

## Comparative Physiological Evaluation of the Potential Use of *Moringa oleifera* Seeds or Leaves as an Alternative Protein Source for the Nile Tilapia *Oreochromis niloticus*

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### ABSTRACT

The animal's need for protein resources for growth is quite expensive. Aquaculture needs a cheap protein source such as plant products. Leaves and seeds of *M. oleifera* have major quantities of proteins, carbohydrates, minerals and vitamins. The present study was carried out to physiologically compare the suitability of moringa seeds or leaves as a partial replacement of the total dietary protein for fish meal in practical diets for the Nile tilapia *Oreochromis niloticus*. For the growth experiment, four isocaloric-isonitrogenous diets contained 0, 2.92, 8.76, 14.6g/ 100g of moringa seed (MSD). Additionally, four isocaloric-isonitrogenous fermented diets (with 35g/ 100g of crude protein, and 1912.27kcal gross energy/ 100g) contained 0, 5, 10 and 20g/ 100g of moringa leaves (MLD). These diets were reared in a water-circulating system. Hematological parameters were measured, such as hemoglobin content, serum glucose, cholesterol and triacylglycerol levels. The specific growth rate of fish decreased significantly ( $P < 0.05$ ) at all concentrations when fish were fed with MSD. However, when fed with MLD, the growth remained unchanged at concentrations of 5 and 10% but decreased at a concentration rate of 20% compared to the control group. The 5 and 10% replacement levels of fish meal with moringa leaves were the optimal levels for the maximum growth performance of the Nile tilapia fingerlings, but moringa seeds inhibited the growth rates of the Nile tilapia at all concentrations. Therefore, moringa leaves are suitable for fish nutrition, while moringa seeds are not recommended in food nutrition, which may be due to the presence of anti-nutritional factors and toxic substances, such as tannins, saponins, and inhibitors of trypsin.

### INTRODUCTION

The Nile tilapia, *Oreochromis niloticus*, is an omnivore fish species of the family Cichlidae. It is recognized as one of the most productive food fish in Egypt and worldwide (Mugwanya *et al.*, 2021). The need of animal protein resources as promoters of growth is quite expensive (Shourbela *et al.*, 2021). Aquaculture is the fastest growing section of food production; nearly 50% of total global food fish production now is from

aquaculture. It is expected that by 2030, the world will need to produce more than 27 million tons of fishery products to cover the growing demand for fish food (FAO, 2016). It is understandable that the increasing aquaculture industry cannot continue to depend on limited stocks of fish meal from the fish catch (NRC, 1999). Therefore, an emergent strategy to replace fish oil and fish meal in feeds is becoming a priority (FAO, 2006). Hence, many research has focused on reducing the cost of the most expensive component in fish feeds, specifically the protein. The progress of aquaculture depends mainly on the cheap feeds that have the most growth rates in a short period. The most possible way to get low finance fish culture is by making artificial feed components from natural plant stuffs (Sikotariya & Yusufzai, 2019).

Cheap protein sources are mostly from plant products, such as leaves, seeds and other agricultural by-products (Afuang *et al.*, 2003; Richter *et al.*, 2003; State of World Fisheries & Aquaculture, 2007; Wang *et al.*, 2016; Sikotariya & Yusufzai, 2019; Moustafa *et al.*, 2020; Kumar *et al.*, 2022). It would be of extra benefit if these plant species are able to grow on degraded and poor lands under stressful environmental conditions requiring lower external energy and still maintain a reasonable production of products rich of nutrient with a potential as feed ingredients (Francis *et al.*, 2001).

Soybean is presently the most commonly used plant protein source in fish feeds (Kari *et al.*, 2023). However, soybean meal has human food usage, therefore it is required to discover other protein-rich plant resources that could be used in fish diets. Another potential alternative plant protein source for fish feeds is moringa (*Moringa oleifera*).

*Moringa oleifera*, a member of the family Moringaceae, is a plant that grows fast and commonly available in the tropics and subtropics with great economic importance for the food and medical industry (Puycha *et al.*, 2017). In Egypt, *M. oleifera* has been grown in Aswan, North Sinai, and Al-Sharkyia. The different parts of such plants have nourishing importance (Leone *et al.*, 2015). Leaves and seeds of *M. oleifera* have major quantities of proteins, carbohydrates, minerals, vitamins (calcium, phosphorus, potassium, iron), beta carotene, and other bioactive compounds (Sahay *et al.*, 2017).

