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Dietary oregano essential oil improved the growth performance via enhancing the intestinal morphometry and hepato-renal functions of common carp (*Cyprinus carpio* L.) fingerlings

Hany M.R. Abdel-Latif[a,*], Mohsen Abdel-Tawwab[b], Asmaa F. Khafaga[c], Mahmoud A.O. Dawood[d,e]

[a] Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Alexandria University, Edfina 22758, Behera province, Egypt
[b] Department of Fish Biology and Ecology, Central Laboratory for Aquaculture Research, Agriculture Research Center, Abbassa, Abo-Hammad, Sharqia, Egypt
[c] Department of Pathology, Faculty of Veterinary Medicine, Alexandria University, Edfina 22758, Behera province, Egypt.
[d] Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt
[e] School of Freshwater Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI 53204, USA

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ABSTRACT

The current work was designed to assess the influences of dietary oregano essential oil (OEO) on the indices of growth and feed utilization, proximate composition, hepato-renal functions, and histomorphological criteria of livers, kidneys, and intestines of common carp (*Cyprinus carpio* L.) fingerlings. Fish (20.3 ± 0.8 g) were randomly divided into five treatments and fed on different levels of OEO at 0, 5, 10, 15, and 20 g/kg diet for 2 months. The results showed that the dietary OEO significantly improved the growth parameters (final body weight, weight gain, weight gain %, specific growth rate, and feed intake) ($P < .05$) in a dose-dependent regime; meanwhile, the feed conversion ratio was not affected with respect to the control group. The body proximate composition and survival rates were not significantly affected. The serum protein profile (total protein, albumin, and globulin), activities of liver function enzymes (alanine transaminase aspartate transaminase, and alkaline phosphatase), and renal markers (creatinine and urea) were not significantly altered ($P < .05$) by OEO supplementation. The histomorphology of hepatopancreatic and renal tissues of common carp fed OEO at varying levels were correlated with the serum hepato-renal functions without any noticed pathologic lesions. Moreover, the intestinal villi with associated goblet cells and crypt, tunica muscularis (both internal and external muscular layers) and submucosal tissues appeared free of inflammatory and/or degenerative changes. All intestinal morphometric measurements revealed a significant improvement ($P < .05$) in OEO-fed fish in comparison with the control one. Conclusively, in common carp, the dietary OEO improved growth performance and intestinal histomorphology with no inflammatory signs, and with potential hepato-protective effects with the optimum level of 15 g/kg diet. These beneficial effects were possibly because of OEO-mediated beneficial improvements in the histomorphometric criteria of the fish intestines.

1. Introduction

In recent years, the aquaculture industry has been regarded as one of the fastest-growing food-producing sectors, which is greatly developed and contributes positively to worldwide food security (Newman, 2000). Common carp (*Cyprinus carpio* L.) is one of the world's most valuable and commercially important freshwater fish species that has been greatly increased in its global production (FAO, 2018). Accordingly, due to the requirements for a high production rate, the farmers applied an intensive aquaculture system that impaired the health status of fish (Dawood et al., 2020a). Therefore, it is highly recommended to apply feed additives as growth promotors to provide the nutritional requirements for the cultured fish (Abdel-Tawwab and Monier, 2018; Dawood et al., 2020b).

Plant-derivatives, or the so-called “phytogenics” (Dügenci et al., 2003) and plant extracts “phytobiotics” which are mainly obtained from aromatic plants and their essential oils, have been greatly utilized to enhance the growth performance of fish (Abdel-Latif and Khalil,

* Corresponding author.
E-mail addresses: hmhany@alexu.edu.eg (H.M.R. Abdel-Latif), mohsentawwab@gmail.com (M. Abdel-Tawwab), soma_path2008@yahoo.com (A.F. Khafaga), mahmoud.dawood@agr.kfs.edu.eg (M.A.O. Dawood).

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2014; Guardiola et al., 2016; Van Doan et al., 2019; Hoseinifar et al., 2020). Among the natural phytobiotic products with the potential of growth-promoting activity is oregano (*Origanum vulgare* L.), which has strong aromatic and medicinal advantages with potential antimicrobial activities (Oniga et al., 2018). The essential oils, obtained from *O. vulgare*, are mainly consisting of carvacrol and thymol; these phytochemicals comprise approximately 780–820 g/kg of the total essential oils (Sivropoulou et al., 1996). Besides, the remaining minor components from the total oil are the carvacrol precursor (ρ-cymene) (~50 g/kg) and the thymol precursor (γ-terpinene) (~70 g/kg) (Adam et al., 1998).

In fish, several researchers examined the beneficial effects of the whole leaves of oregano, *O. vulgare* extract meal or its essential oil (OEO) as dietary supplements to improve fish health and promote its growth rate. In this concern, it was found that dietary OEO-supplementation can considerably improve the growth and immune responses in several species of fish; for instance, channel catfish (*Ictalurus punctatus*) (Zheng et al., 2009), sturgeon (*Huso huso*) (Ahmadifar et al., 2014), Nile tilapia (*Oreochromis niloticus*) (Abdel-Latif and Khalil, 2014; El-Hawarry et al., 2018), rainbow trout (*Oncorhynchus mykiss*) (Ahmadifar et al., 2011), *Tilapia zillii* (Mabrok and Wahdan, 2018), and Yellowtail Tetra (*Astyanax altiparanae*) (Ferreira et al., 2014). Additionally, the oregano leaves can significantly improve the immune and antioxidant status of gilthead seabream (*Sparus aurata*) (Beltrán et al., 2018; Beltrán et al., 2020) and increased protection of Nile tilapia against *Streptococcus agalactiae* (Santo et al., 2019).

The current study hypothesized that the beneficial effects of dietary supplementation of OEO on fish health and growth might be strongly associated with improvement of the hepato-renal functions and the intestinal histomorphology. Thus, this study was carried out to assess the influences of dietary OEO on the growth performance indices, feed utilization parameters, body composition, hepato-renal functions, and the histomorphology of livers, posterior kidneys, and intestines of common carp, to identify the hepato-protective role of OEO and to reveal the relationships between the intestinal morphometry and growth performance in fish fed dietary OEO.

