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Research Article

Effects of Replacement Cold Press Poppy Seed (*Papaver somniferum*) Oil to Fish Oil at Different Proportions on the Growth Performance, Blood Parameters, and Digestive Tracks Histopathology in Juvenile Common Carp (*Cyprinus carpio*)

Osman Sabri Kesbiç,[1] **Ümit Acar**,[2] **Beste Demirci**,[3] **Funda Terzi**,[4] **Rifat Tezel**,[5] **Ali Türker**,[5] **Kenan Güllü**,[5] **and Hüseyin Serkan Erol**[6]

[1]*Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Kastamonu University, Kastamonu, Turkey*
[2]*Bayramiç Vocational School, Department of Forestry, Çanakkale Onsekiz Mart University, Çanakkale, Turkey*
[3]*Faculty of Veterinary Medicine, Department of Anatomy, Kastamonu University, Kastamonu, Turkey*
[4]*Faculty of Veterinary Medicine, Department of Pathology, Kastamonu University, Kastamonu, Turkey*
[5]*Faculty of Fisheries, Department of Aquaculture, Muğla Sıtkı Koçman University, Muğla, Turkey*
[6]*Faculty of Veterinary Medicine, Department of Biochemistry, Kastamonu University, Kastamonu, Turkey*

Correspondence should be addressed to Ümit Acar; umitacar@comu.edu.tr

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The major goal of the study is to determine the potential use of poppy seed oil as a main lipid source in common carp diets. To investigate this, five different experimental diets (PO0, PO25, PO50, PO75, and PO100) with various proportions of fish oil and poppy seed oil blended as oil sources were provided and supplied to common carp for 90 days. In triplicate groups of 60 fish, 300 common carp weighing 1.15 ± 0.06 g were randomly placed in 100 L aquariums. At the end of the feeding trial, blood and tissue samples were collected from the fish after gravimetric measurements of the fish. The growth and feed utilization performance of the fish were calculated by gravimetric measurements, and tissue samples were examined by hematological, micromorphometric, and histopathological methods. According to the results, poppy seed oil enhanced the quantity of linoleic acid (18 : 2 ω–6) in the diet, and fish fed with the diet consisting completely of poppy seed oil (PO100) had the significantly greatest growth and feed utilization performance ($P < 0.05$). Furthermore, no significant differences in hematological or liver pathology were detected between all groups that were fed with experimental diets ($P > 0.05$). However, as compared to the control group, the digestive system micromorphometry and histomorphometric measurements of the fish fed with PO100 group diets showed a significant increase ($P < 0.05$). In conclusion, it was determined that the carp fed with feeds produced by using only poppy oil as the main fat source improved their digestive system, growth, and feed utilization performance without any negative effects.

1. Introduction

Aquaculture is one of the world's fastest increasing sources of animal protein, and it is critical to meet several of the United Nations' sustainable development targets for the near future [1]. Fishmeal and oil were formerly employed as not only protein and fatty acid sources but also palatable and low-cost feed components for farmed aquatic animals [2]. Fishmeal and fish oil, on the contrary, are not among the low-cost items available today. In recent years, the use of substitute options for fish oil has become a very important topic because fish oil is widely utilized as a human nutritional supplement and in the ration of many farm animal diets, particularly poultry [3]. For that reason, various

research studies have focused on plant-origin oils as a possible replacement for fish oil in aquafeeds [4–8]. Studies and industrial trends suggest that fish oil inclusion rates in aquafeeds have decreased as a result. However, due to the general rise in global aquaculture production, total fish oil consumption has continued its rising trajectory. In near future, it is projected that the aquaculture sectors would increase their efforts to replace marine-based components with useful alternative nutrients [9]. Poppy seed oil, a source of unsaturated fatty acids, could be an alternative and/or useful source for aquafeeds.

Since ancient times, the poppy plant (*Papaver somniferum* L.) has been cultivated for its oil-rich seeds and also the opium that is extracted from its punctured seed capsules. According to the Food and Agriculture Organization, global poppy seed output in 2018 was 76,240 tonnes, including 47,879 tonnes in Europe, with Turkey leading the way (26,991 tonnes), followed by the Czech Republic, India, Australia, France, Spain, Hungary, and China [10]. Poppy seeds and straw are heavily utilized in many baked goods; poppy capsules and straw's alkaloids are commonly employed in the practice of pharmacy [11]. Poppy seeds contain a significant amount of oil, with ranges from 45 to 54 percent by weight. The fatty acid profile of this oil varies according to the cultivar. Linoleic acid is the most abundant fatty acid in the poppy seed oil (68 percent). Linoleic acid, oleic acid, linolenic acid, and palmitoleic acid are the unsaturated fatty acids and also stearic acid, palmitic acid, and arachidonic acid are the most common saturated fatty acids found in poppy seed oil [12].

