

## EFFECT OF OLIVE LEAF EXTRACT ON GROWTH PERFORMANCE, FEED UTILIZATION AND PHYSIOLOGICAL STATUS OF NILE TILAPIA

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### SUMMARY

The purpose of this study was to examine how dietary olive leaf extract (OLE) affected the Nile tilapia's growth performance, feed utilization, physiological and histological status. In twelve 1.0 m<sup>3</sup> hapas, 180 Nile tilapia fishes, each weighing 11.82±0.02 g and with a body length of 8.80±0.10 cm, were randomly distributed in four groups. Group one received 1% kg<sup>-1</sup> OLE, Group two received 1.5% kg<sup>-1</sup> OLE, Group three received 2% kg<sup>-1</sup> OLE, and the control group received no olive leaf extract for ninety days. At the end of the trial, group 3 (2% kg<sup>-1</sup> of OLE) had considerably higher weight gain, length gain, and specific growth rate values than other experimental groups. Additionally, the trail groups fed different amounts of OLE showed improvements in feed intake and protein intake values. The group fed the diet with OLE had significantly lower levels of blood albumin, and triglycerides compared to control. Group three (2% kg<sup>-1</sup> of OLE) had significantly greater levels of blood total protein and globulin, as well as alkaline phosphatase, ALT, AST, amylase, lipase, and protease activities when compared to other groups. The results showed some histological alterations in the liver, especially in Group 1, while the tissues of the intestine and spleen appeared normal when treated with OLE. According to the current findings, diets containing 2% kg<sup>-1</sup> of OLE were effective feed additives and may help in enhancement of utilization of food, organo-somatic indices, and some biochemical items of Nile tilapia.

**KEYWORDS:** Hapas, Olive Leaf Extract, Physiological and Histological Status.

### INTRODUCTION

Tilapias are a species of fish that are farmed all over the world; in 2023, they produced about 6.7 million tons. El-Sayed and Fitzsimmons (2023) estimated that this industry is worth more than 14.1 billion USD. However, aquaculture always faces problems in recent years, including a number of infectious diseases. Antibiotics have been used for both medicinal and preventative purposes. Therefore, finding environmentally friendly drugs for aquaculture is essential to ensure the sustainability of aquaculture and preventing infections.

In order to prevent and control the conditions, immune stimulants can also be a great substitute for antibiotics and even immunizations (Dawood *et al.*, 2018). Olives (*Olea europaea*) are evergreen shrubs belonging to the Oleaceae family (Gokdogan and Erdogan, 2018). The most significant natural source of oleuropein is found in olive leaves (OLE). The primary bioactive ingredients in OLE, polyphenolic compounds such oleuropein, hydroxytyrosol, and verbascositol, are known to have a variety of metabolic effects. According to several studies, oleuropein possesses a vast range of pharmacological properties, such as antiviral, antibacterial, anti-

inflammatory, and antioxidant properties (Visioli *et al.*, 2002). In addition to its anti-carcinogenic, anti-atherosclerotic, and anti-inflammatory qualities, oleuropein has a potent antioxidant impact due to its ability to link endogenous peptides (Gikas *et al.*, 2007).

Previous studies have demonstrated a strong link between fish disease resistance and herbal feed additives, which impact the immune system (Mohanty and Sahoo, 2010). Nonetheless, the impact of OLE on carcass composition and growth rate (Sokooti *et al.*, 2021). According to Baba *et al.* (2018), *Oncorhynchus mykiss* survival rate and biochemical parameters are improved by OLE, particularly at 0.1%. OLE was used in the current study to assess its impacts on Nile tilapia growth rate, utilization of food, haematological items, biochemical assays, histological investigation, and carcass composition.

### MATERIALS AND METHODS

#### Experimental design:

This trial was conducted at the fish research centre of Arish University, Faculty of Environmental Agricultural Sciences in Egypt.

After two weeks of acclimatization to the experimental settings, Nile tilapia fingerlings weighing  $11.82 \pm 0.02$  g and measuring  $8.80 \pm 0.10$  cm were fed commercial feed (Skirting Co. 30%) twice a day. Following that, 180 fish of Nile tilapia, *Oreochromis niloticus*, were divided into twelve hapas (three treatments with three replications, plus a control group), each hapa containing fifteen fish, and the volume of each hapa was one m<sup>3</sup>. By using air pumps, the hapas were supplied with compressed air. The fish were fed on food (30% crude protein) three times a day until satiated, and were habituated to the hapas once more for two weeks. For 90 days, the fish were fed a meal containing 1% kg<sup>-1</sup> (Group 1), 1.5% kg<sup>-1</sup> (Group 2), and 2% kg<sup>-1</sup> (Group 3) of OLE three times a day at 09:00, 13.00, and 17.00 h. Half of the water volume in each hapa was siphoned off and replaced every day with another fresh one, during the acclimatization and experimental period. Fluorescent light tubes were used to maintain a 12-hour light-and-dark cycle during the photoperiod. Every two weeks, the average fish's body weight in each hapa was calculated in order to assess their growth and modify the feeding schedule.

Using an automated probe (Hanna HI-2550), water quality parameters such as temperature, dissolved oxygen, and pH were measured every day. The results showed that the values were  $30.20 \pm 2^{\circ}\text{C}$ ,  $5.92 \pm 0.71$  mg/L, and  $6.90 \pm 0.50$ , respectively. We used a DREL 2000 spectrophotometer (Hach

Company, Loveland, CO, USA) to measure the total ammonia ( $0.25 \pm 0.1$  mg/L). According to Boyd and Tucker (2012), all of these items fell within the ideal ranges for fish farming. Fish were gathered from each hapa at the end of the experiment, counted, and weighed in order to determine the parameters of growth and utilization of food.

#### Experimental diet preparation:

In accordance with Pourghorban *et al.* (2022), in a clean, dry flask, 10 g of olive leaf plant were mixed with 50 ml of deionized water and thoroughly stirred. The flask was placed on a magnetic stirrer and swirled for twenty-four hours at 25°C in a dark room. The material was stirred and then filtered into a 50 ml volumetric flask using filter paper. The filtrate was transferred into a pre-sterilized container after passing through a sterile 0.22 µm filter. The filtrate was stored at -20°C in a dark bottle until it was needed.

Table (1) displays the approximate chemical makeup of a control diet that contains 30% crude protein. OLE was added to the experimental meals at concentrations of 0.0 % for the control group, 1%, 1.5%, and 2% kg<sup>-1</sup> for other groups. Then, after evenly spraying, mixing it for 30 minutes, and pelleting it (1–2 mm diameter), it was added to the diet's contents. For later use, the experimental diets were kept in plastic bottles at -4°C.

