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Effect of *Moringa oleifera* Leaf-Based Formulated Feeds on Growth Performance, Feed Utilization, and Intestinal Histology of the Nile Tilapia Fingerlings, *Oreochromis niloticus*

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ABSTRACT

Tilapia fingerlings with a mean weight of 2.51g were used in the study. The fingerlings were collected from fish tanks and randomly distributed into four concrete tanks, each measuring 3 x 2 x 2.5m. The stocking density was 60 fish per hapa, with a total of 180 fish in each tank/treatment. The feeding trial lasted for 8 weeks. Four experimental diets containing 28% crude protein were prepared, with differing levels of *Moringa oleifera* (Mo) meal (0, 4, 6, and 8g/ kg of diet). The fingerlings fed these diets were designated as Mo0, Mo4, Mo6, and Mo8, respectively. Fish sampling was conducted every two weeks to assess growth parameters. A sample of fish from each group was collected for stomach content analysis and promptly preserved in 10% neutral-buffered formalin for histological analysis. The groups fed Mo6 and Mo8 had significantly higher final body weights (FBW), specific growth rates (SGR), protein efficiency ratios (PER), protein productive values (PPV), and feed conversion ratios (FCR) compared to the group fed Mo4. Histological examination revealed that in the control group (Mo0), the intestinal section showed normal villi with a low number of goblet cells. In the Mo4 group, there were reduced intestinal villi and a low number of goblet cells. In the Mo6 group, there was a significant increase in both the number and length of intestinal villi, with a low number of goblet cells. In the Mo8 group, there was a marked increase in the length of intestinal villi, along with notable lymphocytic infiltration. The present study concluded that adding *Moringa oleifera* meal to the feed of the Nile tilapia fingerlings at a rate of more than 6g/ kg of feed improves growth performance, survival rate, and feed utilization. Furthermore, the addition of moringa improves digestion efficiency by increasing the length of intestinal villi and inducing marked lymphocytic infiltration.

INTRODUCTION

The aquaculture sector has experienced continuous growth over the past two decades, making it the fastest-growing agricultural production sector, particularly in fish production **(FAO, 2014)**. In 2018, worldwide aquaculture production was 82.1 million tons. Inland aquaculture accounted for 62% (51.3 million tons) and coastal and marine aquaculture accounted for 38% (30.8 million tons) **(FAO, 2020)**.

In Africa, the fish production sector has great potential for breeding and growth. However, the failure to fully exploit this potential has slowed the sector's growth in recent years **(Msangi & Batka, 2015)**. Tilapia is a popular fish species in the sector due to its high growth rate and good disease resistance **(El-Sayed, 2006)**. It is considered a herbivorous/carnivorous fish, which means that its production cost and feed composition are lower than those of other carnivorous species **(Naylor *et al.*, 2021)**. In addition, tilapia is recognized for its leniency to modest water quality and high resistance to pathogens **(Bhujel, 2014)**.

According to **FAO (2020)**, global tilapia production is probable to reach 7.3 metric tons by 2030. Among the approximately 70 species of tilapia known worldwide, the Nile tilapia (*Oreochromis niloticus*) is the most commonly cultured species **(Thomas & Micheal, 1999; Bentsen *et al*., 2012)**.

Moringa is a plant known for its ability to treat malnutrition diseases. It is used in many food products as a general tonic, without any known undesirable side effects **(Oyeyinka & Oyeyinka, 2018)**. Additionally, moringa has medicinal properties, including treating infections, strengthening nerves, antivirals, preventing and revitalizing liver diseases. It is also known for being antidiabetic and anticancer **(Gopalakrishnan *et al*., 2016; Razzaq *et al*., 2020)**. Research has indicated that *Moringa oleifera* plant improves the growth performance of the Nile tilapia, *Oreochromis niloticus* **(Afuang *et al*., 2003; Elabd *et al*., 2019)**, and rohu, *Labeo rohita* **(Hussain *et al*., 2018)**. Moreover, it increases forage utilization by *Pangasius bocourti* **(Puycha *et al*., 2017)**. It also improves the immune response of *Oreochromis niloticus* (**Abd El-Gawad *et al*., 2020; Monir *et al*., 2020)** and *Sparus aurata* **(García *et al*., 2020; Mansour *et al*., 2020)**.