As it is well known, fish oil is the fundamental and indispensable source of oil in aquafeeds. One of the most important reasons for this is that fish oil contains a high percentage of polyunsaturated fatty acids necessary for fish [13]. Poppy seed oil's effects on trout growth and fillet quality have been researched since it is believed that it may be utilized as the primary source of oil in aquafeeds due to the unsaturated fatty acids it contains [7]. The aim of this study was to clarify the potential of poppy seed oil to modify the fatty acid content in aquaculture feeds formulated for common carp and to determine the changes occurring in growth performance, health status, and digestive tract in common carp that were fed with these experimental feeds.

2. Materials and Methods

2.1. Ethic Statement. All the experiments were conducted within specific protocols and that protocols have been approved by Kastamonu University Animal Experiments Local Ethics Committee (Decision no: 2020.05). In addition to that, all practices on fishes in the study were carried out by European Union Directive no: 2010/63.

2.2. Experimental Design. Three hundred common carp (*Cyprinus carpio*) weighing 1.15 ± 0.06 g were randomly put in a semirecirculating system with 15 aquariums containing 100 L each (20 fish per aquarium). The fishes were acclimatized to the experimental settings for one week prior to the start of the feeding trial. All experimental fishes were fed the control diet during the adaption period. During the 90-day feeding experiment, fishes were given five different experimental diets three times daily until saturated, at 08 : 00, 12 : 00, and 17 : 00 for 90 days, and a 12D : 12 L photoperiod was used throughout the feeding trial. All experimental diets were supplied to groups of common carp with the same number and body weight.

2.3. Preparation of Experimental Diets. In this research, the experimental diets for common carp fry were prepared using the rations shown in Table 1. The same materials were used to produce five isonitrogenous and isolipidic experimental diets. Only cold-pressed poppy seed oil (PSO) and unrefined fish oil (FO) were used as lipid sources in those diets. The experimental diets included PSO, which has been either totally (100% PSO100) or partially (25% PSO25, 50% PSO50, and 75% PSO75) replaced with fish oil, and one of the experimental diets contain full FO (PSO0) to control. Before forming pellets in a laboratory-size pellet machine with a 4 mm size, the materials for the feeds were properly combined, and water was then added and dried in an oven at 40°C. Prior to use, pellets were crushed in the grinder with a size of 400 microns and sieved through a sieve with a mesh opening of 300 microns. The experimental diets were stored at −20°C until the feeding experiment.

2.3.1. Fatty Acid Analysis of Experimental Diets. Fatty acid analyses of experimental diets were performed according to the Ackman [14] method by CG/MS (Shimadzu, GCMS–QP 2010 ULTRA, Japan) device using oil extracted from feeds by Folch et al. [15].

2.4. Calculation of the Growth and Feed Utilization Parameters. All calculations relating to growth and feed utilization performance were carried out according to Kesbiç and Yigit [16].

2.5. Blood Collection and Hematological Analyses. Following the conventional blood sample protocol, fishes were sedated with clove oil, a regularly used approach in research with blood sampling from fish, and hematological parameters were examined according to Blaxhall and Daisley [17]. Wintrobe index (WI) parameters such as mean corpuscular volume (MCV), mean corpuscular Hb (MCH), and mean corpuscular Hb concentration (MCHC) were calculated by Zandecki et al. [18].

2.6. Morphological Examination. At the end of the feeding experiment, common carp (six fishes per group) were euthanized with high-dose clove oil (200 mg L^{-1}), an anesthetic often used in fish studies, and were used and were necropsied for liver and gut collection in the control and PSO-fed groups. The intestine was divided into three loops, namely, proximal, middle, and distal, and each loop was divided into two limbs, namely, ascendens and descendens. Morphometric measurements (width, length, and thickness)

Table 1: Formulation, proximate composition, and fatty acid content of the experimental diets in different proportions of poppy seeds (0% PSO0, 25% PSO25, 50% PSO50, 75% PSO75, and 100% PSO100).