**Table1. The experimental diets ingredients and composition (% as dry mater basis)**

Diets				
Ingredients (%)	Control	1%OLE	1.5%OLE	2%OLE
Fish meal	20	20	20	20
Soybean meal	33	33	33	33
Yellow corn	23	23	23	23
Wheat bran	13	13	13	13
Soybean oil	6	6	6	6
Carboxymethylcellulose (CMC)	1	1	1	1
Vitamin and mineral premix <sup>1</sup>	4	4	4	4
OLE %/kg	0	1	1.5	2
Chemical composition (%)				
Dry matter	90.41	90.48	90.43	90.40
Crude protein	30.21	30.20	30.25	30.23
Crude lipid	3.48	3.49	3.45	3.49
Ash	6.18	6.12	6.14	6.10
Fiber	4.35	4.35	4.38	4.33
Nitrogen free extract	55.78	55.84	55.78	55.85
GE (KJ g <sup>-1</sup> ) <sup>2</sup>	18.31	18.33	18.31	18.33

1. Each kilogram of vitamin and mineral premix contained: (100 mg diet): ascorbic acid (vit C), 15.2 mg; thiamine HCl (vit B6), 1.1 mg; inositol, 39.5 mg; calcium, 1.25 mg; zinc, 1.0 mg; retinol (vit A), 1.5 mg; phosphorus, 3.5 mg; choline chloride, 3.5 mg; magnesium, 2.0 mg; copper, 1.0 mg; pyridoxine (vit B6), 1.3 mg; phospholipids, 3.5 mg; α-tocopherol acetate (vit E), 5.5 mg; folic acid, 0.4 mg; cholecalciferol (vit D3), 7.5 mg; cyanocobalamine (vit B12), 0.006 mg; riboflavin (vit B2), 1.5 mg; menadione sodium bisulphite (vit K3), 0.03 mg; manganese, 2.0 mg; Iodine, 2.0 mg; sodium, 1.0 mg; iron, 1.0 mg; nicotinic acid, 4.3 mg; biotin, 0.35 mg. 2. Gross energy, calculated based on 23.64, 39.54, and 17.57 KJ g<sup>-1</sup> for protein, lipids, and carbohydrates, respectively according to (Brett, 1973).

### **Growth indexes, utilization of food, and carcass formation:**

Total body length and weight of all fish in each hapa were measured separately at the end of the experiment. Weight gain (WG), specified growth rate (SGR), feed efficiency ratio (FER), feed conversion ratio (FCR), condition factor (K), protein efficiency ratio (PER), and survival rate (SR) were among the growth parameters and feed utilization that were assessed for every group.  $WG (\%) = [(WF - WI) / WI] \times 100$ ;  $SGR (\% \text{ body weight} / \text{day}) = [(\ln WF - \ln WI) / t] \times 100$ ;  $K (\%) = [(body \text{ weight (g)} / (body \text{ length (cm)}^3)] \times 100$ ;  $SR (\%) = (\text{final number of remaining fish} / \text{initial number offish}) \times 100$ ,  $FCR = \text{feed intake (g)} / \text{WG (g)}$ ;  $FER = \text{wet WG (g)} / \text{dry feed fed (g)}$ ;  $PER (\%) = \text{WG (g)} / \text{protein intake (g)}$ .  $WI$  = initial body weights,  $WF$  = final body weights (g) and  $t$  = the trial duration.

The AOAC (2005) method was used to analyze the feed and determine the carcass composition, which included moisture, protein, ash, fat, and carbohydrates. Crude protein and lipid levels were measured using the Kjeldahl and Soxhlet methods, respectively. A moisture analyzer was used to determine the amount of moisture. Additionally, the samples were burned for eight hours at 600°C to measure the amount of ash.

### **Organo-somatic indices:**

According to Schreck and Moyle (1990), the hepatosomatic index (HSI) was calculated as follows:  $HSI = 100 \times [\text{liver weight (g)} / \text{total body weight (g)}]$ . According to Ricker (1979), the viscerosomatic index (VSI) was calculated as follows:  $VSI = 100 \times [\text{visceral weight (g)} / \text{total body weight (g)}]$ . In accordance with Al-Hussaini (1947), relative gut length (RGL) was calculated as follows:  $RGL = \text{absolute gut length (cm)} / \text{total body length (cm)}$ .

### **Samples collection:**

A 3-ml sterile syringe was used to draw blood samples from fish heart at the end of the trial. A part of the blood was then put into heparinized vials for haematological purposes, and the remaining blood was centrifuged for 10 minutes at 3000 rpm to extract serum and kept at -20°C for later use.

### **Haematological parameters:**

A hemocytometer was used to count white blood cells (WBCs) and red blood cells (RBCs) in accordance with Brown (1980). The most common method, as described by Brown (1980), was used to determine hemoglobin (Hb) and hematocrit (Hct) values. The platelets count (PLT) was determined using the Brecher *et al.* (1953) method. Additionally, an Olympus microscope was used to count eosinophils, neutrophils, lymphocytes, and monocytes. Using the usual formulas, blood indices mean corpuscular volume (MCV), mean corpuscular

hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated.  $MCV (fl) = (\text{hematocrit value} \times 10) / \text{RBCs (number} \times 10^{12} L^{-1})$ ,  $MCH (pg) = (\text{hemoglobin value} \times 10) / \text{RBCs (number} \times 10^{12} L^{-1})$ , and  $MCHC (g / dl) = (\text{hemoglobin value} \times 100) / \text{hematocrit value}$ .

### **Biochemical analysis:**

The method of Trinder (1969) was used to assess the blood glucose level. The technique reported by Fossati and Prencipe (1982) was used to determine triglycerides levels in blood. The methods of Henry (1964) and Doumas *et al.* (1971) were used to measure total protein (TP) and albumin (ALB), respectively. By deducting the values of ALB from the TP, the serum globulin (GLO) was calculated. Using laboratory kits from Biodiagnostic Co., Giza, Egypt and in compliance with Reitman and Frankel (1957) method, the activities of aminotransferases (ALT and AST) were assessed. The activity of alkaline phosphatase (ALP) was measured in accordance with Belfield and Goldberg (1971).

### **Digestive enzymes activities:**

Diagnostic reagent kits for fish from Cusabio Biotech Co. Ltd., Wuhan, Hubei, China were used to measure the digestive enzyme activity in the serum. Serum amylase in accordance of Bernfeld (1955), lipase (Shihabi and Bishop, 1971), and protease (Ross *et al.*, 2000), activities were assessed.

### **Histopathological examination:**

Each group's liver, spleen, and small intestine tissue samples were carefully removed, labelled, and preserved in 10% neutral buffered formalin. They were prepared for paraffin embedding then sectioned to a four µm thickness. Haematoxylin and eosin (H&E) were used to stain the sections. A LABOMED CxL series light microscope (LABOMED Inc., USA) was used to examine the specimens, and a TouPCam U3CMOS05100KPA digital camera (ToupTek Photonics, Hangzhou, Zhejiang, P. R. China) was used to take pictures. The H & E-stained slides were subjected to blind review for routine histological examination.