The moringa tree is multifunctional. It is globally cultivated due to its rich protein, vitamin, carbohydrate, and mineral content, providing high nutritional value for both livestock and humans. Additionally, its seeds contain edible oil with a high content of 42%, and they are also utilized for medicinal purposes. Moreover, the seeds' coagulation properties are employed in wastewater treatment (Foidl *et al.*, 2001). Moringa is used to fight malnutrition in children, pregnant women, person with HIV, and improve the immune system (Oyeyinka & Oyeyinka, 2018).

The seeds are rich in oil and protein sources and can also be used for the purification of water. Moreover, the roots are a source of spices, and the leaves are rich in carotenoids, ascorbic acid, and iron (Sahay *et al.*, 2017). Essential amino acids found in moringa leaves are cysteine, methionine, typtophan and lysine (Makkar & Becker, 1996). A comparison between the amino acid composition of raw moringa leaf and that of soybean revealed a nearly same pattern of all the essential amino acids (Foidl *et al.*, 2001).

*M. oleifera* has an antioxidant activity due to its phenolic components (Sreelatha & Padma, 2009) and flavonoids (DeiviArunachalam *et al.*, 2021) contents. Although there are several benefits of *M. oleifera* seeds, few studies were done on their toxicity to cultivated fish (Ayotunde *et al.*, 2011b). The most common phytochemical contents present in moringa seeds include flavonoids (13.09%), alkaloids (12.28%), tannin (5.32%), saponins (1.37%) and cyanide (0.05%), while the proximate analysis were reported as: moisture (4.77%), ash (1.71%), protein (31.04%), crude fiber (1.17%), fat (21.25%) and carbohydrate (40.06%) (Olorode *et al.*, 2014). Although the total amount of these essential amino acids is abundant in the leaf, making it suitable for animal feed (Afuang *et al.*, 2003), there is little information regarding the utilization of moringa leaves or seeds in fish feed. Therefore, the present study was carried out to physiologically compare the suitability of moringa seeds or leaves as a partial replacement of the total dietary protein for fish meal in practical diets for the Nile tilapia *Oreochromis niloticus* based on its effects on blood parameters, growth performance and body composition.

## MATERIALS AND METHODS

### Preparation of the fish for experiments

The Nile tilapia *O. niloticus* fingerlings (1.95g average initial weight) were collected from local hatcheries (NIOF hatchery in May, 2022). The fish were stocked into 20L fiberglass tanks in a closed, recirculation indoor system. The tanks were provided with a continuous flow of aerated dechlorinated tap water at 25°C (hardness 5.2mg/ L as CaCO<sub>3</sub>, Ca<sup>2+</sup> 0.045– 0.069mM/ L, Na<sup>+</sup> 0.024mM/ L, dissolved oxygen of 80% saturation, pH range between 7.2– 7.5). The fish were fed daily with fish diet (35% protein) at a rate of 3% body weight; feeding was interrupted 24hr before the start of experiments and throughout their duration. During the experiments, fish were transferred to 12L glass aquaria, and its water was changed every 24hr by siphon technique to minimize disturbance.

### Experimental design

Groups of ten fish were randomly stocked into each aquarium, with four replicates per each treatment. In order to avoid the influence of any systematic stress factors, the

fish groups were randomly redistributed half way through the experiment. In each trial, one aquarium was allocated to fish fed on the diet without moringa to serve as control. Each aquarium was considered as an experimental unit. The fish were fed ad libitum at 09.00, 12.00, and 15.00h. Approximately 15 to 20min after all feeding activity had subsided, the uneaten feed was removed and weighed to determine the amount of eaten feed. Every day, faecal waste was siphoned from each aquarium. Group weight measurements were done at weekly intervals, moreover experimental periods lasted for 9 weeks for all tested diets.