2. Materials and methods

2.1. Origanum vulgare extract and preparation of experimental diets

Ropadiar powder plus® is a commercial product of Ropapharm International BV, the Netherlands was used as a source of oregano essential oil (OEO) (10%) which extracted from *O. vulgare* L (each one kg of this product contains approximately 602.0 g carvacrol (60.2%) and 40 g thymol (4%) and calcium carbonate as a carrier). Five experimental diets (Table 1) were formulated using different levels of Ropadiar powder plus® (as a source of OEO) at 0, 5, 10, 15, and 20 OEO g/kg diet. All diets ingredients were finely grounded, thoroughly mixed, and pelleted through a 1.0 mm diameter diets. The grounded feed pellets were well-mixed with OEO in the presence of water (30–40%) to formulate the five test diets containing 5, 10, 15, and 20 OEO g/kg diet (Table 1). After air-drying, the diets were kept in a refrigerator at −20 °C until further use.

2.2. Fish rearing conditions

A total of one hundred and fifty (150) of common carp (*C. carpio* L.) fingerlings (20.3 ± 0.8 g) were obtained from a local fish hatchery (Saft Khalid, Behera Province, Egypt), transported, and acclimated to the lab conditions for 2 weeks, during which fish were fed on a basal commercial diet (30% crude protein). After acclimation, fish were stocked in 15,100-L glass aquaria (10 fish/aquarium), which were supplied with continuous aeration via compressed air via air pumps. Fish were divided into 5 treatments (with three replicates per each) and fed on the experimental diets up to apparent satiation at 9:00 and 13:00 h for successive 8 weeks. The light was maintained at 12: 12 h light: dark cycle throughout the day using fluorescent light tubes. To maintain clear healthy water, throughout the acclimation period and afterward, a three-quarter of aquarium's water was daily siphoned to remove faeces and uneaten food and replaced with new well-aerated water from the storage tank.

Table 1
Ingredients and proximate chemical composition (g/kg on a dry weight basis) of experimental diets of common carp containing different levels of oregano essential oil.

Ingredients	Oregano essential oil (g/kg diet)				
	0	5	10	15	20
Fish meal (72% crude protein)	85	85	85	85	85
Soybean meal (45% crude protein)	400	400	400	400	400
Wheat bran	190	190	190	190	190
Ground corn	230	225	220	215	210
Cod fish oil	30	30	30	30	30
Corn oil	15	15	15	15	15
Vitamins premix[a]	15	15	15	15	15
Mineral premix[b]	15	15	15	15	15
Starch	20	20	20	20	20
Oregano essential oil	0	5	10	15	20
Total	1000	1000	1000	1000	1000
Proximate chemical analysis (g/kg)					
Dry matter	922	927	924	925	923
Crude protein	294	294	297	292	294
Ether extract	70	72	73	77	73
Total ash	69	67	63	68	71
Crude fiber	49	48	47	46	45
Nitrogen-free Extract[c]	518	519	520	517	517
GE (KJ/g diet)[d]	18.58	18.68	18.81	18.86	18.75

[a] Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamin, 0.005 g; a-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

[b] Mineral premix (g/kg of premix): $CaHPO_4.2H_2O$, 727.2; $MgCO_4.7H_2O$, 127.5; KCl 50.0; NaCl, 60.0; $FeC_6H_5O_7.3H_2O$, 25.0; $ZnCO_3$, 5.5; $MnCl_2.4H_2O$, 2.5; $Cu(OAc)_2.H_2O$, 0.785; $CoCl_3.6H_2O$, 0.477; $CaIO_3.6H_2O$, 0.295; $CrCl_3.6H_2O$, 0.128; $AlCl_3.6H_2O$, 0.54; $Na2SeO_3$, 0.03.

[c] Nitrogen-Free Extract (calculated by difference) = 100 – (protein% + lipid % + ash% + fiber%).

[d] Gross energy (GE) was calculated from NRC (1993) as 16.7, 37.4, and 16.7 kJ/g for protein, lipids, and carbohydrates, respectively.

At the end of the 8-weeks feeding experiment, all fish fasted for a period of 24 h before blood sampling. Five fish/tank were sampled and anesthetized using sodium bicarbonate buffered tricaine methane sulfonate (MS-222; 30 mg/L) (Matsche, 2011). Afterward, fresh blood was drawn from the caudal veins without anti-coagulant, kept in ice for 4 h, serum separated by centrifugation (3000 ×*g* for 15 min at 4 °C) and stored in −20 °C for subsequent biochemical analysis.

2.3. Water quality measurements

Water was sampled every two weeks at 15 cm depth from each aquarium to evaluate the parameters of water quality. Dissolved oxygen (DO) and temperature (°C) were measured on-site with a portable oxygen meter (Jenway, London, UK). The pH was measured using a pH-meter (Digital Mini-pH Meter, USA). The unionized ammonia (NH_3) was measured using special kits (HACH Co., Loveland, CO., USA). The water parameters were within their normal ranges as 27.5–29.2 °C, 5.4–5.7 mg/L, and 7.4–7.6, and 0.074–0.082 mg/L for water temperature, DO, pH, and NH_3, respectively. Throughout the whole experiment, these values are kept-up within ranges acceptable for fish farming regarding to Boyd and Tucker (2012).

2.4. Growth performance and feed utilization parameters

At the end of the experimental period, all fish per each tank were assembled, counted, and group weighed. The growth performance indices and feed utilization parameters were calculated as the following equations:

Weight gain (WG) (g) = W2 − W1;

Weight gain % (WG%) = 100 (W2 − W1)/W1;

Specific growth rate (SGR; %/day) = 100 [Ln W2 (g) − Ln W1 (g)]/T,

whereas W1 = initial body weight of fish, W2 = final body weight of fish, and T is the rearing period (day);

Feed intake (FI) = the total of the given feed (g) to fish during the rearing period.

Feed conversion ratio (FCR) = FI/WG;

Fish survival (%) = 100 (final number of fish / initial number of fish).

2.5. Proximate chemical analysis

At the end of the feeding experiment, the proximate chemical components of the whole-fish body were assessed according to methods of AOAC (1995). The moisture content was evaluated following oven drying at 105 °C up to constant dry weight (Memmert UN110, Buchenbach, Germany). The crude protein (CP) content was measured using the Micro-Kjeldahl apparatus (Foss Kjeltec 2200, Hillerqd, Denmark), meanwhile, the content of total lipids was evaluated by using petroleum ether extraction in Soxhlet apparatus for 16 h. Ash content was further calculated from the weight loss occurred following sample incineration at 550 °C for 6 h in a muffle furnace (Heraeus Instruments K1252, Hanau, Germany). Gross energy (GE) was calculated from NRC (2011) as 16.7, 37.4, and 16.7 kJ/g for protein, lipids, and carbohydrates, respectively.