### **Statistical analysis:**

According to Dytham (2011), the data was examined using a one-way analysis of variance using the SPSS software version 20 (SPSS, Richmond, VA, USA).

## **RESULTS**

### **Growth indexes, utilization of food and carcass composition:**

Growth parameters of *O. niloticus* fed different amount of OLE are presented in Table (2). Weight gain, specific growth rate, and length gain value were

significantly higher ( $P \leq 0.05$ ) in group three (2% kg<sup>-1</sup> of OLE) compared to group 1 (1% kg<sup>-1</sup> of OLE) and group two (1.5% kg<sup>-1</sup> of OLE). In group 3, the WG, SGR, and LG values were 10.88±0.71 g fish<sup>-1</sup>, 0.72±0.08 % day<sup>-1</sup>, and 2.10±0.15 cm fish<sup>-1</sup>, respectively. Whereas, the WG, and SGR values, in group one were 5.75±0.97 g fish<sup>-1</sup>, and 0.43±0.06 % day<sup>-1</sup>, respectively. The minimum value of LG (1.37±0.20 cm fish<sup>-1</sup>) was recorded in group two. On the contrary, FBW, SR, and K were not remarkably affected by the trail diets.

Table (3) shows feed consumption and carcass composition findings for *O. niloticus* fed meals with

various concentrations of OLE. FI, PI, CP, and ash content values were noticeably higher ( $P \leq 0.05$ ) in group three (2% kg<sup>-1</sup> of OLE) than in the other groups. However, there were no discernible changes among treatments in utilization of food. Group three (2% kg<sup>-1</sup>) had the lowest moisture content (71.13±0.18%) compared to (73.62±0.14%) in control group. The carcass compositions (crude protein, moisture, and ash content), were basically different ( $P \leq 0.05$ ). Diets including OLEs had no effect on the levels of FCR, FER, PER and crude lipid.

**Table 2. Means and standard error for growth performance and survival rate of Nile tilapia feed a diet with various levels of OLE for 90 days**

Parameters	Diets			
	Control	1%OLE	1.5% OLE	2%OLE
IBW <sup>1</sup> (g fish <sup>-1</sup> )	11.82±0.05	11.82±0.05	11.82±0.05	11.82±0.05
FBW <sup>2</sup> (g fish <sup>-1</sup> )	18.73±0.47	17.58±0.99	19.83±0.27	22.70±0.73
WG <sup>3</sup> (g fish <sup>-1</sup> )	6.55 <sup>ab</sup> ±0.50	5.75 <sup>b</sup> ±0.97	8.01 <sup>ab</sup> ±0.63	10.88 <sup>a</sup> ±0.71
SGR <sup>4</sup> (%day <sup>-1</sup> )	0.49 <sup>b</sup> ±0.03	0.43 <sup>b</sup> ±0.06	0.56 <sup>ab</sup> ±0.11	0.72 <sup>a</sup> ±0.08
SR <sup>5</sup> (%)	88.89±2.22	93.33±3.84	88.67±2.66	91.11±2.22
IBL <sup>6</sup> (cm fish <sup>-1</sup> )	9.00±0.00	8.73±0.27	8.90±0.10	8.57±0.30
FBL <sup>7</sup> (cm fish <sup>-1</sup> )	10.50±0.00	10.73±0.33	10.27±0.21	10.67±0.40
LG <sup>8</sup> (cm fish <sup>-1</sup> )	1.50 <sup>ab</sup> ±0.00	2.00 <sup>a</sup> ±0.25	1.37 <sup>b</sup> ±0.20	2.10 <sup>a</sup> ±0.15
K <sup>9</sup>	1.58±0.04	1.42±0.07	1.86±0.27	1.91±0.30

The different letters at same row indicate significant difference ( $P \leq 0.05$ ). 1. Initial body weight, 2. Final body weight, 3. Weight gain, 4. Specific growth rate, 5. Survival rate, 6. Initial body length, 7. Final body length, 8. Length gain, 9. Condition factor.

**Table 3. Means and standard error for feed utilization and carcass composition of Nile tilapia feed a diet with various levels of OLE for 90 days**

Parameters	Diets			
	Control	1%OLE	1.5%OLE	2%OLE
FI <sup>1</sup> (g fish <sup>-1</sup> )	9.13 <sup>b</sup> ±0.30	9.31 <sup>b</sup> ±0.12	10.04 <sup>ab</sup> ±0.84	10.43 <sup>a</sup> ±0.19
FCR <sup>2</sup>	1.41±0.12	1.70±0.25	1.39±0.27	1.01±0.17
FER <sup>3</sup>	0.72±0.06	0.62±0.01	0.78±0.17	1.04±0.16
PI <sup>4</sup>	2.74 <sup>b</sup> ±0.09	2.79 <sup>ab</sup> ±0.04	3.01 <sup>a</sup> ±0.14	3.13 <sup>a</sup> ±0.31
PER <sup>5</sup>	2.40±0.19	2.05±0.33	2.61±0.55	3.48±0.48
Moisture (%)	73.62 <sup>a</sup> ±0.14	72.94 <sup>b</sup> ±0.03	72.63 <sup>b</sup> ±0.26	71.13 <sup>c</sup> ±0.18
Crude protein (%)	61.86 <sup>c</sup> ±0.40	62.18 <sup>c</sup> ±0.31	62.98 <sup>b</sup> ±0.01	63.62 <sup>a</sup> ±0.14
Crude lipid (%)	5.47±0.21	5.59±0.22	5.32±0.13	5.79±0.03
Ash (%)	1.43 <sup>c</sup> ±0.04	1.65 <sup>b</sup> ±0.03	1.70 <sup>b</sup> ±0.04	1.88 <sup>a</sup> ±0.06

The different letters at same row indicate significant difference ( $P \leq 0.05$ ). 1. Feed intake, 2. Feed conversion rate, 3. Feed efficiency ratio, 4. Protein intake, 5. Protein efficiency ratio

#### Organo-somatic indices

Table (4) displays the organo-somatic index results for *O. niloticus* fed the different studied experimental meals. In comparison with control group, group 3 (2% kg<sup>-1</sup> of OLE) showed a significant

increase ( $P \leq 0.05$ ) in both the viscero-somatic index (VSI) and relative gut length (RGL). There were no differences in the hepato-somatic index (HSI) among the trail groups.