### **Culture system**

The feeding trials were conducted in 3L plastic aquaria. Water was circulated through biological and mechanical filters. The recirculation system used a 200L vertical screen filter system utilizing high-density polyester screens (FIAB fish technic, GmbH, Germany) to remove particulate matter and provide substrate for nitrosomonas and nitrobacter bacteria. A blower and air stone provided continuous aeration. Water exchange rate for the system was approximately 5% of the total volume per day. Each aquarium was supplied with water at a rate of 200mL/ h and cleaned daily. A PVC screen covered the aquarium bottom to prevent the experimental fish from eating their faeces. A black plastic screen covered the back and sides of all aquaria to minimize disturbances caused by personnel present in the laboratory. Illumination was supplied by fluorescent ceiling lighting, controlled by an automatic electric timer to maintain a photoperiod of 18:6h light: dark per day cycle.

Water temperature and dissolved oxygen levels were daily measured. Total ammonia and nitrite levels were measured twice weekly (spectrophotometer, Hach Company, Loveland, CO). Total alkalinity and hardness were monitored once a week (colorimeter, Orion Company), and pH was monitored daily using an electronic pH meter. Salinity was checked every other day with a salinity refractometer (Erma, Tokyo, Japan). Over the duration of the study, the average values of water-quality parameters (average  $\pm$  S.D.) were as follows: water temperature was  $26.0 \pm 1.0^\circ\text{C}$ , dissolved oxygen was  $7.0 \pm 0.5\text{mg/L}$ , total ammonia was  $0.4 \pm 0.03\text{mg/L}$ , nitrite was  $0.05 \pm 0.01\text{mg/L}$ , total alkalinity was  $107.0 \pm 25.2\text{mg/L}$ , hardness was  $5.2 \pm 1.0\text{mg/L}$ , and pH was  $7.7 \pm 0.3$ .

### **Blood and tissue samples collection**

Three groups of 10 fish were killed at the end of the experiment (9 weeks), for each of the experimental diets. The handled controls were subjected to the same amount of disturbance as the experimental fish, but they were fed a moringa-free diet. Fish were caught by hand net quickly to minimize the disturbance. Then, they were placed upside down and the blood was obtained by making an incision directly into the heart using heparinized glass pipette, then the blood taken was divided for blood hemoglobin analysis using heparinized tubes and serum analysis using tubes without anticoagulant. The serum

was separated directly by centrifugation to avoid hemolysis and stored at  $-20^{\circ}\text{C}$  till the analysis was done. After blood sampling, the fish were decapitated, and the skin was removed. A piece of white epaxial muscle was taken from a specific area below the dorsal fin and then taken and stored frozen as serum at  $-20^{\circ}\text{C}$ . The intestine was dissected and prepared for bacterial analysis. In addition, the muscle was dissected and frozen for further analysis of the body composition.

### **Analytical techniques**

Hemoglobin content in blood was determined by using the commercial kit diamond diagnostic hemoglobin kit. The serum glucose concentration was determined using the glucose-liquizyme GOD-PAP kit provided by (Spectrum, MDSS GmbH Schiffgraben 41 30175 Hannover, Germany) (Caraway & Watts, 1987). The determination of cholesterol in serum was determined by using the CHOD/POD method kit (Meiattini *et al.*, 1978).

The determination of triacylglycerol in serum was by using the GPO/PAP method kit (Stein, 1987). Muscle protein concentration was measured by the method of biuret (Gornall *et al.*, 1949). The muscle lipid content was determined with an adaptation of the sulphophosphoanilin method, as described by Knight *et al.* (1972). Moreover, the muscle water content was analyzed by taking a piece of a specific weight of white epaxial muscle and drying it to a constant weight at  $100^{\circ}\text{C}$  for 24h. It was then reweighed after drying, and then the water content was measured as a percentage to the muscle weight.