2.6. Haemato-biochemical measurements

Total proteins (TP) and albumin (ALB) values were evaluated colorimetrically using commercial diagnostic kits (Bio-diagnostics, Giza, Egypt). TP and ALB values were determined according to the methods described by Henry (1964) and Wotton and Freeman (1982), respectively. Meanwhile, Globulin (GLO) values were gotten by the subtraction of ALB from T P values.

Serum levels of creatinine were evaluated according to the methods of Henry (1974), while urea levels (blood urea nitrogen, BUN) were assessed according to Coulombe and Favreau (1963). The activities of serum aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined according to the methodology of Reitman and Frankel (1957), and alkaline phosphatase (ALP) according to Tietz et al. (1983).

2.7. Histomorphological examination

2.7.1. Histomorphology of livers and posterior kidneys

At the end of the feeding experiment, fish (six fish per group) were necropsied for collection of livers, and posterior kidneys of fish in the control group and OEO-fed groups were collected. For intestine samples, cross-section cuts (3 cm in length) were sampled from the midgut (2 cm below the pyloric region). Next, the collected specimens were immediately fixed for 48 h in 10% neutral buffered formalin solution. After fixation, tissue specimens were processed via the paraffin embedding technique according to the methodology described in Bancroft and Gamble (2013). Concisely, tissues were dehydrated in ascending grade of ethyl alcohol, cleared in xylene, blocked in paraffin wax, cut to several 5 μm thick sections, and finally stained with hematoxylin and eosin (H & E stain). Afterward, several representative photomicrographs were captured from the prepared sections with a digital camera (Leica EC3, Leica, Germany) connected to a microscope (Leica DM500).

2.7.2. Intestinal morphology and histomorphometry analysis

Following blood sampling, fish were incised, and the whole intestinal tracts were carefully separated. Mid parts of the intestinal tracts were further dissected into segments. The intestinal analysis was conducted in two fish from each aquarium (n = 6 fish/each treatment). The computerized quantitative histomorphometry analysis was done on the images captured from the intestinal tissues of fish by a digital camera attached to a bright field microscope (Nikon E−200, Tokyo, Japan) at ×400 magnification. The effects of OEO dietary supplementation were evaluated by light microscopic (LM) observations in terms of (1) villus height (μm, from the tip to the base of villus), (2) villus width (μm, at the tip, and the crypt/villus junction), (3) crypt depth, (4) Villus height/crypt depth ratio, and (5) tunica muscularis thickness. The measurements were done using a computerized image analysis system (Image J software; Bethesda, MD, USA) (Schneider et al., 2012). The villi height, crypt depth and the villi width at crypt/villus junction and villi tips were measured (mean value was calculated for 15 villi/ sample, x10).

2.8. Data analysis

Data collected from the current feeding experiment (growth indices, body composition, haemato-biochemical parameters, intestinal morphometric measurements) were statistically analyzed using one-way analysis of variance (ANOVA) to assess the effects of the dietary inclusion of different levels of OEO in diets for common carp. The differences between means were tested at a 5% probability level using the Duncan test as a post-hoc test. The polynomial regression analysis was applied to reveal the linear and quadratic effects of OEO on the measured variables (Yossa and Verdegem, 2015). All the statistical analyses were done via SPSS program version 22 (SPSS, Cengage Learning Australia) (Allen et al., 2014).

3. Results

3.1. Growth performance and feed utilization

The effects of experimental diets on the growth performance indices and feed utilization parameters are presented in Table 2. There were no significant differences in the initial body weight (IBW) of the fish at the start of the feeding trial ($P < .05$) (20.3 ± 0.8 g). After the 8-week of the feeding experiment, all fish grew approximately by a triple of their IBW. No mortalities were observed during the whole feeding trial. It was noticed that dietary OEO has a positive influence on the growth performance and feed utilization parameters (FBW, WG, WG %, FI, and SGR) in a dose-dependent regime ($P < .05$; Table 2) with regard to the control group. Additionally, it was observed that FBW, WG, WG %, FI, and SGR had the highest values in OEO15% and OEO20% levels.

Moreover, fish fed on OEO-supplemented diets consumed more feed than that fed the control basal diet. The maximum FI noticed in the fish group fed 15–20 g OEO/kg diet (94.7–97.9 g feed/fish, respectively), whereas fish in the control diet consumed lowest feed amount (65.5 g feed/fish). Among all groups, values of FCR were not significantly affected ($P < .05$) by the dietary OEO supplementation to common carp and its range was 1.67–1.68. Considering the fish survival in all experimental groups, it was observed that, throughout the feeding period, fish in all experimental groups were in good health status as noticed from their general activity with no mortalities among all the experimental groups ($P < .05$; Table 2).

3.2. Body composition

The effects of experimental diets on the proximate chemical

Table 2
Growth performance indices and survival rates of common carp fed diets containing different levels of oregano essential oil for eight weeks.

	Oregano essential oil (g/kg diet)					SEM	P value	
	0	5	10	15	20		Linear	Quadratic
Initial body weight (g)	20.3	21.0	20.7	20.8	21.0	0.206	0.513	0.801
Final body weight (g)	59.7c	65.6b	69.4b	77.3a	79.3a	2.051	0.001	0.001
WG (g)	39.4d	44.6c	48.7b	56.5a	58.3a	1.999	0.001	0.001
WG %	194.1c	212.4bc	235.3b	271.6a	277.6a	9.555	0.001	0.001
SGR (%g/day)	1.926c	2.034c	2.160b	2.344a	2.372a	0.051	0.001	0.001
FI (g feed/fish)	65.5c	73.2b	80.2b	94.7a	97.9a	3.609	0.001	0.001
FCR	1.67	1.64	1.67	1.68	1.68	0.022	0.873	0.976
Fish survival (%)	100	100	100	100	100	0.000	1.00	1.00

Data were presented as mean ± SE. Means having the same letter in the same row are significantly differed at $P < .05$.
WG: Weight gain, WG%: Weight gain %, SGR: Specific growth rate, FI: Feed intake, FCR: Feed conversion ratio.

Table 3
Proximate chemical composition (g/kg on fresh weight basis) of the whole body of common carp fed diets containing different levels of oregano essential oil for eight weeks.

