28
Micro elements (mg /kg)		
Fe	321	172
Zn	32	48
Mn	65	41
Cu	13	15

Table 2. Ingredients and chemical composition of the experimental fish diets.

Ingredients	Experimental Diets (%)			
	D1 (Control)	D2	D3	D4
Corn grains	34	34	34	34
Soya bean meal	19	17.1	16.2	14.3
Moringa leaves meal*	-	3	4.4	7.4
Wheat flower	10	10	10	10
Fish meal	16	16	16	16
Poultry meat meal	14	14	14	14
Palm oil	5.5	4.4	3.9	2.8
Premix* *	1.5	1.5	1.5	1.5
Chemical composition as fed basis (%)				
Moisture	9.09	9.21	9.3	9.45
Crude protein	32.2	32.35	32.44	32.3
Crude fat	5.05	4.12	4.21	3.4
Crude ash	10.74	11. 26	11.98	11.75
Crude fiber	6.25	6.4	6.75	7.52
NFE	36.67	36.66	35.32	35.58
Gross energy Kcal/Kg	4060	3990	4000	3915

*On the basis that protein content in soya bean meal (SBM) and moringa leaves meal (MLM) were 42.79 and 27.40 %, respectively.
** Premix: each 1 kg of premix contained: vitamin A, 550,000 IU; vitamin D, 110,000 IU; vitamin E, 11,000 mg; vitamin K, 484 mg; vitamin C, 50 g; vitamin B1, 440 mg; vitamin B2, 660 mg; vitamin B3, 13,200 mg; vitamin B5, 1100 mg; vitamin B6, 1045 mg; vitamin B9, 55 mg; choline, 110,000 mg; biotin, 6.6 mg; iron, 6.6 g; copper, 330 mg; manganese, 1320 mg; zinc, 6.6 g; selenium, 44 mg; iodine, 110 mg.

The improvement in growth performance indices with fish fed 3.0% or 4.4% MOLM diets compared with those fed the control diet could be attributed to the presence of several nutrients in the moringa leaves that stimulate growth and increase the nutrients bioavailability and feed utilization such as high quality protein, vitamins, minerals, antioxidants, cytokinins-type hormones and antimicrobial compounds (Yang *et al*.,

2006; Abd El-Rahim, 2014; Rapatsa and Moyo, 2014; Sherif *et al*., 2014; Abdel-Nabey *et al*., 2015; Gopalakrishnan *et al*., 2016; Brilhante *et al*., 2017; Chatepa and Mbewe, 2018; Ibrahim *et al*., 2019; Kaleo *et al*., 2019).

The lowest growth indices for fish fed 7.4 % MLM diet may be due to the decrease in feed intake as a presence of high levels of tannins, saponins and phenolic compounds in moringa leaves. These compounds have bitter and pungent taste and make the diet less palatable when its quantity increases with increasing the level of MOLM in the diet (Makkar and Becker, 1996; Becker and Makkar, 1999; Hlophe and Moyo, 2014; Karthivashan *et al*., 2015; Laheng *et al*., 2022).

The obtained results (Table 3) indicated that *Moringa Oleifera* leaves meal (MLM) can be safely included in the diet of Nile tilapia fish from 3.0 to 4.4% in replacement for 10 to 15% soya bean meal in the diet without any adverse effects on growth performance indices of fish.

Parveen *et al.* (2021) used different levels of MLM (0, 4, 8 and 10%) in the diet of Nile tilapia for 10 weeks and found that diet with 10% MLM significantly (P<0.05) increased the growth rate, survival Rate (SR), specific growth rate (SGR) and feed conversion efficiency (FCE) compared to the control and other groups.

In this concern, most of the previous studies in the literature showed that MOLM can be used safely in the diet of fish at level from 3–15% (Ozovehe and Nzeh, 2013; Sherif *et al*., 2014; Magouz *et al*., 2016; Mahboub *et al*., 2018; Laheng *et al*., 2022). However, in other studies, MOLM was included successfully at level up to 30% (Manuel *et al*., 2019).

The variation and the discrepancy in the results of these studies may be due to the variation in number and age of fish used, duration of the experiment and the differences in the chemical analysis of MOLM, which lead to unbalanced formulation of the experimental diets.

Haematological and biochemical blood parameters of Nile Tilapia fish

Table 4 show some of haematological and biochemical blood parameters of Nile Tilapia fish fed for 6 weeks diets containing different levels of MLM as a partial replacement for SBM.