%	PSO0	PSO25	PSO50	PSO75	PSO100	*P* value
Fish meal	21	21	21	21	21	
Soybean meal	38	38	38	38	38	
Corn starch	16	16	16	16	16	
Wheat flour	15	15	15	15	15	
Vit.-min. Mix	4	4	4	4	4	
Fish oil	6	4.50	3	1.50	—	
Poppy seed oil	—	1.50	3	4.50	6	
g/100 g diet	Nutritional content					
Crude protein	35.08	35.12	35.14	35.06	35.14	
Crude lipid	8.56	8.61	8.42	8.55	8.51	
Crude ash	5.20	5.16	5.17	5.24	5.16	
Crude cellulose	3.53	3.51	3.50	3.50	3.53	
NFE	47.63	47.60	47.77	47.63	47.66	
%	Fatty acid content[a]					
C8:0	0.33 ± 0.02^{a}	0.26 ± 0.00^{b}	0.22 ± 0.02^{bc}	0.18 ± 0.02^{c}	0.23 ± 0.01^{bc}	0.002
C10 : 0	n.d	n.d	n.d	n.d	n.d	n.d
C12 : 0	1.08 ± 0.07^{a}	0.83 ± 0.02^{b}	0.64 ± 0.05^{c}	0.52 ± 0.04^{c}	0.60 ± 0.02^{c}	<0.001
C13 : 0	1.27 ± 0.08	0.84 ± 0.01	0.66 ± 0.06	0.54 ± 0.05	n.d	<0.001
C14 : 0	7.70 ± 0.07^{a}	2.67 ± 0.16^{b}	4.12 ± 0.24^{c}	2.63 ± 0.17^{d}	1.03 ± 0.02^{e}	<0.001
C16 : 0	20.35 ± 0.25^{a}	18.18 ± 0.24^{b}	15.87 ± 0.88^{c}	14.87 ± 0.74^{c}	10.06 ± 0.01^{d}	<0.001
C16 : 1 (n−7)	5.63 ± 0.10^{a}	4.32 ± 0.06^{b}	3.12 ± 0.21^{c}	2.01 ± 0.09^{d}	0.78 ± 0.10^{e}	<0.001
C17 : 0	1.93 ± 0.05^{a}	1.45 ± 0.00^{b}	1.11 ± 0.08^{c}	0.82 ± 0.06^{d}	0.78 ± 0.05^{d}	<0.001
C18 : 0	5.35 ± 0.03^{a}	4.57 ± 0.02^{b}	3.89 ± 0.25^{c}	3.57 ± 0.20^{c}	2.80 ± 0.02^{d}	<0.001
C18 : 1 (n−9 cis)	8.53 ± 0.37	8.89 ± 0.12	5.60 ± 4.86	6.13 ± 5.83	9.21 ± 0.16	0.748
C18 : 2 (n−6 cis 9,12)	3.17 ± 0.12^{e}	20.57 ± 0.10^{d}	39.44 ± 1.84^{c}	51.43 ± 3.50^{b}	66.52 ± 0.45^{a}	<0.001
C20 : 0	1.89 ± 0.10^{a}	1.44 ± 0.00^{b}	1.15 ± 0.08^{c}	0.92 ± 0.07^{c}	0.99 ± 0.02^{c}	<0.001
C18 : 3 (n−3)	2.12 ± 0.06^{a}	1.72 ± 0.01^{b}	1.46 ± 0.07^{c}	1.28 ± 0.07^{cd}	1.19 ± 0.02^{d}	<0.001
C20 : 1 (n−9)	1.53 ± 0.00^{a}	1.19 ± 0.09^{ab}	1.10 ± 0.09^{bc}	0.89 ± 0.17^{bc}	0.77 ± 0.03^{c}	0.004
C21 : 0	1.40 ± 0.09^{a}	1.04 ± 0.00^{b}	0.83 ± 0.07^{bc}	0.65 ± 0.03^{c}	0.80 ± 0.02^{c}	<0.001
C22 : 0	1.43 ± 0.09^{a}	1.02 ± 0.02^{b}	0.85 ± 0.06^{bc}	0.68 ± 0.05^{c}	0.78 ± 0.02^{c}	<0.001
C20 : 3 (n−3)	1.12 ± 0.00^{a}	0.83 ± 0.00^{b}	0.66 ± 0.04^{c}	0.52 ± 0.04^{d}	n.d	<0.001
C20 : 4 (n−6 cis)	1.42 ± 0.02	1.08 ± 0.00	0.82 ± 0.06	0.58 ± 0.00	n.d	<0.001
C23 : 0	1.25 ± 0.08^{a}	0.93 ± 0.01^{b}	0.75 ± 0.07^{bc}	0.62 ± 0.05^{c}	0.75 ± 0.02^{bc}	<0.001
C20 : 5 (n−3 cis)	9.55 ± 0.12^{a}	7.30 ± 0.00^{b}	5.24 ± 0.27^{c}	3.26 ± 0.21^{d}	0.91 ± 0.00^{e}	<0.001
C24 : 0	1.16 ± 0.07^{a}	0.88 ± 0.00^{b}	0.70 ± 0.05^{bc}	0.57 ± 0.05^{c}	0.67 ± 0.00^{c}	<0.001
C24 : 1 (n−9 cis)	1.59 ± 0.13^{a}	0.85 ± 0.02^{b}	0.79 ± 0.03^{bc}	0.52 ± 0.04^{c}	n.d	<0.001
C21 : 5 (n−3 cis)	1.38 ± 0.03^{a}	1.09 ± 0.04^{b}	0.77 ± 0.02^{c}	0.56 ± 0.01^{d}	n.d	<0.001
22 : 6 (n−3 cis)	18.87 ± 0.01^{a}	14.75 ± 0.31^{b}	10.14 ± 0.48^{c}	6.15 ± 0.12^{d}	1.07 ± 0.00^{e}	<0.001
$\sum\omega 3$	23.38 ± 0.26^{a}	18.24 ± 0.32^{b}	13.02 ± 0.69^{c}	8.58 ± 0.30^{d}	3.02 ± 0.05^{e}	<0.001
$\sum\omega 6$	4.60 ± 0.15^{e}	21.66 ± 0.10^{d}	40.27 ± 1.91^{c}	52.02 ± 3.50^{b}	66.52 ± 0.45^{a}	<0.001
$\sum\omega 9$	5.08 ± 0.11^{a}	0.84 ± 0.02^{b}	0.32 ± 0.00^{c}	0.16 ± 0.00^{cd}	0.04 ± 0.00^{d}	<0.001