Parameters	Oregano essential oil (g/kg diet)					SEM	P value	
	0	5	10	15	20		Linear	Quadratic
Moisture	719.3	715.7	718.3	718.0	719.0	0.925	0.805	0.711
Crude protein	176.0	178.0	176.7	176.3	175.6	0.565	0.752	0.535
Total lipids	90.0	92.3	91.0	91.0	91.3	0.920	0.846	0.933
Total ash	11.7	12.3	13.7	13.7	13.3	0.331	0.064	0.057

Data were presented as mean ± SE.

composition of the whole-fish body are presented in Table 3. It was found that the supplementation of diets with different levels of OEO did not significantly affect the whole-fish body composition (moisture, CP, total lipids, and total ash) ($P < .05$). Nonetheless, the values of moisture, crude protein, total lipids and total ash in all OEO-supplemented groups were elevated in comparison with the control group, but with no significant differences reported ($P < .05$; Table 3).

3.3. Haemato-biochemical measurements

The effects of experimental diets on serum protein profile (TP, ALB, and GLO), hepatic enzymes (ALT, AST, and ALP), and renal functions (creatinine and BUN) of common carp are presented in Table 4. Among all experimental groups, it was found that serum TP, ALB, and GLO values significantly increased as OEO levels increased in fish diets and maximum values were observed by feeding OEO and OEO at 15 and 20% levels. On the other hand, activities of serum ALT, AST, and ALP, as well as, creatinine and BUN levels were not significantly affected ($P < .05$) in experimental groups with respect to the control group.

3.4. Intestinal, liver, and kidney histomorphology

The effects of dietary OEO on common carp livers (Fig. 1), posterior kidneys (Fig. 2), and mid intestine samples (Fig. 3) were evaluated.

3.4.1. Histomorphology of fish livers

LM observations showed that the hepatopancreatic tissues from the control fish (Fig. 1A) had a normal gross histomorphology of the hepatic parenchyma with polygonal hepatocytes, round-shape nucleus, a quite homogeneous cytoplasm, and glycogen granules. Besides, portal vein, central vein, and sinusoid appeared in a normal structure. Also, the acinar cells appeared with normal acidophilic cytoplasm and basophilic portion with the nucleus. OEO-fed fish groups (Fig. 1B-E) had a similar structure of fish liver in the control group without any noticed pathologic lesions.

3.4.2. Histomorphology of carp posterior kidneys

Tissues of the posterior kidneys in the control fish (Fig. 2A), as well as OEO-fed fish groups (Fig. 2B-E), showed similar histologic limits of proximal and distal tubules, glomerulus, parietal epithelium of

Table 4
Changes in blood protein profile and hepato-renal indicators of common carp fed diets containing different levels of oregano essential oil for eight weeks.

Parameters	Oregano essential oil (g/kg diet)					SEM	P value	
	0	5	10	15	20		Linear	Quadratic
Serum protein profile								
TP (g/dL)	4.19d	5.35c	6.02b	6.73a	6.86a	0.271	< 0.001	< 0.001
ALB (g/dL)	1.80c	2.58b	2.96ab	3.13a	3.23a	0.147	< 0.001	< 0.001
GLO (g/dL)	2.40c	2.76b	3.06ab	3.60a	3.64a	0.160	< 0.001	< 0.001
Hepato-renal functions								
ALT (IU/L)	55.1	56.6	55.9	56.9	57.8	0.567	0.140	0.349
AST (IU/L)	12.78	12.93	13.10	13.24	13.27	0.105	0.081	0.219
ALP (IU/L)	11.66	11.99	11.69	11.71	11.90	0.157	0.451	0.645
Creatinine (mg/dL)	0.73	0.76	0.76	0.75	0.78	0.028	0.463	0.759
BUN (mg/dL)	5.60	5.75	5.83	5.93	6.19	0.103	0.157	0.168

Data were presented as mean ± SE.
TP: Total protein, ALB: Albumin, GLO: Globulin, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, BUN: Blood urea nitrogen.