This study aimed to assess the impact of varying levels of dietary *Moringa oleifera* on the growth performance, feed utilization, and intestinal histology of the Nile tilapia *Oreochromis niloticus* fingerlings.

MATERIALS AND METHODS

Experimental fish and nutrition protocol

The experiment was accomplished in concrete tanks located in the Lake Nasser Development Authority in Sahara, Aswan City. Tilapia fingerlings with mean weight of 2.51g were used. Fingerlings were collected from fish tanks and were then randomly stored in four concrete tanks, with dimensions of 3 x 2 x 2.5m. The stocking density was 60 fish/happa with a total of 180 fish in each tank/treatment. The feeding trial spanned over 8 weeks.

The tilapia fingerlings were placed in the experimental tanks for acclimatization before introducing the experimental diets. Four experimental diets containing 28% crude protein were prepared, with differing levels of *Moringa oleifera* (Mo) meal (0, 4, 6 and 8g/ kg of diet). The fingerlings fed these diets were labelled as Mo0, Mo4, Mo6 and Mo8, respectively. Composition and chemical analyses of the experimental diets are presented in Table (1).

The feed components were blended utterly, and adequate water was added to certify smooth pelleting. Pellets of 2mm size were then shaped using a local pelleting machine and were stored in airtight packaging till use. The Nile tilapia fingerlings were fed twice a day at 8:30 AM and 2:30 PM. The feed amount was calculated as 6% of the total body weight, and it was adjusted every two weeks. Diets were designated as:

- **Mo0** = control diet
- **Mo4** = 4g Mo/kg diet
- **Mo6** = 6g Mo/kg diet
- **Mo8** = 8g Mo/kg diet

Collection of *Moringa oleifera* and processing:

Moringa oleifera leaves were gathered from Aswan University Campus. The leaves were cleaned with water and were dried in the sun before being ground into a powder using an electrical blender.

Fish and feed analyses

Feed samples were collected to analyze their proximate composition (Table 1) including moisture, protein, lipid, and ash contents according to **AOAC (2012)**. NFE was calculated by the difference according to the following equation: NFE = 100 - [Moisture % + Ash % + % lipid + % protein + Fiber %]. The dietary gross energy (GE) content was estimated using the following factors: (5.64, 9.44 and 4.11 kcal/100g) for protein, lipid and NFE, respectively **(McDonald *et al*., 1973)**.

Determination of growth parameters of fish

Fish sampling was conducted every two weeks to calculate fish growth parameters. Indices were determined to evaluate growth performance as follows:

Weight Gain (WG, g) = FBW – IBW

Specific Growth Rate (SGR, %/day) = (Ln FBW-Ln IBW) 100 / days

Where: **IBW**= initial body weight; **FBW**= final Body weight.

Survival rate (SR) =100× (final number of fish/initial number of fish)

Feed conversion ratio (FCR) = feed intake/ weight gain

Protein efficiency ratio (PER) = protein gain / protein intake

Protein productive value (PPV, %) = 100 × (protein gain/protein intake)

Table 1. Composition (g\kg) and chemical analyses (% on dry mater basis) of the diets with the four levels of moringa meal used to feed the Nile tilapia fingerlings