Results of blood analysis (Table 4) show that dietary inclusion of different levels of MLM significantly (p≤ 0.05 or 0.01) increased levels of RBCs, GPx, platelets (PLT), nitric oxide (NO), IgM and lysozymes, with exception that levels of NO and GPx were decreased in blood of fish when level of MLM elevated in the diet to 7.4%. The levels of WBCs and SOD were not affected significantly in the blood serum by inclusion different levels of MLM in the diet compared with those fed a diet without MLM (control).

The increased levels of GPx, nitric oxide (NO), IgM and lysozymes in blood serum of fish fed 3.0 or 4.4% MLM indication to the improvement of antioxidant status and immunity response with inclusion of MLM in the diet which reflected on the increase levels of RBCs and PLT and improvement of growth indices and the feed utilization efficiency.

The decrease levels of GPx and No in the blood serum of fish fed in the diet 7.4% MLM compared to the control fish, indication to the decrease of antioxidant capacity which could be attributed to that moringa

Table 3. Growth performance parameters (X ± SE) of Nile Tilapia fish fed for three and six-weeks diets containing different levels of MLM as a partial replacement for SBM.

Parameters	Experimental groups			
	G1 (control)	G2 (3.0 % MLM)	G3 (4.4% MLM)	G4 (7.4% MLM)
		After three weeks		
Initial body weight (g)	28.23±0.14	28.43±0.08	27.33±1.06	28.4±0.05
Final body weight (g)	36.60±0.45^{b}	38.63±0.18^{a}	37.77±0.41^{b}	35.03±0.52^{c}
Body weight gain (g)	8.36±0.31^{ab}	10.20±0.25^{a}	10.44±0.87^{a}	6.63±0.53^{b}
Feed intake (g)	15.43±0.38^{ab}	16.11±0.29^{a}	15.62±0.43^{a}	14.05±0.59^{b}
Feed conversion ratio (FCR)	1.84±0.02^{b}	1.58±0.01^{c}	1.50±0.02^{c}	2.13±0.08^{a}
		After six weeks		
Initial body weight (g)	28.23±0.14	28.43±0.08	27.33±1.06	28.40±0.05
Final body weight (g)	66.86±1.12^{c}	72.30±0.43^{b}	76.56±0.99^{a}	60.03±0.89^{d}
Body weight gain (g)	38.63±1.08^{c}	43.86±0.50^{b}	49.23±1.31^{a}	31.63±0.92^{d}
Feed intake (g)	71.32±1.01^{a}	67.68±0.94^{b}	72.78±0.55^{a}	69.48±0.24^{b}
Feed conversion ratio (FCR)	1.84±0.02^{b}	1.54±0.04^{c}	1.48±0.02^{c}	2.20±0.05^{a}
Survival rate (%)	75.5	82.2	83.5	68.9

*3.0, 4.4 and 7.4 % of moringa leaves meal (MLM) equal replacement of 10, 15 and 25 % of soya bean meal (SBM) in the diet, respectively. a, b, c = Means in the same row with different superscript differ significantly (P<0.05); * P= <0.05; ** P= <0.01; and NS= Not significant.

Table 4. Some of haematological and biochemical blood parameters (X ± SE)* of Nile Tilapia fish fed for 6 weeks diets containing different levels of MLM as a partial replacement for SBM.

Blood components	Experimental groups				Sig.
	G1 (Control)	G2 (3% MLM)	G3 (4.4 % MLM)	G3 (7.2 % MLM)	
WBC (x 10^{3}/µL)	6.87±1.08	6.90±0.32	6.92±0.27	5.29±0.68	NS
RBC (x10^{6}/µL)	1.27±0.09^{b}	1.36±0.06^{b}	1.54±0.14^{a}	1.65±0.01^{a}	*
PLT (x10^{3})	138.0±5.78^{d}	363.0±1.16^{a}	260.67±6.23^{b}	204.0±7.51^{c}	**
IgM (µg/ml)	8.34±0.15^{b}	9.34 ± 0.29^{a}	9.67±0.09^{a}	8.34±0.14^{b}	*
LYZ (U/L)	14.00±1.53^{b}	19.50±0.87^{a}	20.67±1.85^{a}	19.00±2.52^{a}	**
NO (µmol/l)	124.34±2.97^{b}	138±3.22^{a}	143.34±5.47^{a}	99.67±2.97^{c}	**
GP_{X} (U/L)	196.34±2.03^{c}	231±0.58^{b}	245.0±5.72^{a}	188.34±4.92^{d}	**
SOD (U/ml)	3.37±0.17	3.06±0.15	3.50±0.22	3.11±0.09	NS

a, b, c = Means in the same row with different superscript differ significantly (P<0.05); * P= <0.05; ** P= <0.01; and NS= Not significant.
* Average of 3 blood samples for each group.