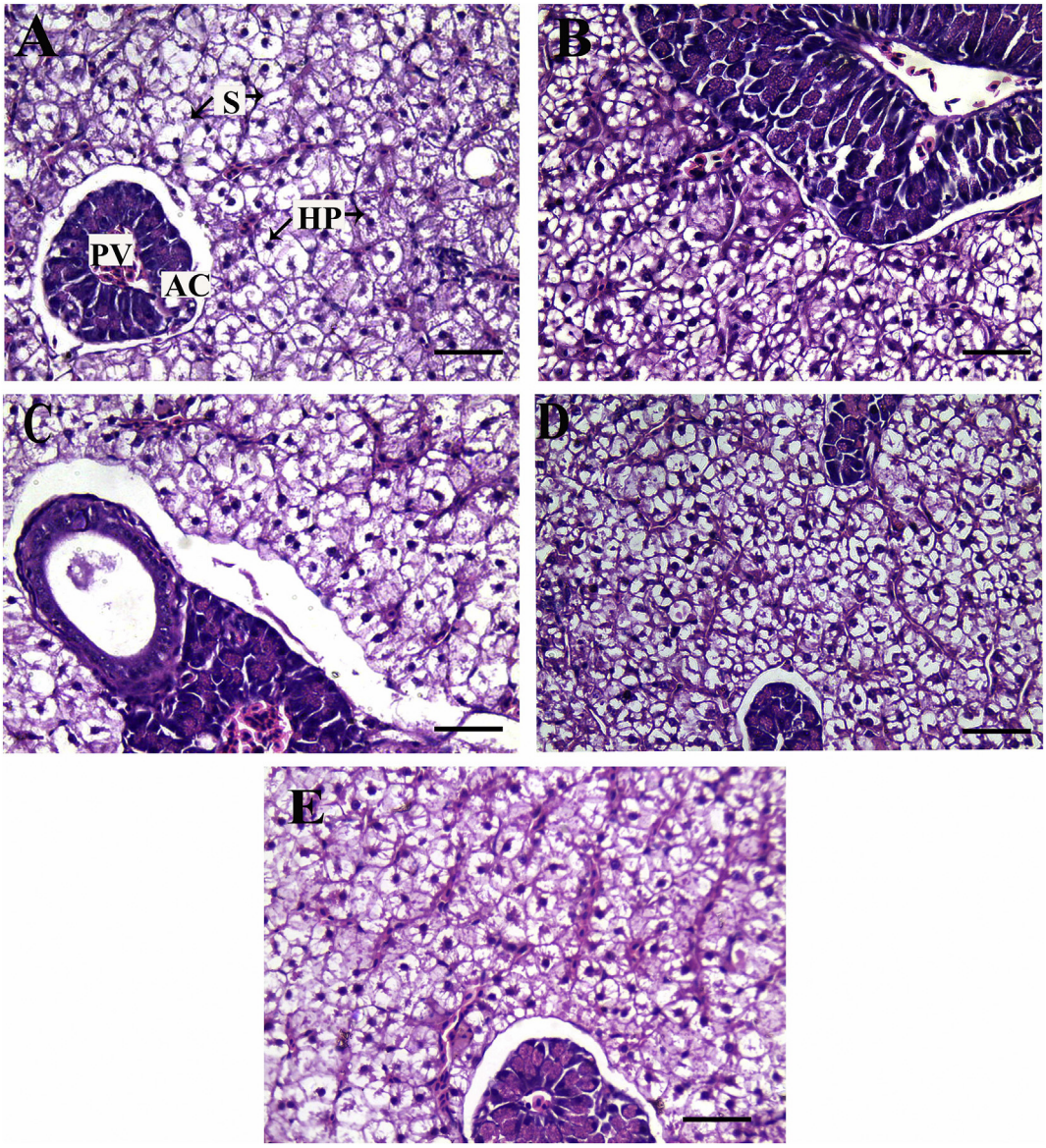

Fig. 1. Comparative photomicrographs (Hematoxylin & Eosin (H & E) staining) of hepatopancreas of common carp (*C. carpio*) fed on control diet (A) and experimental diets supplemented with different levels of oregano essential oil (OEO) (5, 10, 15, and 20 g/kg diet) (B, C, D, & E respectively) for 8 weeks. Tissues from all groups showed normal histologic structures of the portal vein (PV), sinusoid (S), and hepatocytes (HP). The acinar cells (AC) appeared in normal structure with normal acidophilic cytoplasm and basophilic portion with nucleus (Scale bar = 50 μm).

Bowman's capsule, normal wide bowman spacing, and hematopoietic tissue.

3.4.3. Histomorphology and intestinal morphometry of carps

Histologically, the intestinal wall of common carp consists of 4 layers from the lumen (mucosa, sub-mucosa, internal, and external muscular layers (tunica muscularis interna and externa), and serosa. Fig. 3 showed that the mucosa and submucosa of the mid intestines of caps in the control group (Fig. 3A), as well as in OEO-fed fish groups (Fig. 3B-E) had a normal histomorphology. Furthermore, the intestinal villi and associated crypt, tunica muscularis (internal and external muscular layers) and submucosal tissues appeared free of inflammatory and/or degenerative changes. Additionally, the columnar epithelium and goblet cells appeared properly arranged. Concerning the histomorphometric analysis of the intestines, results revealed a significant improvement ($P < .05$) in villus height, villus width (at the tip and the crypt/villus junction), and the crypt depth in OEO-fed fish groups in a dose-dependent manner (Table 5). However, the villus to crypt ratio was significantly ($P < .05$) elevated in the control fish as well as those fed 5 and 10 g OEO/kg ration (OEO5 and OEO10), meanwhile, its values were significantly decreased in OEO15 and OEO20 treatments. Interestingly, there was no significant difference observed in the thickness of both layers of tunica muscularis in both the control and OEO-supplemented fish groups.

4. Discussion

Nowadays, there is an increasing interest in the usage of dietary phytobiotics as natural growth promotors and immunostimulants in practical fish diets to improve growth, health status, immune responses, and protection against bacterial diseases (Abdel-Latif and Khalil, 2014; Abdel-Tawwab, 2016; Hoseinifar et al., 2018; Ahmadifar et al., 2019). OEO has proven itself to be a promising alternative to dietary antibiotics with potential biological properties (Zhang et al., 2020).