Ingredient	Mo0	Mo4	Mo6	Mo8
Fish meal	120	120	120	120
Wheat flour	100	100	100	100
Wheat bran	150	150	150	150
Soybean meal	346	346	346	346
Corn meal	200	196	194	192
Moringa	0	4	6	8
Corn oil	30	30	30	30
Sunflower oil	20	20	20	20
Vit. and Min. Mix*	30	30	30	30
Ascorbic acid	4	4	4	4
Proximate analysis on dry matter basis				
Dry mater	89.71	89.63	89.72	89.52
Moisture	10.29	10.37	10.28	10.48
Crude protein (N × 6.25)	32.29	32.32	31.37	31.82
Crude fat	7.26	7.61	7.38	7.18
Crude fiber	6.19	6.86	6.83	6.18
Ash	6.5	6.87	6.59	6.15
Carbohydrate (NFE)**	37.47	35.97	37.55	38.19
Gross energy (GE) kcal/100g3 ***	405.92	403.23	402.14	405.44

*Each 100 gram of vitamin and mineral contained: Mineral: Zn, 2.50 mg; Mn, 16.00 mg; Fe, 31.50 mg; Cu, 5.50; I, 0.55 mg; CA, 1.15 gm and P, 450 mg. Vitamins: A, 7500000 IU; Bi, 100 mg; B3, 500 mg; B6, 150 mg; B12, 2.5 mg; E, 100 mg; K, 100 mg; Pantothenic acid, 275 mg; Folic acid, 100 mg and vit. D3, 7500 IU.

**Nitrogen free extract (NFE) = 100 − (protein percentage + lipid percentage + ash percentage + fiber percentage).

***GE (kcal/100 g DM) = CP x 5.64 + EE x 9.44 + NFE x 4.11 calculated according to **McDonald *et al.* (1973)**.

Histopathological examination

A fish sample was collected from each group for embedding in paraffin sections. The fish were immediately washed with distilled water, dissected, and eviscerated. The stomach was collected and immediately fixed in 10% neutral-buffered formalin for histological examination, following the methods of **Abdelhakeem *et al.* (2022)** and **Bancroft and Gamble (2002)**. Three segments from each organ were subjected to ascending grades of alcohol (70, 80, 90, and 100%) for dehydration, then cleared in methyl benzoate and embedded in paraffin wax. Sections were cut using a semi-automated sliding microtome at 3 to 5µm thickness. The paraffin sections were mounted on glass slides, dried, deparaffinized in xylene, rehydrated in a graded alcohol series (100, 90, 80, and 70%), and stained with Harris's Hematoxylin and Eosin (H&E). The stained sections were visualized and examined using a DMLS light microscope (Leica, Germany) outfitted with an MC120 HD camera (Leica).

Statistical analysis

The growth, feed utilization, and body composition data were analyzed using the analysis of variance (ANOVA) to determine significant differences ($P<0.05$) among the treatment means. All statistical analyses were performed with IBM-SPSS version 28 software.

RESULTS AND DISCUSSION

1. Growth performance and survival rate

The results of final body weight (FBW), weight gain (WG), survival (SUR) and specific growth rate (SGR) of fingerlings fed on moringa levels for eight weeks are presented in Table (2) and illustrated in Fig. (1).

Tabel 2. Final body weight (FBW), weight gain (WG), survival and specific growth rate (SGR) of Nile tilapia fingerlings (*Oreochromis niloticus*), fed four levels of *Moringa oleifera*

Parameter	Mo0	Mo4	Mo6	Mo8
IBW	2.52±1.66	2.52±1.66	2.52±1.66	2.52±1.66
FBW	7.69±0.22^{c}	9.59±0.5^{b}	15.52±.42^{a}	15.0±.23^{a}
Gain	5.17±0.22^{c}	7.07±0.50^{b}	13.0±0.42^{a}	12.48±0.23^{a}
SUR	93.33±2.55^{a}	88.89±2.42^{b}	95.56±1.47^{a}	94.44±.56^{a}
S GR	1.49±0.04^{c}	1.78±0.07^{b}	2.42±0.04^{a}	2.38±0.02^{a}

- Values with different superscripts in the same raw indicated significant differences ($P\leq 0.05$)
- Data expressed as average ±SE

Increasing the amount of moringa in tilapia feed leads to better growth in tilapia fingerlings. The group that was fed with Mo6 and Mo8 had significantly higher final body weights (15.52 and 15g, respectively) than the group fed with Mo4 (9.59g), while the group fed with Mo0 had lowest FBW (7.69g).