[a], data represent mean ± SD from two replicates for each analyses ($n = 10$). Values with different letters in the same row indicate significant differences between their groups ($P < 0.05$).

of the tissue and organs were made with a digital caliper (Insize). The measured tissues were stored in histological cassettes in a 10% formalin solution for histopathological examination.

2.7. Histopathological Examination. After fixation, tissue cassettes were dehydrated with an increasing degree of ethyl alcohol, cleared in xylene, and blocked in paraffin wax. Following this, sections of 5 μm thickness were cut on the rotary microtome (Leica RM 2255). Finally, the sections were stained with hematoxylin-eosin (H&E stain) and the photographs of the sections were viewed with a camera (Leica DM 400B) attached to the microscope. The proximal, middle, and distal loops of the intestine of control and poppy oil experimental group fish were photographed using x200 microscope objective lens. The effects of poppy oil dietary supplementation, villus height (μm, from the tip to the base of villus), villus width (μm, in the upper 1/3 of the villus), and width of tunica muscularis (Figure 1(f)) were analyzed using software (Image J software; Bethesda, MD, USA) to evaluate pathological changes in the liver, which were scored using the method described by Demirci et al. [5].

2.8. Statistical Analysis. Data collected from carp experimental groups (growth and feed utilization performance, intestinal morphometric, and histological measures) were statistically analyzed using one-way analysis of variance (ANOVA) to evaluate the effects of dietary inclusion of different levels of poppy oil. The differences between means