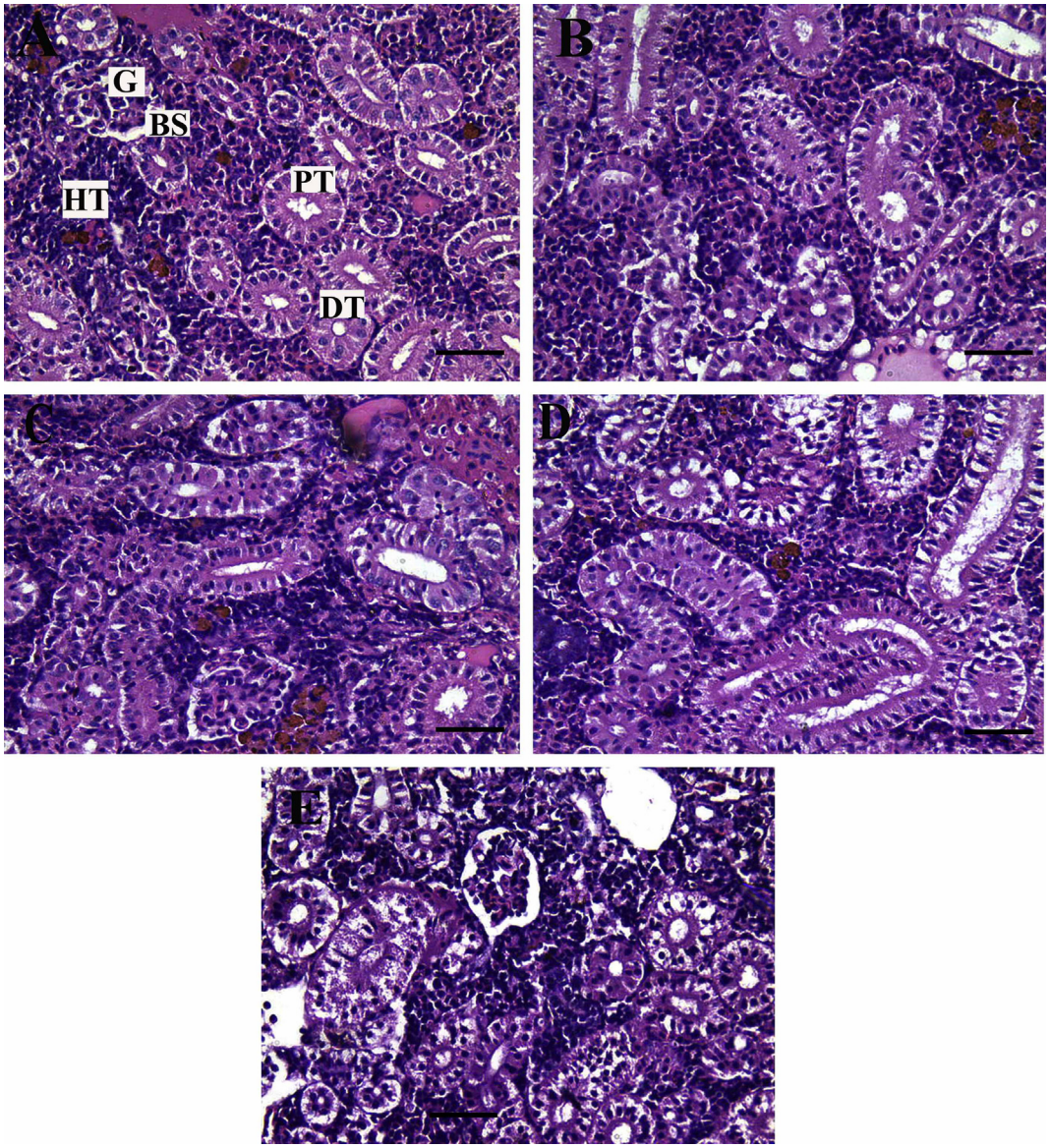

Fig. 2. Comparative photomicrographs (H & E staining) of the posterior kidney of common carp (*C. carpio*) fed on control diet (A) and experimental diets supplemented with different levels of oregano essential oil (OEO) (5, 10, 15, and 20 g/kg diet) (B, C, D, & E respectively) for 8 weeks. Renal tissues showed similar histologic limits of proximal (PT) and distal (DT) tubules, glomerulus (G), parietal epithelium of Bowman's capsule, normal wide bowman spacing (BS), and hematopoietic tissue (HT) (Scale bar = 50 μm).

4.1. Growth performance and feed utilization

The current study illuminated that dietary OEO significantly improved growth performance and feed utilization parameters (FBW, FI, WG, WG%, and SGR) of common carp with an optimum level of 15 g/kg diet.

The growth stimulation effects of OEO-fed groups could be accredited to several factors; 1) the distinctive aromatic flavor of OEO which makes it a strong appetizer, which subsequently leads to increase the levels of feed intake and diet palatability, which results in improving the fish FBW and WG (Abdel-Latif and Khalil, 2014), 2) the growth-promoting effects of oregano and its advantageous properties on fish health status were attributed to its constituents of beneficially valuable phytochemicals, where Zheng et al. (2009) proposed that the synergistic effects between carvacrol and thymol potentiated and triggered the FBW of channel catfish, 3) another possible reason for the improvement in the growth performance can be accredited to the potent effects of carvacrol and thymol contents which considerably promote the secretion of the digestive enzyme and improve their vital activities (Hernandez et al., 2004; Puvača et al., 2013). Furthermore, these bioactive phytochemicals extracted from OEO might increase the secretion of digestive enzymes, which in turn, lead to the increased food consumption (Radhakrishnan et al., 2015; Zhang et al., 2020), and 4) the dietary supplementation of thymol and carvacrol can modulate the structure of the intestinal bacterial communities, which in turn will improve the digestion and absorption of nutrients. Ran et al. (2016) suggested that both carvacrol and thymol beneficially change the composition of the gut bacterial communities of Nile tilapia. Similarly, Zhang et al. (2020) reported that OEO improved both the digestive enzymes (protease, lipase, and amylase) and modulated the diversity intestinal microbiota of the koi carp (*Cyprinus carpio*) via increased communities of beneficially important *Propionibacterium*, *Brevinema*, and *Corynebacterium*.

Consistent with our results, Abdel-Latif and Khalil (2014) recorded significant enhancement of the growth of Nile tilapia fed on 10% OEO. In previous studies used thymol and/or carvacrol in fish diets, Zheng