**Table 4. Means and standard error for organo-somatic indices of Nile tilapia feed a diet with various levels of OLE for 90 days**

Parameters	Diets			
	Control	1%OLE	1.5%OLE	2%OLE
HSI <sup>1</sup>	2.25±0.05	2.62±0.44	2.61±0.22	2.64±0.18
VSI <sup>2</sup>	6.15 <sup>c</sup> ±0.09	6.19 <sup>c</sup> ±0.06	6.99 <sup>b</sup> ±0.06	7.85 <sup>a</sup> ±0.08
RGL <sup>3</sup>	296.66 <sup>c</sup> ±2.40	299.66 <sup>c</sup> ±2.60	358.33 <sup>b</sup> ±2.87	462.66 <sup>a</sup> ±0.66

The different letters at same row indicate significant difference ( $P \leq 0.05$ ). 1. Hepato-somatic index, 2. Viscero-somatic index and 3. Relative gut length

**Haematological parameters:**

Table (5) displays the haematological parameters of *O. niloticus* fed the studied experimental diets of OLE. WBCs, monocytes, eosinophils, and MCH were significantly difference ( $P \leq 0.05$ ) among the trail groups. Group one (1% kg<sup>-1</sup> of OLE) recorded the maximum values of WBCs count ( $19.89 \pm 0.04$ ) ( $\times 10^3$  cell/ mm<sup>3</sup>) and monocytes value ( $5.75 \pm 0.60\%$ ), while the minimum value of WBCs count was recorded in group two (1.5% kg<sup>-1</sup> of OLE) Of ( $19.34 \pm 0.05$ ) ( $\times 10^3$  cell/ mm<sup>3</sup>) and the lowest value

of monocytes value ( $3.74 \pm 0.01\%$ ) was recorded in group three. The values of eosinophil and MCH rose in group three relative to other groups. The lowest eosinophil value ( $1.15 \pm 0.05\%$ ) was recorded in group one and the minimum value of MCH ( $16.79 \pm 0.16$  pg.) was recorded in group two. The current trail diets had no effect on *O. niloticus* RBCs count, platelet count, lymphocytes, neutrophils, haemoglobin, haematocrit, MCV, and MCHC values.

**Table 5. Means and standard error for haematological parameters of Nile tilapia feed a diet with various levels of OLE for 90 days**

Parameters	Diets			
	Control	1%OLE	1.5%OLE	2%OLE
Red blood cells ( $\times 10^6$ cell/ mm <sup>3</sup> )	1.53 $\pm$ 0.10	1.39 $\pm$ 0.15	1.53 $\pm$ 0.16	1.28 $\pm$ 0.02
White blood cells ( $\times 10^3$ cell/mm <sup>3</sup> )	19.55 <sup>ab</sup> $\pm$ 0.25	19.89 <sup>a</sup> $\pm$ 0.04	19.34 <sup>b</sup> $\pm$ 0.05	19.62 <sup>ab</sup> $\pm$ 0.02
Lymphocytes (%)	84.70 $\pm$ 2.13	82.75 $\pm$ 0.60	89.65 $\pm$ 0.37	89.00 $\pm$ 0.57
Neutrophils (%)	8.40 $\pm$ 0.67	10.35 $\pm$ 0.97	4.70 $\pm$ 0.06	5.33 $\pm$ 0.51
Monocytes (%)	5.70 <sup>a</sup> $\pm$ 0.80	5.75 <sup>ab</sup> $\pm$ 0.60	4.35 <sup>ab</sup> $\pm$ 0.54	3.47 <sup>b</sup> $\pm$ 0.01
Eosinophils (%)	1.20 <sup>b</sup> $\pm$ 0.00	1.15 <sup>b</sup> $\pm$ 0.05	1.30 <sup>b</sup> $\pm$ 0.11	2.20 <sup>a</sup> $\pm$ 0.06
Platelet count	57.50 $\pm$ 0.28	55.00 $\pm$ 0.73	56.00 $\pm$ 0.15	56.50 $\pm$ 0.86
Haemoglobin (g/dl)	2.75 $\pm$ 0.09	2.52 $\pm$ 0.25	2.57 $\pm$ 0.10	2.85 $\pm$ 0.02
Haematocrit (%)	16.73 $\pm$ 0.13	16.71 $\pm$ 0.29	16.90 $\pm$ 0.23	16.45 $\pm$ 0.03
MCV (fl)	109.45 $\pm$ 8.41	120.22 $\pm$ 5.14	110.46 $\pm$ 3.71	128.51 $\pm$ 1.96
MCH (pg)	17.97 <sup>b</sup> $\pm$ 0.81	18.13 <sup>b</sup> $\pm$ 0.28	16.79 <sup>b</sup> $\pm$ 0.16	22.27 <sup>a</sup> $\pm$ 0.52
MCHC (g/dl)	16.44 $\pm$ 0.38	15.08 $\pm$ 0.51	15.21 $\pm$ 0.80	17.33 $\pm$ 0.14

The different letters at same row indicate significant difference ( $P \leq 0.05$ )

**Biochemical analysis and digestive enzymes activities:**

Table (6) displays the biochemical parameter and digestive enzyme values that were obtained at the end of the experiment. Parameters values showed that the glucose levels in all experimental groups did not differ significantly. Nevertheless, 2% OLE (Group 3)

had significantly greater levels of total protein and globulin, as well as ALT, AST, alkaline phosphatase, amylase, lipase, and protease activities ( $P \leq 0.05$ ) than the other groups. In comparison with control group, all groups fed OLE meals showed lower serum albumin and triglyceride levels.

**Table 6. Means and standard error for biochemical analysis and digestive enzymes activities of Nile tilapia feed a diet with various levels of OLE for 90 days**

Parameters	Diets			
	Control	1%OLE	1.5%OLE	2%OLE
Glucose (mg/dl)	2.26 $\pm$ 0.99	2.32 $\pm$ 0.15	2.52 $\pm$ 0.17	2.16 $\pm$ 0.03
Triglycerides (mg/dl)	190.50 <sup>a</sup> $\pm$ 0.29	171.50 <sup>b</sup> $\pm$ 0.87	155.50 <sup>c</sup> $\pm$ 0.28	144.50 <sup>d</sup> $\pm$ 0.28
Total protein (mg/dl)	3.60 <sup>b</sup> $\pm$ 0.06	3.14 <sup>c</sup> $\pm$ 0.01	3.54 <sup>b</sup> $\pm$ 0.01	3.75 <sup>a</sup> $\pm$ 0.29
Albumin (mg/dl)	2.38 <sup>a</sup> $\pm$ 0.02	1.66 <sup>c</sup> $\pm$ 0.01	2.10 <sup>b</sup> $\pm$ 0.05	1.71 <sup>c</sup> $\pm$ 0.08
Globulin (g/dl)	1.22 <sup>b</sup> $\pm$ 0.06	1.48 <sup>b</sup> $\pm$ 0.05	1.44 <sup>a</sup> $\pm$ 0.03	2.04 <sup>a</sup> $\pm$ 0.03
ALT (U/l)	112.00 <sup>c</sup> $\pm$ 0.58	101.50 <sup>d</sup> $\pm$ 0.86	156.50 <sup>b</sup> $\pm$ 0.28	251.50 <sup>a</sup> $\pm$ 0.86
AST (U/l)	133.50 <sup>c</sup> $\pm$ 0.28	111.00 <sup>d</sup> $\pm$ 0.58	219.00 <sup>b</sup> $\pm$ 0.58	247.50 <sup>a</sup> $\pm$ 0.87
Alkaline phosphatase (mg/dl)	35.65 <sup>b</sup> $\pm$ 0.14	31.15 <sup>c</sup> $\pm$ 0.28	31.95 <sup>c</sup> $\pm$ 0.84	52.20 <sup>a</sup> $\pm$ 0.98
Amylase (U/l)	48.75 <sup>c</sup> $\pm$ 0.15	43.50 <sup>d</sup> $\pm$ 0.29	76.68 <sup>b</sup> $\pm$ 0.76	83.20 <sup>a</sup> $\pm$ 0.17
Lipase (U/l)	32.95 <sup>c</sup> $\pm$ 0.26	27.90 <sup>d</sup> $\pm$ 0.29	34.80 <sup>b</sup> $\pm$ 0.11	36.00 <sup>a</sup> $\pm$ 0.58
Protease (U/l)	14.54 <sup>d</sup> $\pm$ 0.30	17.24 <sup>c</sup> $\pm$ 0.06	20.50 <sup>b</sup> $\pm$ 0.30	33.60 <sup>a</sup> $\pm$ 0.10