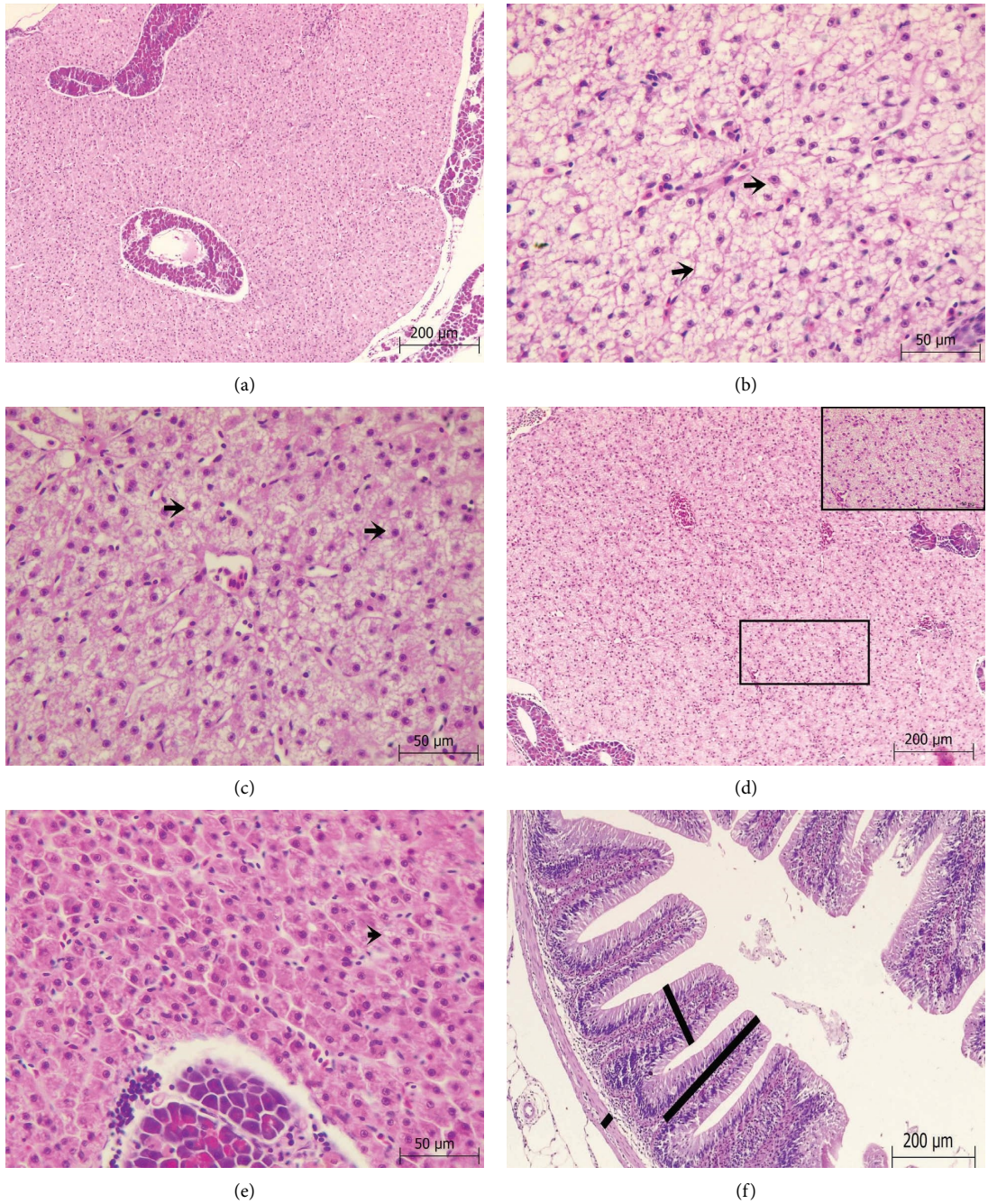

FIGURE 1: Hematoxylin-eosin staining. (a) Normal appearance of hepatocytes (bar: 200 μm). (b) and (c). Hydropic and vacuolar degeneration of hepatocytes (black arrows) in liver (bar: 200 and 50 μm). (d) Hydropic degeneration of hepatocytes in liver (bar: 200 and 50 μm). (e) Swelling in the hepatocytes in liver (bar: 50 μm). (f) Intestine section fish fed the diet. Fold height, HF; tunica muscularis, TMH; height, line; villus width, VW (bar: 200 mμ).

were determined by the post hoc test Tukey ($P < 0.05$). All values are presented as mean ± standard deviation. Statistical significance of liver histopathological scores was evaluated using IBM SPSS Statistics 25.0 software, and histopathological data were analyzed using nonparametric statistics using the Kruskal–Wallis test followed by the Mann–Whitney U test as post hoc. The value of $P < 0.05$ is considered statistically significant.

3. Results

3.1. Growth and Feed Utilization. The growth and feed utilization performance of the common carp were presented in Table 2. The growth and feed utilization performance of fishes fed with experimental diets showed significant influences ($P < 0.05$) on growth performance and feed utilization of common carp.

3.2. Hematological Parameters. Table 3 shows the hematological parameters in the blood of the experimental fish induced by the oil source in the experimental diets. Although all parameters except MCH were not significantly affected ($P < 0.05$) by the oil source used in the experimental diets, the MCH value showed a significant decrease ($P < 0.05$) in common carp fed with the PSO75 group compared to the control.