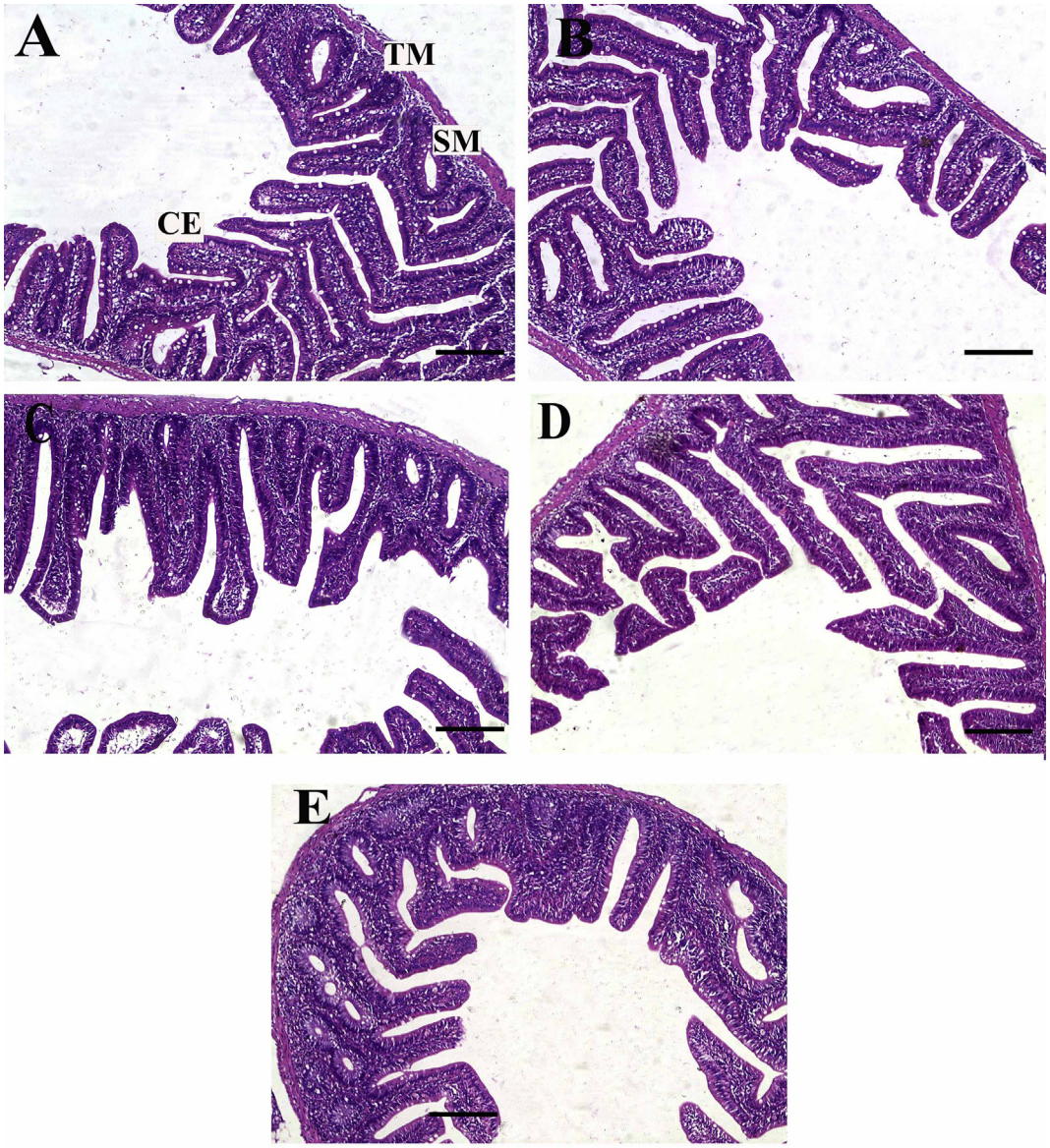

Fig. 3. Comparative photomicrographs (H & E staining) of the intestinal tissues of common carp (*C. carpio*) fed on control diet (A) and experimental diets supplemented with different levels of oregano essential oil (OEO) (5, 10, 15, and 20 g/kg diet) (B, C, D, & E respectively) for 8 weeks. Tissues from all groups showed normal histologic structures of intestinal villi and crypt, tunica muscularis (TM), submucosa (SM), together with the normal arrangement of columnar epithelium (CE), and goblet cells (Scale bar = 100 μm).

et al. (2009) illustrated that the combination of thymol and carvacrol has a positive effect on the growth parameters of the channel catfish. Moreover, Ahmadifar et al. (2011) illuminated similar enhancement of the growth performance indices in rainbow trout fed on thymol-carvacrol diets. Additionally, Ahmadifar et al. (2014) indicated that dietary supplementation of thymol–carvacrol significantly enhanced FBW, WG, SGR, and FCR of sturgeons. Amer et al. (2018) and Abd El-Naby et al. (2020) showed that dietary thymol supplementation

Table 5
Histomorphometric measurements of mid intestines of common carp fed diets containing different levels of oregano essential oil for eight weeks.

Item (μm)	Oregano essential oil (g/kg diet)				
	0	5	10	15	20
Villus height	527.67 ± 3.93d	562.00 ± 6.11c	601.67 ± 6.01b	625.00 ± 2.89b	658.33 ± 17.40a
Villus width at the tip	37.00 ± 1.15e	45.00 ± 1.73d	55.33 ± 1.67c	63.00 ± 1.53b	69.33 ± 1.76a
Villus width at the crypt/villus junction	53.33 ± 1.20c	57.33 ± 1.45bc	58.00 ± 1.53bc	59.00 ± 2.52b	64.67 ± 0.88a
Crypt depth	59.00 ± 2.08d	65.00 ± 2.65 cd	69.33 ± 1.76c	80.67 ± 2.33b	96.67 ± 4.41a
Villus height / crypt depth ratio	8.96 ± 0.25a	8.67 ± 0.38a	8.69 ± 0.24a	7.76 ± 0.19b	6.82 ± 0.13c
Tunica muscularis thickness	213.33 ± 12.02a	217.67 ± 11.57a	222.33 ± 6.94a	215.33 ± 12.98a	213.33 ± 18.78a

Data represent mean ± SE from two replicates per each group (n = 10).
Values with different letters in the same row indicate significant differences between different test groups at $P < .05$.

significantly stimulated the growth performance of Nile tilapia. On the other hand, other studies used OEO as a feed supplement and found a significant improving effect of OEO on WG, SGR, protein efficiency ratio (PER) and carcass weight (CW) of Yellowtail Tetra (Ferreira et al., 2014). El-Hawarry et al. (2018) observed a significant improvement of the growth of Nile tilapia fed diets supplemented with different levels of OEO and reared under low or medium stocking densities. Besides, Diler et al. (2017) observed a significant increase in the growth of rainbow trout fed on diets supplemented with essential oil of *Origanum onites*.

In contrast, Yilmaz et al. (2015) recorded no significant differences in the FBW, SGR, and FCR among rainbow trout fed diets supplemented with carvacrol. Furthermore, dietary supplementation of oregano leaves in Nile tilapia diets did not affect the FI, diet palatability, diets consumption, or growth performance parameters after 30 days of feeding (Santo et al., 2019). Similarly, dietary supplementation of carvacrol or thymol as feed additives in the diets of rainbow trout did not significantly affect the WG of rainbow trout after 56 days of feeding experiment but lowest FCR was observed in both groups of thymol or carvacrol with relation to the control group (Giannenas et al., 2012).

The differences in growth performance parameters obtained herein and other studies in relation to feeding OEO may be attributed to several factors related to fish species, sizes, immune responses, organum meals (dietary form and organum species), or to the experiment conditions (inclusion doses, feeding period, or experimental setup conditions) among others.

4.2. Proximate body composition

On studying the proximate composition of the whole body of the fish, the obtained results illustrated that OEO supplementation to common carp did not significantly affect moisture, crude protein, total lipids, and total ash of the whole-fish body. These findings are similar to previous results in Nile tilapia (Abdel-Latif and Khalil, 2014). Additionally, Zheng et al. (2009) elucidated no significant differences in proximate body composition of channel catfish fed diets supplemented with either carvacrol, thymol, a combination of carvacrol and thymol, and Orego-Stim® (containing essential oil of *O. heracleoticum*). Besides, Yilmaz et al. (2015) reported no significant difference in the proximate composition of rainbow trout fed with diets supplemented with the carvacrol when compared with the control group. Amer et al. (2018) found that there were no significant differences in contents of dry matter, crude protein, fat, and ash content of the whole body of fish fed thymol-enriched diets.

On contrary, Ahmadifar et al. (2011) illustrated that the values of lipids, protein and ash contents in the whole-fish body were significantly elevated in rainbow trout fed on thymol-carvacrol diets more than those of the control group. Similarly, in sturgeon, it was found that protein and ash contents in the body of fish groups fed on 1–2 g/kg diet of dietary thymol–carvacrol (concentration %) were significantly increased with respect to the control group (Ahmadifar et al., 2014). Besides, it was found that the highest value for carcass crude protein content was observed in Yellowtail Tetra fed a diet containing 2.5 g/kg diet of OEO (Ferreira et al., 2014). Changes in protein and lipid content in the fish body are known to be linked to changes in their synthesis, deposition rate in muscle and/or different growth rate (Abdel-Tawwab et al., 2006).