### **Experimental diets**

#### **Moringa seed diets (MSD) and moringa leaves diets (MLD)**

Four isocaloric-isonitrogenous diets (35g/100g of crude protein, and 1912.27kcal gross energy/ 100g) contained 0, 2.92, 8.76, 14.6g/ 100g moringa seed, referred as A, B, C, D diets, and four isocaloric-isonitrogenous fermented diets (35g/ 100g of crude protein, and 1912.27kcal gross energy/ 100g) contained 0, 5, 10 and 20g/ 100g moringa leaves. All diets were prepared as follows: All feed ingredients were grounded and mixed in a commercial mixer (Spar mixer, 3 HP, Taiwan) for 20min. Vitamin and mineral mixes were gradually added with continuous mixing. Distilled water ( $60^{\circ}\text{C}$ ) was slowly added while mixing to achieve a consistency suitable for pellet production. The wet mix was then passed through a kitchen meat grinder and dried for 24h at  $60^{\circ}\text{C}$  in a forced air drying oven. The dried diet was chopped into pellets in a blender and then passed through laboratory test sieves (mesh 2.00 and 0.88mm) to ensure homogeneity of particle size. All feeds were stored at  $-20^{\circ}\text{C}$  until used.

**Table 1.** Content of experimental diets with moringa seed (MSD) (g 100 g<sup>-1</sup> diet)

Content	A	B	C	D
Soybean meal	63	60.4	55.01	49.67
Fish meal	6	5.75	5.25	4.74
Moringa seed	0	2.92	8.76	14.6
Corn	24	24	24	24
Soybean oil	2.49	2.51	2.56	2.57
Decalium phosphate	1.4	1.4	1.4	1.4
Vitamin %	1	1	1	1
Mineral %	2.02	2.02	2.02	2.02
Total	100	100	100	100

**Table 2.** Content of experimental diets with moringa leaf (MLD) (g 100 g<sup>-1</sup> diet)

Content	0%	5%	10%	20%
Fish meal	58	56.3	55.5	53.08
Moringa leaf	0	5	10	20
Corn	34	31	27.09	20.042
Soybean oil	4	3.7	3.41	2.882
Vitamin & mineral %	4	4	4	4
Total	100	100	100	100

**Table 3.** Proximate composition of diet (g 100 g<sup>-1</sup> diet)

Protein	35	35	35	35
Total fat	4.42	4.86	5.76	6.65
Total ash	5.226	5.38	5.61	5.832
Total crude fiber	2.74	3.061	3.68	3.009
Carbohydrate	52.88	51.7	49.95	49.5
Total energy	1912.27 kcal	1909.52 kcal	1915.28kcal	1942.95kcal

### Growth experiment

Experimental diets were fed to triplicate groups of fish (1.95g) till satiation twice a day for 9 weeks. At the end of the experiment, all fish from each tank were separately weighed, killed, grounded in a commercial blender and stored at -20°C for subsequent body composition analysis. Feed efficiency performance, including fish weight gain percentage (WG), feed conversion ratio (FCR), protein deposition value (PDV), calculated energy deposition value (EDV), protein efficiency ratio (PER), and specific growth rates (SGR) were calculated with the following equations:

$$WG = (W_f - W_i) / W_i \times 100.$$

Where,  $W_f$  is the mean final weight (g) per fish, and  $W_i$  is the mean initial weight (g) per fish.

$$\text{SGR} = (\text{LN } W_2 - \text{LN } W_1) / (T_2 - T_1)$$

Where,  $W_2$  is weight (g) at time  $T_2$

$W_1$  is weight (g) at time  $T_1$

FCR = feed (dry) intake (g)/wet weight gain (g).

### Statistical analysis

Statistical analysis was performed using SPSS (SPSS 20.0 for Windows, SPSS Inc., USA). Normal distributions were checked by Shapiro–Wilk test and homogeneity of variances by Levene test. One-way analysis of variance (ANOVA) was carried out to evaluate the effect of moringa seeds and leaves amendment on blood hemoglobin, serum biochemical variables, and growth performance. Data were analyzed using student's t-test. A value of  $P < 0.05$  was considered to be statistically significant.

## RESULTS

Changes of the parameters during feeding with diets contained 0, 2.92, 8.76, 14.6g/ 100g MSD, referred as A, B, C, D diets consequently (MSD), and diets contained 0, 5, 10 and 20g/ 100g MLD, are given as 0, 5, 10 and 20.