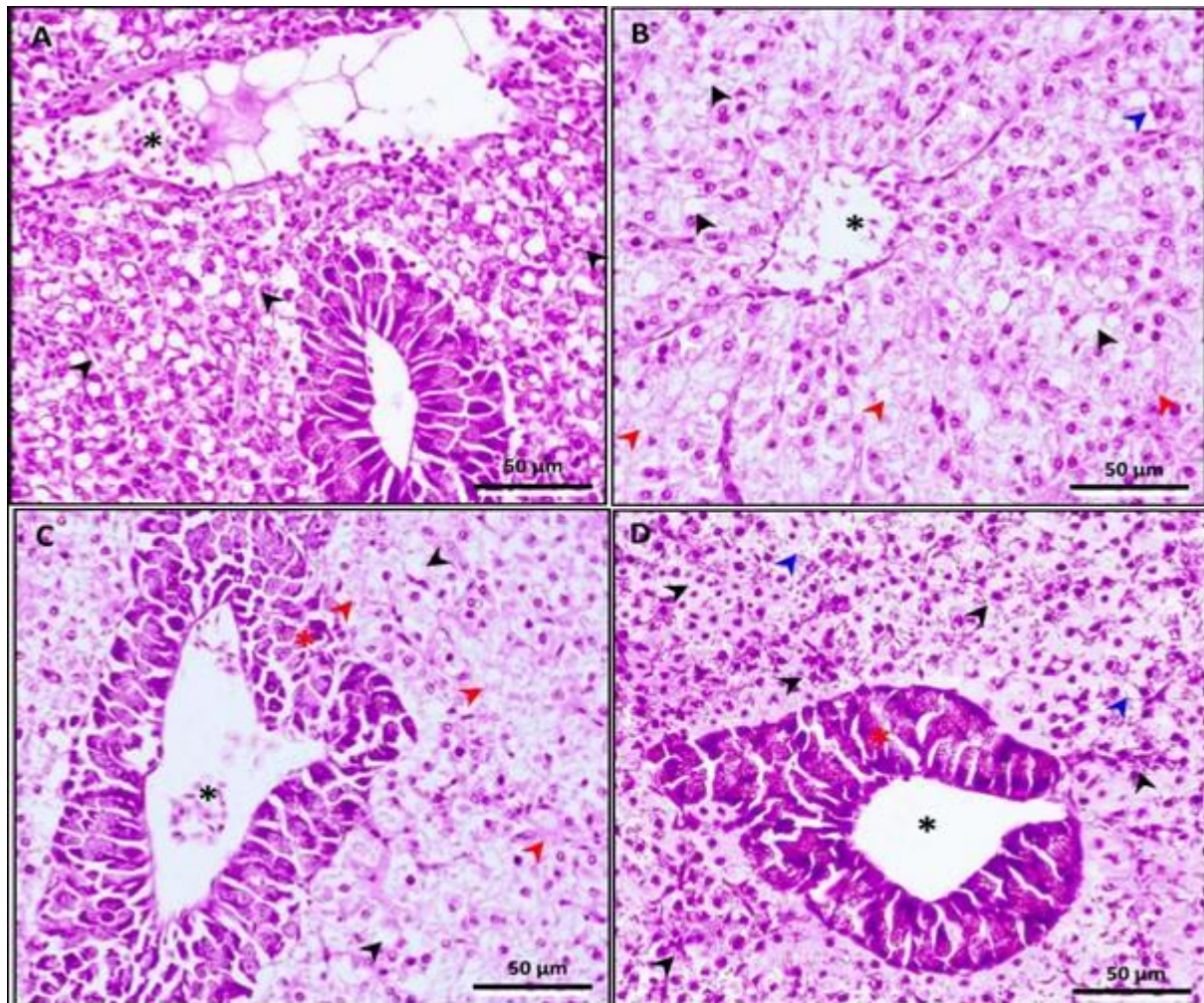
The different letters at same row indicate significant difference ( $P \leq 0.05$ ).

**Histological examination:****Liver:**

Liver samples from each group are shown in Figure (1). The control group displayed moderate hepatocyte vacuolation and normal hepatic vein anatomy (Fig. 1A).

Group 1 (1% OLE) displayed moderate inflammatory cell infiltration, moderate necrosis, and moderate hepatocyte vacuolation (Fig. 1B). Figures 1C (1.5% OLE group 2) and figure 1D (2% OLE group 3) showed moderate necrosis and lipid storage and normal hepatopancreatic tissue surrounding a hepatic vein.