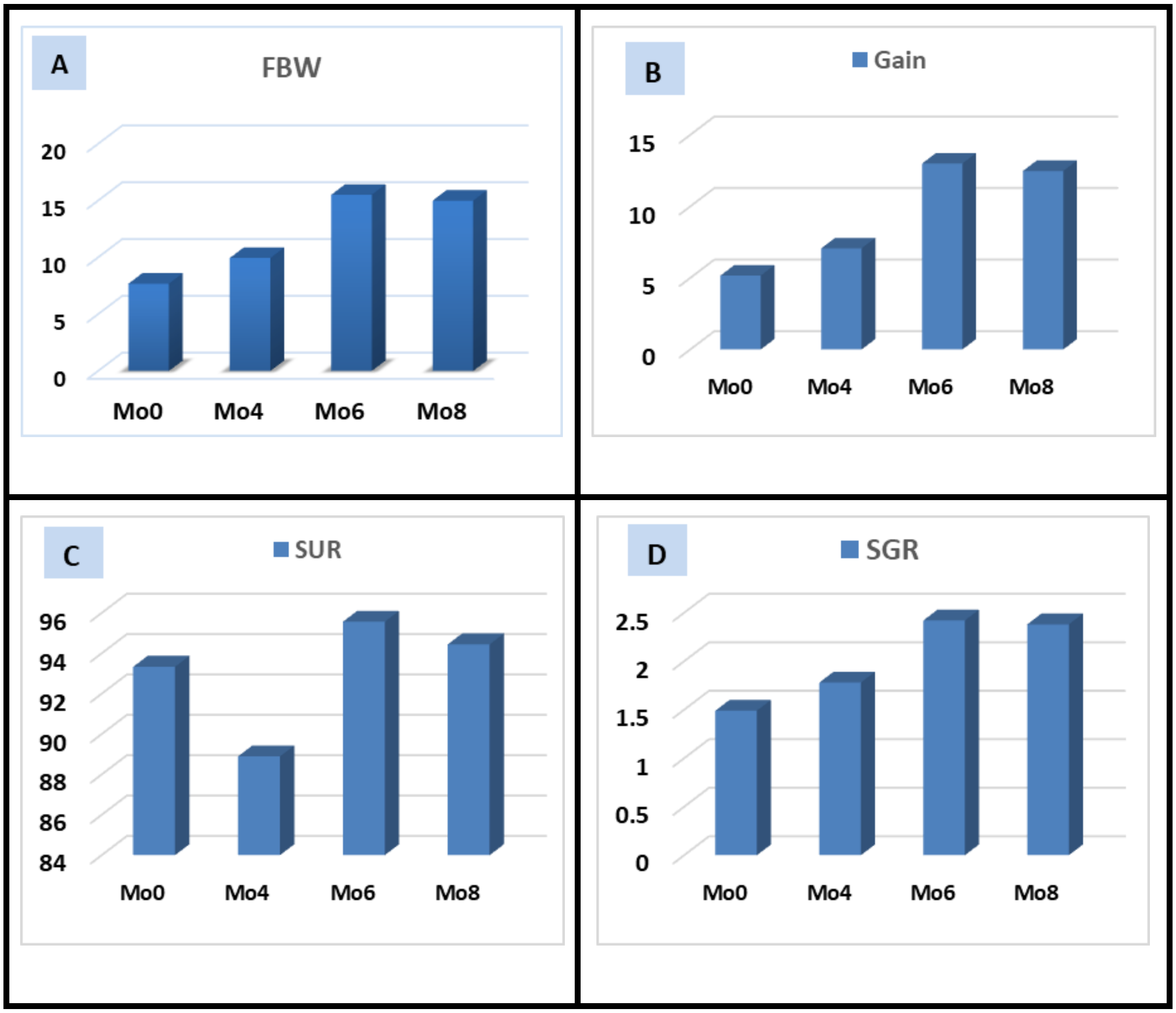


Fig. 1. Effect of four levels of moringa meal (0, 4, 6 and 8g/ kg) added to fish meal in the basal on **A**: Final Body weight (FBW) **B**: Weight gain (WG) **C**: Survival and **D**: specific growth rate (SGR) of the Nile tilapia (*Oreochromis niloticus*), 2.52g initial BW.

Much research has been conducted on natural additives in fish diets, such as *Moringa oleifera*. In a recent study, **Elabd *et al.* (2019)** found that the Nile tilapia grew faster when fed a diet containing moringa leaves. This aligns with the findings of the current study. The increased body weight and weight gain may be attributed to the high digestibility of moringa leaves, which could enhance nutrient absorption **(Backer, 1995)**. These results can also be attributed to the high nutritional value and antibacterial properties of moringa **(Fahey *et al.*, 2001)**. *Moringa oleifera* contains a natural enzyme that aids in the digestion of fibrous food in animals **(Backer, 1995)**, which may help explain these findings. The high nutritional content and antibacterial capabilities of the moringa plant might account for these results **(Fahey *et al.*, 2001)**. Reports indicate that

Moringa oleifera contains a natural enzyme that helps animals digest fibrous food **(Backer, 1995)**. The findings are consistent with the results of **El-Moustafa *et al*. (2015)**, who conducted an experiment using four levels of *Moringa oleifera* leaf meal (MOLM) (0, 0.2, 0.4, and 0.6%) fed over a 6-week period.They found that the Japanese quail chicks treated with MOL at levels of 3, 5, and 7g/ kg diet showed significantly higher body weight, body weight gain, and feed intake compared to the control group. On the other hand, **El-Kassas *et al.