### 1. Growth performance and feed utilization

Specific growth rate of fish during feeding decreased significantly ( $P < 0.05$ ) at all concentrations when fish were fed with MSD, and remained unchanged during feeding with MLD at concentrations of 5 and 10%, then the growth rate of fish decreased at concentration of 20% compared to the control. Weight gain decreased significantly ( $P < 0.05$ ) by increasing moringa seed concentration. Additionally, weight gain decreased at all concentrations of moringa leaves except at a concentration of 10%, where it increased significantly ( $P < 0.01$ ). Fish in the control group fed actively on the experimental diet throughout the experiment, while other groups of MSD were slightly reluctant to consume the feed.

There was no feed-related mortality observed through the whole period of the experiment. FCR showed an increasing trend with all moringa seeds content and reached its highest level at concentration B, also FCR increased significantly ( $P < 0.05$ ) at concentrations of 5 and 20% of MLD, but remained unchanged at concentration of 10% when compared to control (Table 4, 5).

### 2. Hematological and biochemical parameters

Hemoglobin content of fish during feeding with MSD showed no significant changes at all concentrations when compared to control. Moreover, when fish were fed

with MLD, they showed no significant change at all concentrations except at concentration of 20%, it decreased significantly ( $P < 0.01$ ). Serum glucose levels decreased significantly in fish fed with MSD at the concentrations B and C compared to the control ( $P < 0.05$  and  $P < 0.01$ ) and remained unchanged at concentration D. Additionally, it decreased significantly in fish fed with MLD at the all concentrations compared to the control showing its lowest level at concentration of 5% compared to the control group ( $P < 0.01$ ). Cholesterol level decreased with high significance ( $P < 0.05$ ) in serum during feeding with MSD at all concentrations, but cholesterol level increased with high significance ( $P < 0.05$ ) in serum during feeding with dietary MLD at concentrations 10 and 20%, and showed no significant change at concentration of 5%. Serum triacylglycerol increased with high significance ( $P < 0.01$ ) during feeding with MSD. Furthermore, serum triacylglycerol increased significantly during feeding with MLD at all concentrations. No significant change of muscle water content during feeding with MSD or MLD. Muscle water content was not changed through the whole experimental diets (Tables 4 and 5).

**Table 4.** Biochemical profile and growth performance for *Oreochromis niloticus* fed experimental diets contained 0, 2.92, 8.76, 14.6g/ 100g moringa seed (MSD), referred as A, B, C, D diets consequently (MSD)

MS	Hemoglobin (g/dl)	Glucose (mg/dl)	Triacylglycerol (mg/dl)	Cholesterol (mg/dl)	Moisture (%)	Weight gain (g/g)	FCR	SGR
A	7.07±1.0	36.3± 1.4	55.12±2.0	160.5±10.4	75.4±2.0	3.64±0.4	1.67±0.05	2.74±0.04
B	7.37±0.9	31.5±0.8**	39.6±3.1**	94±21.1*	75.9±3.2	3.29±0.2*	2.68±0.1**	2.03±0.02*
C	7.17± 1.2	33.7±0.6*	42.0±4.0**	100±23.2**	75.8±2.5	2.88±0.6**	2.02±0.07*	2.45±0.06*
D	6.67±0.7	36.8±1.2	41.7±1.8**	122±31.7**	26.1±2.6	2.55±0.3**	2.05±0.04*	2.35±0.02*

Values are means of 10 replicates for each treatment ± standard error of the mean (Mean values are significant at the level of \* $P < 0.05$  or \*\* $P < 0.01$ )

**Table 5.** Biochemical profiles and growth performance for *Oreochromis niloticus* fed experimental diets contained 0, 5, 10 and 20g/ 100g moringa leaves (MLD)

MS	Hemoglobin (g/dl)	Glucose (mg/dl)	Triacylglycerol (mg/dl)	Cholesterol (mg/dl)	Moisture (%)	Weight gain (g/g)	FCR	SGR
0%	7.98± 0.03	48.5±1.4	58±10.4	159±11.4	75.4±2.0	6.77±0.4	1.18±0.01	3.71±0.06
5%	7.98±0.9	32.7±1.8**	118± 22.1*	152±21.1	75.9±3.2	5.75±0.2*	1.34±0.02*	3.50±0.02
10%	7.95±0.05	34.7±2.6*	105±26.2**	183±12.2*	75.8±2.5	7.15±0.6**	1.21±0.03	3.76±0.08
20%	6.42±0.04**	43.6±1.2*	118±31.7**	184±13.7**	76.1±2.6	5.47±0.3**	1.37±0.04*	3.34±0.05*

Values are means of 10 replicates for each treatment ± standard error of the mean (Mean values are significant at the level of \* $P < 0.05$  or \*\* $P < 0.01$ ).