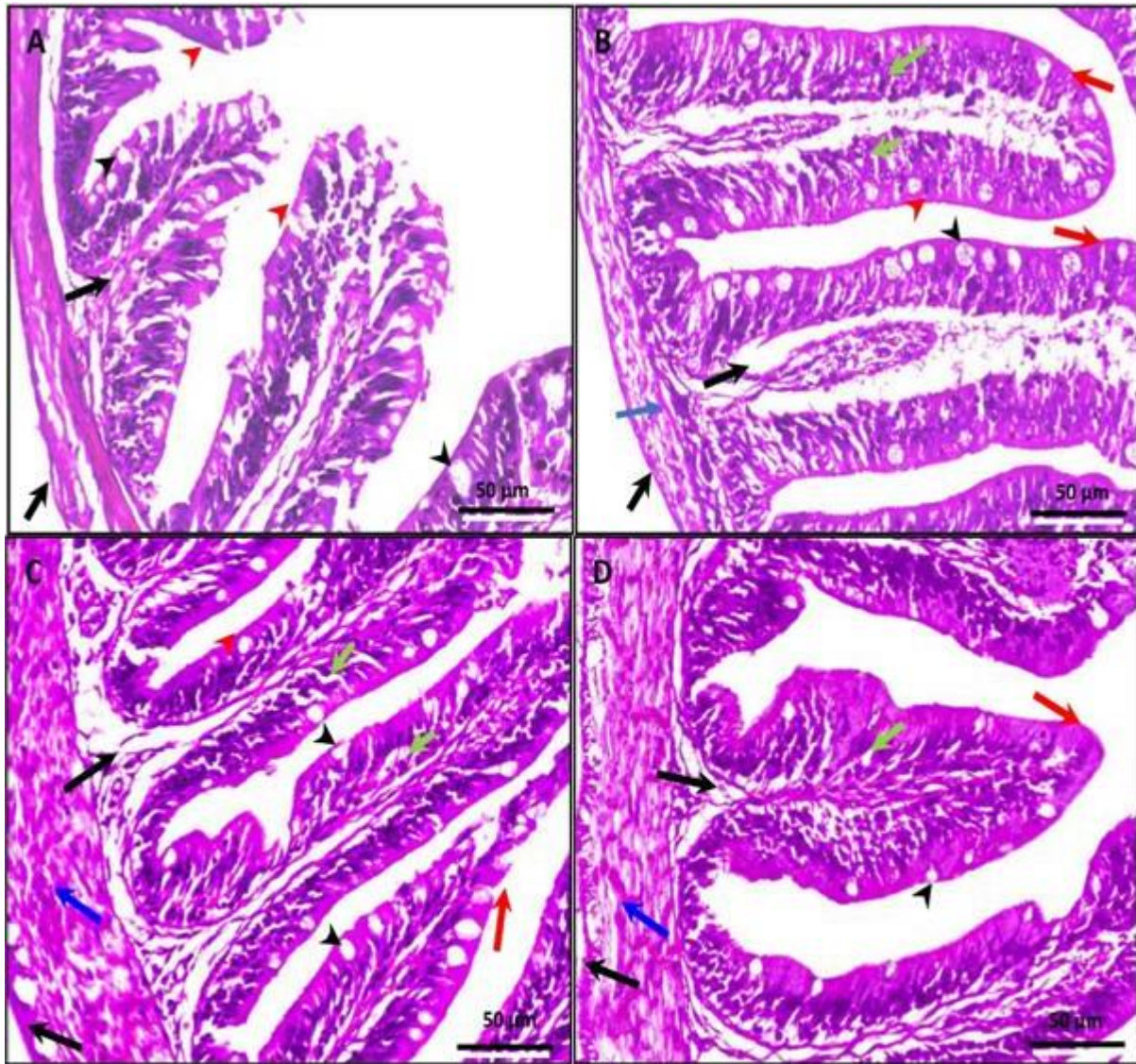
**Figure 1:** Histopathological alterations observed in the liver of *O. niloticus* showed moderate hepatocyte vacuolation (black arrowhead), hepatic veins (black star), moderate inflammatory cell infiltration (blue arrowhead) and moderate necrosis (red arrowhead), lipid storage along with normal consistent hepatopancreatic tissue (red star) surrounding a hepatic vein (black star); A control, B (1% OLE group), C (1.5% OLE group), D (2% OLE group), (H&E stain,  $\times 400$ , scale bar 50  $\mu\text{m}$ ).

#### **Intestine:**

The small intestine tissue architecture was normal in all the current treated groups. A well-organized epithelium with intact villi and a regular distribution of goblet cells is preserved by the

mucosal layer. The anatomy of the muscularis, serosa, and submucosa layers is entirely typical (Fig. 2).





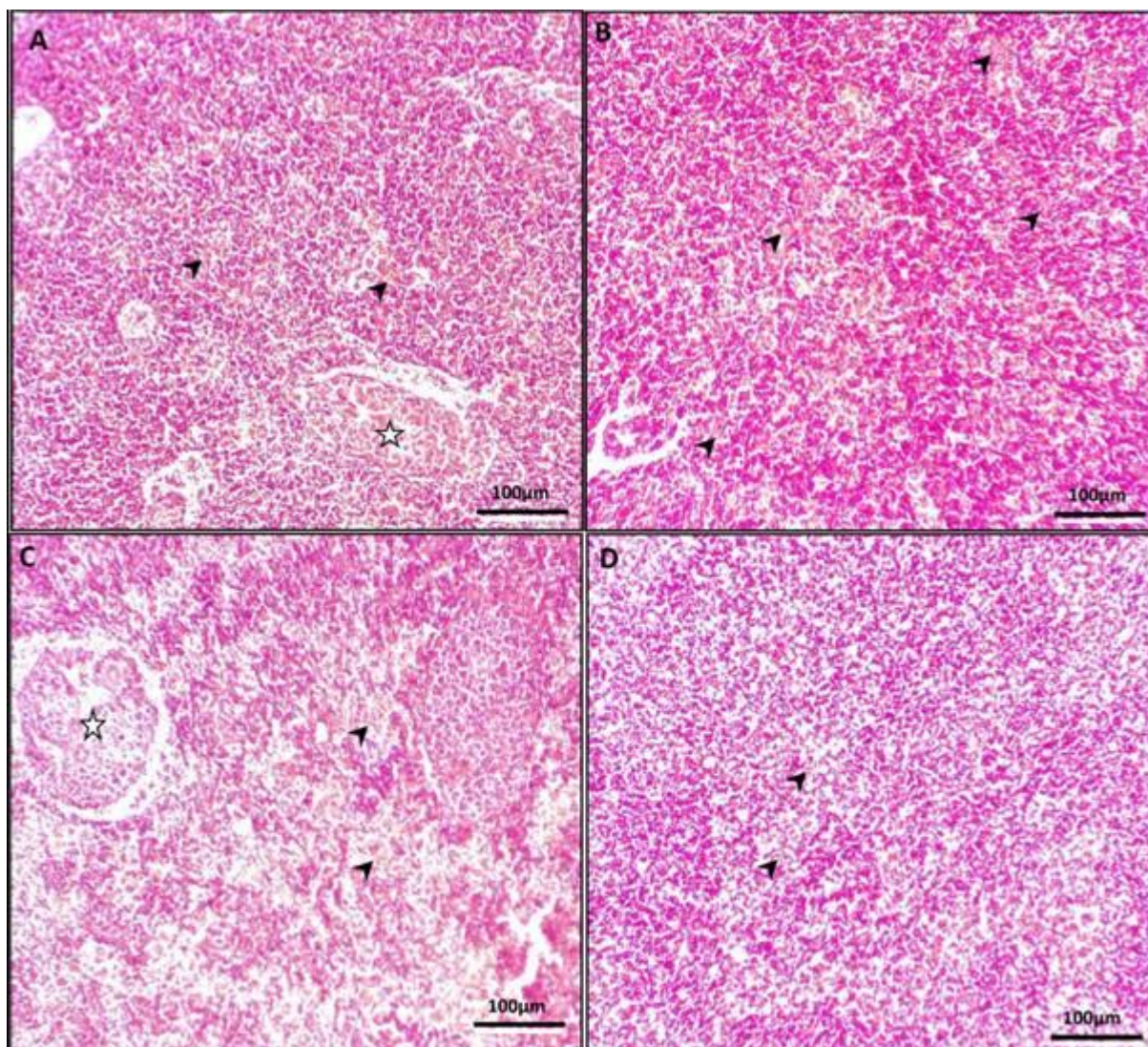
**Figure 2: Histopathological sections in the intestine of *O. niloticus*. Illustrates moderate goblet cell proliferation (black arrowhead) and notably moderate villi (red arrowhead) (A). Presents normal intestinal tissue architecture, where the mucosal layer maintains a well-structured epithelium (green arrow) with intact villi (red arrow) and a normal distribution of goblet cells (black arrowhead), the submucosa (black arrow), muscularis (blue arrow) and layers all exhibit typical structural integrity (B), (C) and (D). A control, B (1% OLE group), C (1.5% OLE group), D (2% OLE group), (H&E stain,  $\times 400$  magnification, scale bar 50  $\mu\text{m}$ ).**