* (2020)** found that after an 8-week feeding experiment, food enrichment with 0, 5, and 10% MOLM improved the growth performance of mono-sex tilapia. They observed that a diet containing 5% MOLM significantly enhanced growth performance. There were no significant differences in survival rates between the groups fed Mo0, Mo6, and Mo8, which were 93.33±2.55, 95.56±1.47, and 94.44±0.56, respectively. However, the lowest survival rate (88.89±2.42) was recorded in the Mo4 group. **Parveena *et al*. (2021**) demonstrated that including moringa in the Nile tilapia diets at a rate of 10% resulted in the highest survival rate. Similarly, **Puycha *et al.* (2017)** found that the survival rate remained consistent across all treatments when different amounts of moringa (0, 100, 150, and 200g/ kg fish) were fed to the catfish over 60 days.

In the current study, the specific growth rate (SGR) of tilapia fingerlings increased as the level of *M. oleifera* in the feed increased. The fry fed Mo6 and Mo8 had the highest SGR ($P< 0.05$), followed by those fed Mo4. The fry in the control group (Mo0) recorded the lowest SGR. These findings are consistent with research by **El-Son *et al.* (2022)**, which involved feeding the Nile tilapia in the Habas system with different levels of moringa supplementation (0.0, 5.0, 7.0, and 9.0g/ kg). The study showed that a diet containing 5g of moringa resulted in the highest specific growth rate compared to all other treatments.

2. Protein and energy utilization

Protein efficiency ratio (PER), protein productive value (PPV) and feed conversion ratio (FCR) (feed /gain) are shown in Table (3) and Fig. (2).