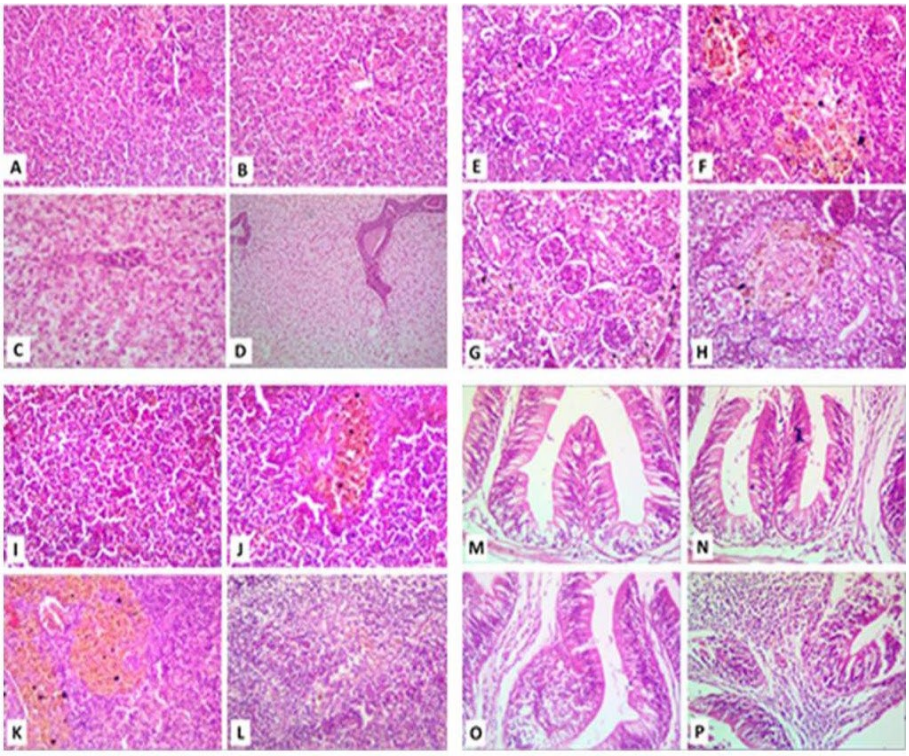

Fig. 1. Histopathological changes in internal organs cells of Nile tilapia fish fed in the diet different levels of moringa leaves meal (MLM) as partial replacement for soya bean meal (SBM). A: liver of control fish, showing normal hepatic parenchyma (polygonal hepatocytes with central nuclei and arranged in two-cell thick cords. Their cytoplasm had variable amounts of glycogen and/or lipid contents. The portal veins were surrounded by scattered islands of pancreatic tissue forming hepatopancreas), H&E. X40. B: liver of fish fed 3% moringa leaves meal (MLM), showing normal histological picture, H&E. X40. C: liver of fish fed 4.4 % MLM, showing mild cytoplasmic vacuolation, H&E. X40 H&E. X10. D: liver of fish fed 7.4 % MLM, showing severe diffuse cytoplasmic vacuolation with cholestasis. E: kidney of control fish, showing normal renal parenchyma (glomeruli and renal tubules), H&E. X40. F: Kidney of fish fed 3 % MLM, showing normal histological picture with hyperplasia of the melanomacrophage aggregates, H&E. X40. G: Kidney of fish fed 4.4 % MLM, showing vacuolations of the epithelial lining of the renal tubules with single-cell necrosis, H&E. X40. H: Kidney of fish fed 7.4 % MLM, showing vacuolations of the epithelial lining of the renal tubules with single-cell necrosis, and hyperplasia of the melanomacrophage aggregates, H&E. X40. I: spleen of control fish, showing normal histological picture (mixed white and red pulps with ellipsoids), H&E. X40. J: Spleen of fish fed 3 % MLM, showing normal histological picture with mild hyperplasia of melanomacrophage aggregates, H&E. X10. K: Spleen of fish fed 4.4 % MLM, showing vascular congestion with hyperplasia of melanomacrophage aggregates, H&E. X10. L: Spleen of fish fed 7.4 % MLM, showing lymphoid depletion with necrosis melanomacrophage centers, H&E. X10. M: Intestine of control fish, showing normal histological picture, H&E. X40. N: Intestine of fish fed 3 % MLM, showing normal histological picture, H&E. X10. O: Intestine of fish fed 4.4 % MLM, showing mild mononuclear cell infiltration, H&E. X10 P: Intestine of fish fed 7.4 % MLM, showing extensive mononuclear cell infiltration, H&E. X10.

leaves meal (MLM) contain anti-nutritional materials (saponins, terpenes, steroids, alkaloids, phytates and tannins) which increase with increasing level of MLM in the diet of fish (Rapatsa andMoyo, 2014; Karthivashan *et al*., 2015; Laheng *et al*., 2022; Ibrahim *et al*., 2023).