### Spleen:

The red pulp and white pulp in the spleen tissue did not clearly separate in any of the treatment groups. Numerous congested sinusoids packed with red blood cells and a network of splenic cords made up the red pulp. The splenic parenchyma displays a variety of melanomacrophage centers at different

reaction stages, with varying quantities of OLE. The white pulp was distinguished by lymphoid sheaths, which were infiltrated and encircled by tiny branches of the central arteries. Melanomacrophage centers are quite abundant, taking up a lot of space in the spleen and aggregating widely (Fig. 3).





**Figure 3:** Histopathological sections in spleen of *O. niloticus* revealed a spectrum of melanomacrophage centers at various response stages, with differing concentrations of (olive leaf extract). The exhibits an extensive positive presence of melanomacrophage centers, occupying substantial areas of the spleen (black arrowhead) and forming large aggregates (star); A control, B (1% OLE group), C (1.5% OLE group), D (2% OLE group). (H&E stain,  $\times 100$ , scale bar 100  $\mu\text{m}$ ).

## DISCUSSION

The current study showed that, after 90 days, meals containing various amounts of OLE had a positive effect on growth parameters and utilization of food. Fish fed 2%  $\text{kg}^{-1}$  of OLE (Group 3) in the present study had an appreciable increase in their WG, SGR, and LG compared to group one and group two. Additionally, group three showed a notable improvement in FI, PI, CP, and ash content values. These findings suggest that OLEs may increase fish growth and utilization of food indices.

Baba *et al.* (2018) found no discernible differences in the growth indices of *Oncorhynchus mykiss* fed diets with amounts of OLE. Fish species treated with varying amounts of OLE may all have an impact on the disparate findings from different studies on fish performance (Sokooti *et al.*, 2021). The majority of studies also found that adding herbal extract to the food enhances the growth performance of Mozambique tilapia (Acar *et al.*, 2015), mrigal carp (Kumar *et al.*, 2017), and Nile tilapia (Aanyu *et al.*, 2018).



Since OLE has anti-nutritional effects, Zemheri-Navruz *et al.* (2020) noticed that weight gain decreased as the amount of OLE increased in diets.

The current results aligned with the findings of Baba *et al.* (2018) and Zemheri-Navruz *et al.* (2019, 2020), who verified enhanced protein, fat, and carbohydrate digestibility in fish given 0.1% OLE. For this reason, fish development performance in relation to food derived from feed and digestive enzymes are often helpful instruments (Furne *et al.*, 2005). To enhance growth efficiency rate, and survival rate, farmed fish are commonly fed a variety of additional additives including algae and plant extracts (Moustafa *et al.*, 2020; Dawood *et al.*, 2021, and Elbially *et al.*, 2021).

In the present study, all groups' carcass lipid contents, however, were not substantially affected. Group three (2% kg<sup>-1</sup> of OLE) has a noticeably greater VSI and RGL. However, OLE treatments had no effect on HSI. According to Sokooti *et al.* (2021), nutritional supplementation with OLE reduced body fat in *Cyprinus carpio* while having no effect on protein, hydration, or ash. Xu *et al.* (2020) discovered combination of Chinese herbal medications had no effect on ash, protein, or moisture in *Lateolabrax japonicas*.

In the current study, WBCs count, monocytes, eosinophils, and MCH were significantly difference in the trail groups. Fish health, stress, and illness are typically evaluated using haematological indicators (Yilmaz *et al.*, 2019). Thus, monitoring haematological indicators can reveal how well fish's immune systems are responding to dietary changes (Fazio, 2019).

In this study, we observed that group 1 has higher WBC counts compared to group two. Baba *et al.* (2018) discovered that all three groups of 0.1, 0.25 and 0.50 % had considerably higher WBC counts, with the 1% OLE group experiencing the greatest rise. White blood cells are thought to play a crucial role in innate or non-specific immunity. Aquatic creatures' health can be determined by their quantity or activity (Secombes, 1990). Thus, it is hypothesized that the use of OLE diets may be the cause of this increase in WBC stimulation. Numerous research, including those by Fayed *et al.* (2019); Ebrahimi *et al.* (2020), and de Souza *et al.* (2020), have indicated that herbal extracts have a favourable impact on fish haematological parameters. In the comparison with each other, the obtained haematological parameters in our study demonstrate that feeding fish diets containing extracts from olive leave groups different significantly, but still around the haematological levels of control group.

In all the current experimental groups, the biochemical parameters were altered by the OLE extracts employed in the present investigation. Significant information about fish health can be obtained from biochemical markers. Diets with varying amounts of OLE had no effect on the serum glucose levels of *O. niloticus* in this study. The results are comparable to those reported by Baba *et al.* (2018), who documented that no considerable change was noticed in the blood glucose levels of *O. mykiss* fed on different levels of OLE diets. On the other hand, Zemheri-Navruz *et al.* (2019) found that *C. carpio* fed meals with 0.5% and 1% OLE had significantly higher blood glucose levels. Utilizing natural products as a potential immunostimulant in the diet showed a lower serum glucose levels in many fish species (Acar *et al.*, 2015). During stressful situations, glucose levels were thought to be particular markers of sympathetic activity.

The current study demonstrated a considerable decrease in triglyceride levels compared to control group. According to the same line, the *C. carpio* cholesterol and triglyceride levels were found to be low, suggesting that feeding them OLEs likely had a hypolipidemic effect (Sokooti *et al.*, 2021). Because of its phenolic components, olive oil can inhibit the liver's capacity to produce lipids (Jemai *et al.*, 2008). Thus, the present study in agreement with Mohamed *et al.* (2020) who concluded that the decrease in triglycerides was probably due to the enhanced flow of fatty acids from the adipose tissue to the liver.