Table 3. Protein efficiency ratio (PER), protein productive value (PPV) and feed conversion ratio (FCR) of the Nile tilapia (*Oreochromis niloticus*), 2.52g initial BW fed with four levels of *Moringa oleifera*

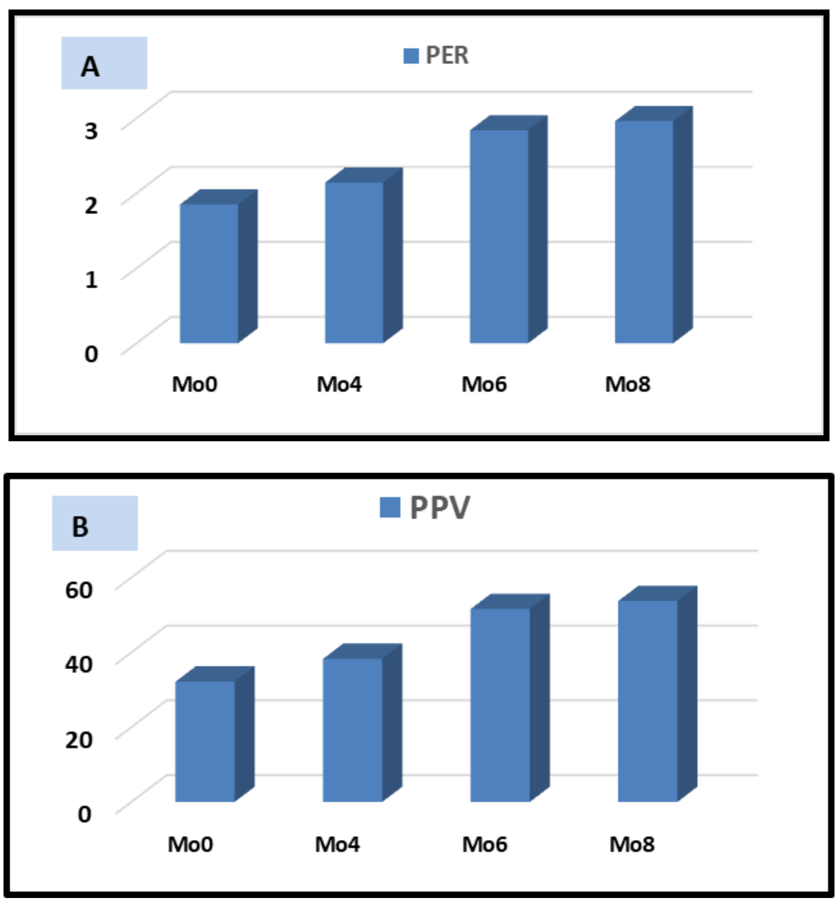
Parameter	Mo0	Mo4	Mo6	Mo8
PER	$1.85{\pm}0.02^{c}$	$2.14{\pm}0.09^{b}$	$2.84{\pm}0.06^{a}$	$2.96{\pm}0.08^{a}$
PPV	$32.18{\pm}0.76^{c}$	$38.28{\pm}1.93^{b}$	$51.60{\pm}1.47^{a}$	$53.74{\pm}1.84^{a}$
FCR	$1.8{\pm}0.02^{a}$	$1.57{\pm}0.07^{b}$	$1.17{\pm}0.02^{c}$	$1.13{\pm}0.03^{c}$

- Values with different superscripts in the same raw indicated significant differences ($P \leq 0.05$)
- Data expressed as average ±SE

Clearly, the protein efficiency ratio (PER) (gain/protein fed), protein productive value (PPV) (100 × retained protein/protein fed), and feed conversion ratio (FCR) of the Nile tilapia fry with an initial body weight of 2.52g were significantly affected by the different moringa levels ($P < 0.05$). The highest PER was observed in the Nile tilapia fed Mo8, with no significant difference between Mo8 and Mo6. However, the lowest PER was observed in the control group (Mo0). The Mo8 group also had the highest PPV, while the Mo0 group had a significantly lower PPV ($P < 0.05$).