The increase of RBCs counts in fish fed moringa leaves meal (MLM) diets could be attributed to the presence of numerous amino acids, many trace elements (copper, iron, zinc, and selenium), multiple vitamins (Vitamins; B12, B6, E, C and riboflavin) and biochemical constituents (such as flavonoids) in moringa leaves (Anwar *et al*., 2007; Abo El-Haded, 2017).

WBCs are essential components of the immune system, and they are influenced by several physiological and environmental variables (Ibrahim *et al*., 2022). In the present study, all fish groups were not exposed to any stress, therefore WBCs counts showed statistically no significant differences between groups.

The improved antioxidant enzyme levels (GPX and NO) could be attributed to the presence of antioxidant components in Moringa leaves, such as vitamin C, β-carotene, α- and γ- tocopherol, β-sitosterol, and vitamin A, as well as phenolic components, such as quercetin, kaempferol, flavonoids, and anthocyanins (Valavanidis *et al*., 2006).

The modulation of immune parameters, lysozyme and IgM could be attributed to the high concentration of monounsaturated fatty acids (MUFAS), particularly oleic acid and saturated fatty acids (SFAS), mainly palmitic and stearic acids in moringa leaves, which contribute in the structure of cell membrane (De Pablo and De Cienfuegos, 2000). Vitamins A, K, and C were also found in Moringa leaves; these vitamins improve immunoglobulin synthesis, boosting the immune response (Moyo *et al*., 2011). Additionally, the amino acids found in *M. Oleifera* leaves are required to produce immunoglobulin (Mora *et al*., 2008).

In this concern, Ibrahim *et al*. (2023) reported that immunological parameters (IgM and lysozymes) and antioxidant capacity parameters (SOD, GPx and NO) were remarkably increased ($P<0.05$) in blood serum of Nile tilapia fed for 60 days 3 or 6% MLM compared with those fed diet without MLM (control) and feeding 6% MLM recorded the best parameters. Also, Sherif *et al*. (2014) used for 6 weeks different levels of MLM in the diet (0, 5, 10, 15, 20 and 25%) of Nile tilapia which injected with pathogenic A. hydrophilia. The authors reported that MLM must not exceed 15% in the diet of fish, where, higher than this level reduced RBCs and WBCs counts and immune response parameters. The authors added that 10% MLM in the diet recorded the best results for improvement of growth performance and immune response parameters.

Histopathological examination

The histopathological changes in internal organs (liver, kidney, spleen and intestine) of fish fed in the diet different levels of moringa leaves meal (MLM) as a partial replacement for soya bean meal (SBM) compared to the control fish are shown in Figure 1.

Liver cells showed normal structure with control fish fed diet without MLM and those fed 3.0% MLM, while it showed mild cytoplasmic vacuolation with those fed 4.4% MLM and severe diffuse cytoplasmic vacuolation with cholestasis with fish fed 7.4% MLM diet.

Kidney cells showed normal structure of renal parenchyma and renal tubules with control fish and those fed 3.0% MLM diet and 4.4% MLM, while it showed vacuolations of the epithelial lining of the renal tubules with single-cell necrosis and hyperplasia of the melano- macrophage aggregates with those fed 7.4% MLM.

Spleen cells showed normal histological picture with fish fed the control diet and normal histological structure with mild hyperplasia of melano-macrophage aggregates with fish fed 3.0 and 4.4% MLM. Spleen cells of fish fed 7.4% MLM diet showed lymphoid depletion with necrosis melano-macrophage centers.

Intestinal cells demonstrated normal histological structure with fish

fed control diet and those fed 3.0 % MLM and it showed normal structure with mild mononuclear cell infiltration with those fed 4.4% MLM. Intestinal cells cleared extensive mononuclear cell infiltration with fish fed 7.4% MLM diet.

The indicated histological findings support the results of blood analysis that moringa leaves meal (MLM) could be safely included in the diet of fish from 3.0 to 4.4% to substitute about 10 to 15% of soya bean Meal (SBM).

Conclusion

Moringa leaves meal have high nutritive value and could be included successfully from 3.0 to 4.4% in the diet of Nile tilapia fish as enhancer for growth, immunity, and antioxidant capacity.

Conflict of interest

The authors declare that they have no conflict of interest.

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