The physical state of aquatic animals, stress levels, and overall health are assessed using total protein as a clinical indicator (Hoseini *et al.*, 2018). The two main serum proteins that play a significant role in the immune system are albumin and globulin (Wiegertjes *et al.*, 1996). Similar findings in (Sokooti *et al.*, 2021) showed that *C. carpio* fed meals containing OLE had higher levels of total protein, and globulin, which was likely a sign of an improved non-specific immune response. The current findings are in agree with those of Baba *et al.* (2018), who discovered that feeding *O. mykiss* a meal containing 0.5% OLEs considerably raised its levels of total protein. Oleuropein is one of the key components of OLE. By enhancing anti-inflammatory, antioxidant, antiviral, and antibacterial properties, this plant polyphenol contributes significantly to immune system promotion (Thawonsuwan *et al.*, 2010).

ALP activity increased in the fish treated with OLE (Group 3), according to the current data. In parallel with our research, Wang *et al.* (2015) reported that ALP plays immunological roles in zebra fish. The liver enzyme levels in Nile tilapia are positively impacted; nevertheless, in some OLE groups, the ALT and AST levels in Baba *et al.* (2018) study have significantly decreased.

Andreeva (1999) reported the level of ALT decreased in *Acipenser baerii* fed bovine lactoferrin. According to a study on the reduction of hepatic enzymes, rainbow trout's nutritional status was unaffected by OLE food supplementation (Baba *et al.*, 2018).

OLE secoiridoids, particularly oleuropein and its derivatives, are potent antioxidant chemicals, according to Assar *et al.* (2023).

The 2% kg<sup>-1</sup> OLE (Group 3) in this study exhibited the highest levels of the digestive enzymes lipase, protease, and amylase activity. Similar results were documented by Sokooti *et al.* (2021), who reported the OLE 0.1 group on *C. carpio* had the highest activity of lipase, protease, and amylase. In a different study, *Quillaja saponin* raised activity of amylase and trypsin in common carp (*C. carpio*) (Francis *et al.*, 2002). According to Wenk (2002), phenolic chemicals have the ability to eradicate harmful microorganisms from the digestive tract. Through typical, consistent hepatopancreatic tissue around a hepatic vein, the addition of OLE inclusion demonstrated a hepatoprotective role in the current investigation. The mucosal layer in the intestine keeps the epithelium well-structured, with intact villi and a regular distribution of goblet cells. The anatomy of the muscularis, serosa, and submucosa layers is all typical. Melanomacrophage centers are found in considerable quantity in the spleen, taking up a lot of space and aggregating into enormous clusters (Zapata and Amemiya, 2000).

Assar *et al.* (2023) found histopathological changes in the hepatopancreas in fish groups fed OLE with *Aeromonas hydrophila* infection. Other research have documented the antiapoptotic properties of olive leaf phenolics. It has been demonstrated that OLE rich in oleuropein and hydroxytyrosol decreases apoptosis of liver and lipid metabolism in rats (Mahmoudi *et al.*, 2018).

Furthermore, by positively affecting inflammation and gut microbiota, the OLE altered intestinal epithelial homeostasis (Deiana *et al.*, 2018). By lowering the production of intracellular ROS and nitrites caused by lipopolysaccharides, the unsaponifiable fraction of extra virgin olive oil showed anti-inflammatory and antioxidant qualities (Cardeno *et al.*, 2014).

## CONCLUSION

Nile tilapia may be able to use food better if they are fed a diet with 2% kg<sup>-1</sup> olive leaf extract for ninety days. This will improve their organo-somatic indices and some biochemical items. Future research would be need to employ a high concentration of OLE in many fish species and with varying protein

diet concentrations, as this study used only one experimental paradigm.

## ETHICS STATEMENT

All national and institutional guideline for the use and care of lab animals was adhered to. Arish University institutional ethics committee granted consent for this work under approval number #ARU/agri.27#.

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## تأثير مستخلص أوراق الزيتون على أداء النمو والإستفادة من الغذاء والحالة الفسيولوجية لأسماك البلطي النيلي

هبة السيد عبد النبي<sup>١</sup>، وداد الشريف<sup>٢</sup>، تبارك محمد<sup>٢</sup>، رواء حسني<sup>٢</sup>، غدير علي دقنق<sup>١</sup>

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تهدف الدراسة إلى معرفة تأثير مستخلص أوراق الزيتون على أداء النمو ومدى الإستفادة من الغذاء والحالة الفسيولوجية والنسجية لأسماك البلطي النيلي. تم وضع ١٨٠ سمكة بلطي نيلية بشكل عشوائي، بمتوسط وزن جسم  $11.82 \pm 0.02$  جم ومتوسط طول جسم  $8.80 \pm 0.10$  سم في اثني عشر هابا بحجم واحد متر مكعب، تلقت المجموعة الأولى ١٪ من مستخلص أوراق الزيتون لكل كجم، وتلقت المجموعة الثانية ١.٥٪ من مستخلص أوراق الزيتون لكل كجم، وتلقت المجموعة الثالثة ٢٪ من مستخلص أوراق الزيتون لكل كجم، ولم تتلق المجموعة الكنترول أي كميات من مستخلص أوراق الزيتون وإستمرت التجربة لمدة تسعين يوماً. وفي نهاية التجربة، كان لدى المجموعة الثالثة زيادة في الوزن المكتسب وزيادة في الطول المكتسب وقيم معدل النمو النوعي أعلى مقارنة بمجموعات التجربة الأخرى. بالإضافة إلى ذلك، أظهرت جميع مجموعات التجربة التي تغذت على كميات مختلفة من مستخلص أوراق الزيتون تحسناً في قيم تناول الغذاء وتناول البروتين. وكانت لدى المجموعات التي تغذت على النظام الغذائي في التجربة مستويات أقل للآلبومين والدهون الثلاثية في المصل والخلايا الأحادية مقارنة بالمجموعة الكنترول. وعند مقارنة مجموعات التجربة ببعضها، أظهرت المجموعة الثالثة (٢٪ / كجم مستخلص أوراق الزيتون) مستويات أعلى لقيم البروتين الكلي والجلوبيولين والفوسفاتيز القلوية ونشاط إنزيمات الكبد ALT وAST ونشاط إنزيمات الأميليز والليباز والبروتياز. كما أظهرت النتائج بعض التغيرات النسيجية في الكبد، وخاصة في المجموعة الأولى (١٪ / كجم مستخلص أوراق الزيتون)، في حين بدت أنسجة الأمعاء والطحال طبيعية عند معالجتها بمستخلص أوراق الزيتون. وفقاً للنتائج الحالية، كانت الوجبات التي تحتوي على ٢٪ / كجم من مستخلص أوراق الزيتون إضافات غذائية فعالة وقد تساعد في تعزيز الإستفادة من الغذاء، والمؤشرات العضوية الجسدية، وبعض العناصر البيوكيميائية لأسماك البلطي النيلي.