A study by **Puycha *et al.* (2017)** found that feeding 10% MOL in the diet of the Bocourti's catfish (*Pangasius bocourti*) decreased SGR, while PER remained the same as the control treatment. Increasing the amount of moringa in the diet led to a decrease in PER values, which may be attributed to the higher levels of anti-nutritional compounds that increase as the moringa content rises **(Francis *et al.*, 2001)**.

In the current study, FCR (feed/gain) ranged from 1.13±0.03 to 1.8±0.02. Generally, FCR improved as the level of moringa increased. The protein efficiency ratio (PER), protein productive value (PPV%), and feed conversion ratio (FCR) are shown in Fig. (2).



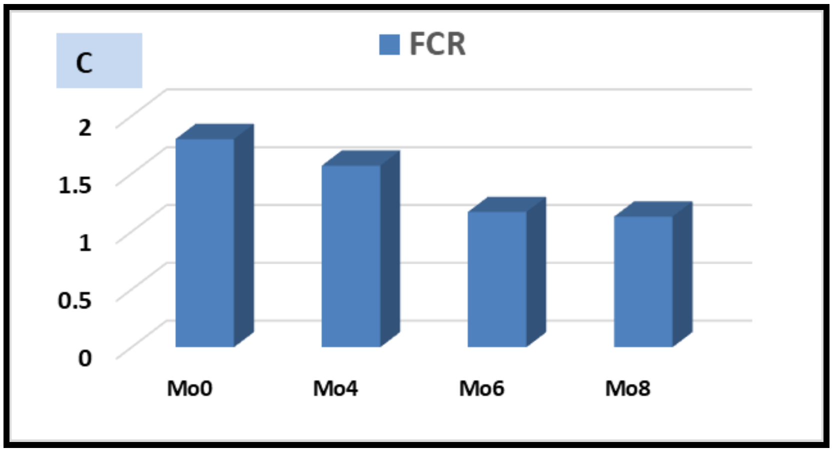


Fig. 2. Effect of four levels of moringa meal (0, 4, 6 and 8g/ kg) added to fish meal in the basal on **A**: Protein efficiency ratio (PER), **B**: Protein productive value (PPV), **C**: Feed conversion ratio (FCR) of the Nile tilapia (*Oreochromis niloticus*), 2.52g initial BW

3. Physic-chemical parameters of water

Dissolved oxygen (DO) was measured *in situ* using an oximeter, while water temperature (T) was assessed with a Celsius glass thermometer, and pH levels were determined with a pH meter. In all treatments, dissolved oxygen concentrations ranged from 5.0 to 5.9mg/ L, temperature ranged from 25 to 31°C, and pH levels ranged from 6.4 to 7.5.

4. Intestinal histopathology

The results revealed that in the control group (Fig. 3a), the intestinal section stained with HandE stain showed normal villi with a low number of goblet cells, while in the (Mo4) group (Fig. 3b), there were low intestinal villi and a low number of goblet cells. In the (Mo6) group (Fig. 3c), there was a significant increase in the number and length of intestinal villi and a low number of goblet cells. As for treatment Mo8, it showed a noticeable increase in the length of the intestinal villi and a noticeable lymphocytic infiltration (Fig. 3d).

In a study by **Magdy *et al*. (2024)**, the researchers described the morphological structure of the mid-intestinal villi of the Nile tilapia and their distinct cellular distribution using scanning electron microscopy. The study found that the mid-intestine of the Nile tilapia is a tubular organ that consists of several layers: tunica mucosa, submucosa, muscularis, and serosa.

Farrag *et al*. (2020) mentioned that an important function of the relatively long, closely spaced mucosal folds in the foregut in *Mugil cephalus* is to allow feed to be retained for a longer time. The greater the exposed surface area, the longer the feed retention and absorption time, thus improving effective digestion and absorption processes. **Mokhtar *et al*. (2015)** reported that the wavy folds in the intestine increase the period of exposure of semi-digested food to the intestinal mucosa, which enhances the absorption of nutrients.