4.3. Haemato-biochemical measurements

Considering the haemato-biochemical parameters of the experimental groups, there were significant increases observed in serum TP, ALB, and GLO values in OEO-fed fish over these of the control group. Consistent with our results, Haghighi and Rohani (2015), Pourmoghim et al. (2015) and Haghighi et al. (2018) reported the dietary supplementation of dried *O. vulgare* extract in fish diets significantly enhanced TP, ALB, and GLO values with respect to the control group. Moreover, Yilmaz et al. (2015) reported significant elevation of TP, ALB and GLO values of trout groups fed diets supplemented with different levels of carvacrol. In contrast, Santo et al. (2019) reported that values of TP were not significantly affected in Nile tilapia fed on diets supplemented with different levels of oregano leaves.

Liver function enzymes are considered as a biological mirror of the health status of the body and indicators of any hepatic lesions and dysfunction (Sloss and Kubler, 2009). In our study, liver function enzymes (ALT, AST, and ALP) were not significantly affected by the supplementation of OEO in the diets of carps. These findings were following the results of Rafieepour et al. (2019) who illustrated that supplementation of oregano extract in diets of rainbow trout resulted in non-changed serum levels of AST, ALT, and lactate dehydrogenase (LDH) in comparison to the control group. Similarly, Hoseini and Yousefi (2019) illustrated that dietary supplementation with thyme (*Thymus vulgaris*) extract beneficially maintained the liver function enzymes of *O. mykiss* to the normal levels.

In fish, Folmar (1993) correlated the elevated levels of serum creatinine and BUN as indicators of renal dysfunction with kidney damage of fish exposed to chemical contaminants. In the present study, it was found that the serum creatinine and BUN levels of fish were not significantly affected among OEO-fed groups with respect to the control group. This result suggested that dietary OEO help in maintaining the normal healthy structure of the renal system of fish. Abd El-Naby et al. (2020) reported that thymol supplementation to Nile tilapia significantly reduced creatinine. From the obtained results herein, it could be assumed that dietary supplementation of OEO in fish diets has no toxic effects on their hepato-renal tissues.

4.4. Histomorphology of fish tissues

Histomorphological changes may provide insight into the impacts of unhealthy feeds on various fish tissues and organs (Yigit et al., 2017). In the current study, the hepatopancreatic tissues, posterior kidneys, and intestines had a normal gross histomorphology of OEO-fed fish groups compared with the control group without any noticeable pathologic lesions in the hepatic and renal tissues. Moreover, all experimental groups did not have any inflammatory scores of the intestinal tissues. On contrary to these findings, Sönmez et al. (2015) demonstrated that supplementation of herbal oils in diets of rainbow trout resulted in mild hydropic degeneration of liver and necrosis of kidney tubules. Furthermore, Yigit et al. (2017) illustrated that supplementation of the essential oil of *O. onites* to diets of rainbow trout resulted in cytoplasmic vacuolation and necrosis of hepatocytes, and slight necrotic changes renal epithelium with cytoplasmic vacuolation in the posterior kidney. Taken together, the results of serum markers of hepato-renal functions and illustrations of the histomorphological criteria of the hepatopancreatic tissues and the execratory part of renal tissues (posterior kidneys), it could be assumed that dietary supplementation of OEO can be beneficially taken into account as a potential hepatoprotective product with beneficial capabilities to improve fish health status.

4.5. Intestinal morphometry

The small intestine plays an important role in fish growth, since it is the primary site for absorption of nutrients (Cheng et al., 2012; Elsabagh et al., 2018; Abdel-Tawwab et al., 2018; Adeshina et al., 2019; Dawood et al., 2019; Dawood et al., 2020a). In fish, long intestinal villi are usually linked with improved gut health, greater efficiency of nutrient absorption, and subsequently, the healthier fish intestinal tracts are leading to enhanced performance (Sklan et al., 2004; Lauriano et al., 2016; Huerta-Aguirre et al., 2019). Relating to the intestinal morphometry, the obtained results herein indicated significant improvement in villus height, villus width (at the tip and the crypt/villus junction), and the crypt depth in OEO-fed fish groups with a dose-dependent manner. In the study of Abd El-Naby et al. (2020), the

histomorphometry analysis revealed that the length of the intestinal villus increased dramatically with thymol addition to diets for Nile tilapia. Likewise, other essential oils obtained from phytogenic sources have been demonstrated to increase the length of the intestinal villi in Nile tilapia (Valladão et al., 2017) and African catfish (*Clarias gariepinus*) (Abdel-Tawwab et al., 2018; Adeshina et al., 2019). On contrary with these results, no significant alterations of the height of the intestinal villi (average of 596.8 μm) of Nile tilapia fed on diets supplemented with different levels of dry oregano leaves with respect to the control group (Santo et al., 2019).

The significant enhancement of intestinal morphometry in the present study has been suggested to increase the surface area of absorption through increasing the length the intestinal height as well as the width of intestinal villi, which will subsequently lead to improvement of nutrients absorption from the fish intestine (Abdel-Mohsen et al., 2018; Abdel-Tawwab et al., 2018; Adeshina et al., 2019; Zhou et al., 2019). Collectively, these results suggest that dietary OEO positively increased the ability of common carp to digest their diets more efficiently, which in turn stimulated its general health status, growth performance, and productivity.

5. Conclusions

In conclusion, the obtained results herein illustrated that dietary supplementation with OEO considerably improved growth performance indices and feed utilization parameters of common carp fingerlings. Additionally, OEO maintained their normal hepato-renal features (serum markers and histomorphological structures). Moreover, dietary administration of OEO at a dose rate of 15 g/kg diet can beneficially improve the intestinal histomorphological and morphometrical criteria. The beneficial effects of dietary OEO were possibly accredited to its hepatoprotective function and its vital role in improving fish guts. However, further research is required for better understanding of the mechanisms of dietary OEO in improving fish health and productivity.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of fish were followed by the authors.

Author contributions

Hany M.R. Abdel-Latif

1. Experimental setup and follow up the fish during the experimental period.
2. Weekly sampling water and examine water quality parameters.
3. Daily observation of fish.
4. Weighing fish before and after the end of the experiment.
5. Blood sampling and serum collection.
6. Double-check with ithenticate plagiarism to avoid similarity in text.
7. Revising the manuscript before submission.
8. Submission of the manuscript and follow up on the peer-review procedures.

Mohsen Abdel-Tawwab

1. Collection of the raw data.
2. Statistical analysis.
3. Revision of the manuscript before submission.

Asmaa F. Khafaga

1. Collection and preparation of samples for intestinal morphometric studies.
2. Histopathological examination of hepatopancreatic tissues as well as posterior kidney.

Mahmoud A.O. Dawood

1. Writing the manuscript and prepare it according to the journal guidelines.
2. Revision of the manuscript before submission.

Declaration of Competing Interest

Authors declare that they have no conflict of interest.

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