## DISCUSSION

The statistical increase in demand for fish showed the need for aquaculture scientists to produce high quality fish that would reach an adult size within the possible shortest time and diminish the production cost, especially the feeding cost. Cost of feeding was estimated to be 40- 60% of the total production cost (**Fagbenro, 2005**). In order to attain a more economically sustainable and environmentally friendly production, research interest has been directed toward the evaluation and use of unconventional protein sources, particularly from plant products, such as seeds, leaves and other agricultural byproducts (**Moustafa et al., 2020; Kumar et al., 2022**).

In this study, specific growth rate of the Nile tilapia remained unchanged at concentrations of 5 and 10% during feeding only with MLD, but decreased at all concentrations at fish fed with MSD, and MLD. This finding agrees with that of **Puycha et al. (2017)** who monitored a reduction in the growth performance as the moringa leaf inclusion increased in the diets of Bocourti's catfish. Moreover, **Adeshina et al. (2018)** recommended a 10% level of moringa leaves to be incorporated in the diets of common carp at juvenile stage for maximum growth performance. On the contrary, **Emam et al. (2021)** found that the dietary *Moringa oleifera* seed aqueous extract at 200mg/ kg diet of the Nile tilapia is recommended to improve the growth performance, however caution must be considered when using *Moringa oleifera* aqueous extract at a concentration over 200mg/ kg diet. However, the current study disagrees with the conclusion of **Tabassum et al. (2021)** concerning the moringa leaves having the potential to be used as a feeding substitute in *C. mrigala* fish diet. Their study suggests that fish fed with a diet at a 10% replacement level showed a maximum growth performance and improved digestibility of nutrients compared to fish fed on the control diet and other test diets. Additionally, these results vary from those of **Hussain et al. (2018)** who revealed that, the maximum weight gain (304%) values were noted at 10% replacement level for moringa leaves in the diet. This study indicated that *Labeo rohita* fingerlings were in a healthier condition compared to the control and other test diets. Furthermore, these results oppose the conclusion of **Hussein (2017)**, who suggested that moringa leaves meal can be added to the diets for the Nile tilapia without any antagonistic properties. This discrepancy may be attributed to the differences in fish species and metabolic responses.

The inhibition of growth upon using moringa seed diets in the Nile tilapia may be attributed to high anti-metabolites, which are toxic for fish, especially tannin (**Dienye & Olumuji, 2014**) where it is revealed that the most common phytochemical contents present in moringa seeds include flavonoids (13.09%), alkaloids (12.28%), tannin (5.32%), saponins (1.37%) and cyanide (0.05%) (**Olorode et al., 2014**), which have inhibition effect on metabolism and thus, reduce the fish growth rate. Additionally, the inhibited growth rates may be due to the relatively high total phenolics (0.7 and 1%), nonhaemolytic saponin (1.5 and 2.3%) and phytic acid (0.5 and 0.8%), as well as NDF

(3.8 and 5.7%), and ADF (3.0 and 4.5%) in the diets (**Richter et al., 2003**). **Ben Salem and Makkar (2009)** suggested that defatted moringa seed meal has the potential to improve sheep growth since the defatted seed cake is free of most plant secondary metabolites, such as tannins, saponins, alkaloids, inhibitors of trypsin and amylase, lectin and cyanogenic glucosides, but contains glucosinolates.