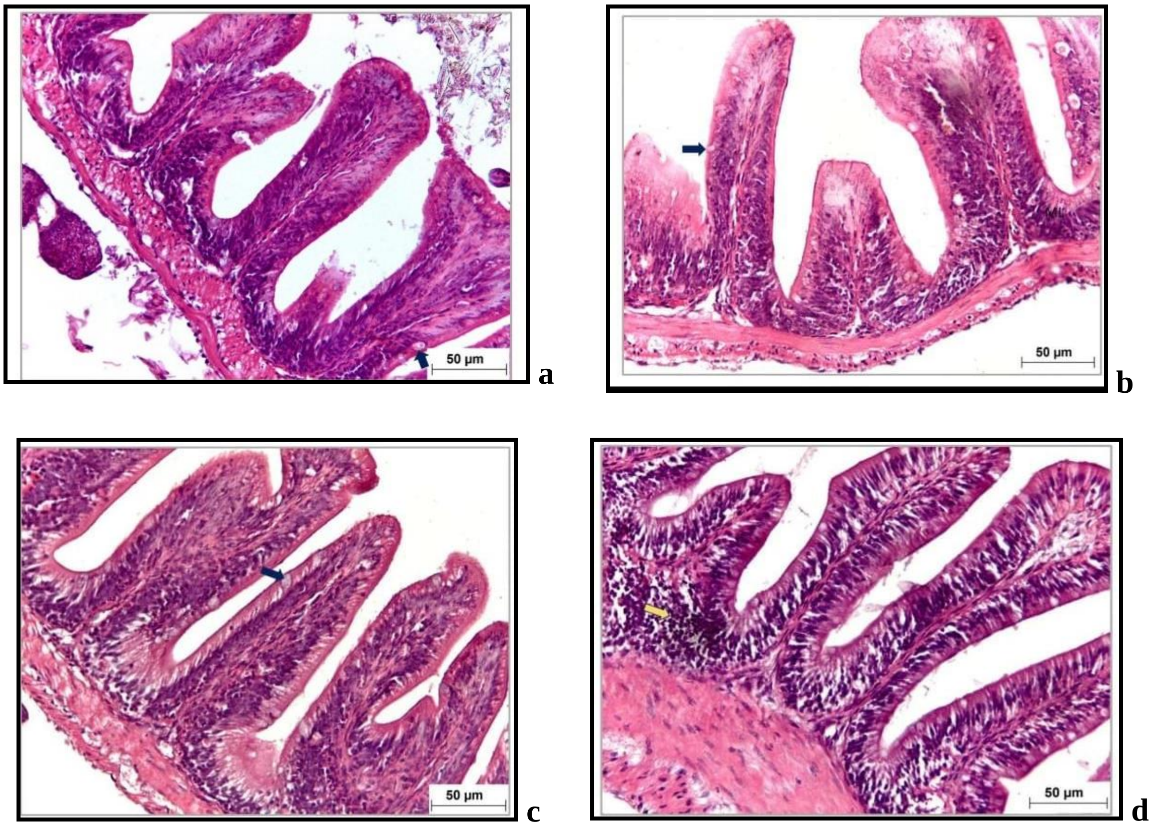


Fig. 3. The intestinal sections stained with HandE stain show the following: **a**. Group Control: Normal villi with a low number of goblet cells (arrow), **b**. Group Mo4: Low intestinal villi and a low number of goblet cells (arrow), **c**. Group Mo6: A remarkable increase in the number and length of intestinal villi and a low number of goblet cells (arrow), and **d**. Group Mo8: A remarkable increase in the length of intestinal villi and marked lymphocytic infiltration (yellow arrow).

CONCLUSION

Incorporating *Moringa oleifera* meal into the diet of the Nile tilapia fingerlings at a rate of 6g/ kg of feed enhances their growth performance, survival rate, and feed utilization. Additionally, the addition of moringa improves digestion efficiency by increasing the intestinal villi length and promoting marked lymphocytic infiltration.

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