The FCR showed an increasing trend with all seeds and leaves content but remained unchanged at a concentration of 10% when compared to the control, which is consistent with the finding of **Richter et al. (2003)**, who observed a similar increasing trend with increasing moringa leaf content. Conversely, **Puycha et al. (2017)** showed poor feed utilization. This finding contrasts with those of **Hussain et al. (2018)** who revealed that the FCR of *L. rohita* fingerlings, where the maximum weight gain (304%) and minimum FCR (1.33) values were noted at a 10% replacement level for the above-mentioned diet, indicating that the fish were in a healthier condition compared to the control and other test diets. Serum glucose levels decreased significantly in fish fed with MSD and MLD diets at all the concentrations compared to the control ( $P < 0.05$ ). The maintenance of the blood sugar is due to the same dietary sugar supply as the control. This results matches that of **Adeshina et al. (2018)** who recorded that, glucose levels decreased significantly with increasing MLD concentrations to be incorporated in the diets of common carp at juvenile stage. Furthermore, **Hussein (2017)** noticed that the inclusion of moringa leaves meal reduced the glucose in the blood serum content significantly; this may be due to the inverting effects of the components of moringa.

Hematological parameters are usually considered as a major indicator for determining the health condition of fish (**Hrubec et al., 2000**). Hemoglobin content of fish during feeding with moringa showed no significant change at all concentrations, except at concentration of 20% of MLD where it decreased. No anemic condition was noticed. Additionally, **Abd-Elgawad et al. (2020)** recorded that Hb amount was not increased significantly in the Nile tilapia. However, in accordance to our results **Tabassum et al. (2021)** revealed that *C. mrigala* fed on moringa leaves based diet at 10% replacement level showed maximum values of Hb (8.47g/ 100ml), then it decreased with the increase in moringa leaves concentration. Similarly, **Adeshina et al. (2018)** recommended 10% level of moringa leaves to be incorporated in the diets of common carp at juvenile stage; for maximum haematological indices such as increased Hb amount. This contradiction to our work and the other researchers may be due to the difference, fish species and its size, time of allocation of diets or fish health status. There is no anaemic condition was noticed, and this may reflect the adaptation of fish to toxicity of seeds.

The lipid metabolism of the liver includes the secretion, transport, and uptake of lipid. A balance between the secretion and uptake of lipids occurred under normal physiological conditions. **Karavia et al. (2013)** used rats as an experimental animal and

attributed the hepatic lipid accumulation to the blocking of lipid secretion. Cholesterol level decreased with high significance ( $P < 0.05$ ) in serum during feeding with MSD at all concentrations, but during feeding with MLD, the cholesterol level increased with high significance ( $P < 0.05$ ) in serum at concentrations of 10 and 20% and showed no significant change at concentration of 5%. Agreeing with **Adeshina *et al.* (2018)**, cholesterol levels decreased significantly with increasing MLD concentrations in the diets of common carp at juvenile stage. Furthermore, serum triacylglycerol increased with a high significance during feeding with MSD and MLD at all concentrations. The increment of TG may be traced back to increasing the absorption of lipids by gut at moringa diets. Moreover, the serum TG concentrations may indicate a more active endogenous lipid transport (**Du *et al.*, 2005; Gatesoupe *et al.*, 2014**). These results might indicate that the ability for transporting lipids out of the liver increased after the intake of a high FDP diet. In the contrary, **Hussein (2017)** noticed that the inclusion of moringa leaves meal for the Nile tilapia reduced cholesterol and triacylglycerol in the blood serum content significantly. This decrease in the lipid profile of moringa seed diets than in the leaves confirm the depletion in growth rates and this may be due to the presence of anti-metabolic factors and toxic substances.

Remarkably, no significant change of muscle water content was detected during feeding with MSD and MLD upon applying the whole experimental diets, which agrees with the observation of **Adeshina *et al.* (2018)** concerning the relative stability of the moisture content.

## CONCLUSION

This study concluded that the 5 and 10% replacement level of fish meal with moringa leaves is the optimal level for maximum growth performance of the Nile tilapia fingerlings. Meanwhile, caution must be considered when using moringa leaves at a concentration over 10% diets.

On the other hand, this study confirms that moringa seeds inhibited the growth rates of the Nile tilapia at all concentration. This may be due to the presence of anti-nutritional factors and toxic substances, such as tannins, saponins, and inhibitors of trypsin. Therefore, moringa leaves are suitable for fish nutrition; however, moringa seeds are not recommended in food nutrition.

It is recommended that moringa seeds should be defatted before use in fish feeding. Defatting the seeds removes most plant antimetabolites, such as tannins, saponins, and inhibitors of trypsin and amylase, making the extract more tolerable for fish consumption.

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