3.3. Histopathology Results of Fish Liver. There was no significant difference ($P > 0.05$) in histopathological findings such as hydropic-vacuolar degeneration, lipidosis, congestion, and dilatation in hepatocytes in the liver between fish fed with all experimental and control diets (Table 4). Hydropic/vacuolar degeneration in hepatocytes was observed severely in the livers of PO25 and PO50 group carps (Figures 1(b)–1(c)), moderately severe in the PO75 group (Figure 1(d)), and similar to the control group in the livers of PO100 diet fed group carps (Figures 1(a)–1(e)).

3.4. Histomorphology Results of Fish Intestine. Villus width, villus length, and tunica muscularis width of the intestines were measured, and statistically significant differences between the groups are given in Table 5. The villus width in the proximal, middle, and distal lobes increased statistically significantly in the poppy oil groups compared to the control group ($P < 0.05$). Proximal loop villus width was significantly increased in PO25, medial loops was significantly increased in PO75, and distal loop was significantly increased in the PO100 group compared to other groups ($P < 0.05$). The villus length of the proximal, middle, and distal loops of common carp fish in the PO100 group increased compared to the other experimental groups ($P < 0.05$). Tunica muscularis thickness increased statistically significantly ($P < 0.05$) in the proximal, middle, and distal loops in the groups in which poppy oil was added compared to the control group, and the highest tunica muscularis thickness was measured in the PO100 group.

3.5. Morphological Results. Table 6 shows morphometric measurements of the spleen, gallbladder, proximal (ascendens and descendens limbs), middle (ascendens and descendens limbs), and distal (ascendens and descendens limbs) loops of the intestine. In the morphometric measurement of the spleen, the width of the spleen was not statistically significant in the control and poppy seed oil groups ($P > 0.05$). The length of the spleen tended to increase in the poppy seed oil-replaced groups compared to the control group, but a significant increase was observed just in the PO100 group ($P < 0.05$). The width and length of the gallbladder were statistically lowest in the PO25 group, while the PO100 group showed the highest increase ($P < 0.05$). The width of the ascendens limb of the middle loop and the width and length of the descendens limb of the distal loop were significantly increased in the groups in which poppy seed oil was replaced, while the highest increase was observed in the PO100 group ($P < 0.05$). There was no statistically significant difference between the control and experimental groups in the width and length of intestinal swelling and other intestinal parts ($P > 0.05$).

4. Discussion

Fish oils give significant nutritional benefits to farmed fish species in terms of growth, health, and maintenance, as well as nutritional benefits to fish and fresh fish consumers. Fish oil is less required in the diets of freshwater species such as carps, catfish, and tilapias than carnivorous fish species. As a result, investigations on oil sources in diets to be employed in the production of these species, particularly carp, are quite fruitful [19–21]. Soybean, linseed, rapeseed, sunflower, palm oil, and olive oil are the most frequent plant-origin oils, efficiently used in the formulation of fish diets, and the main reason for these oils' performance in fish diets is their fatty acid composition, particularly PUFAs, such as linoleic (18 : 2 ω–6) and oleic acid (18 : 1 ω–9), they contain. As an alternative to these oil sources, poppy oil has the potential to be used in fish feeds due to its PUFAs, especially linoleic and oleic acid profiles [12]. This was clearly seen in the fatty acid profiles, especially ω3 and ω6 group of the experimental diets. The quantity of linoleic acid in the diet raised significantly ($P < 0.05$) as the fraction of poppy seed oil in the feed increased (Table 1). The combination of poppy seed oil with fish oil in rainbow trout feeds increased growth performance, according to a study on the growth performance and flesh quality of trout [7]. The current study yielded similar results; it was observed that increasing the quantity of poppy seed oil in the experimental diet formulation improved carp growth and feed utilization performance.

This study showed that replacing fish oil in the diet with poppy seed oil had no adverse effect on the growth and survival of juvenile common carp. The relative fatty acid profiles that satisfy the needs of this species may be responsible for the growth performance of common carp on a diet containing poppy seed oil. A similar pattern was seen in earlier investigations, indicating that freshwater or omnivorous fish species can often utilize a complete plant oil utilization [22, 23]. Although growth and feed utilization performance are the most essential economic factors for fish farming, fish health is extremely important for sustainable production, and hematological analyses are one of the important tools used in recent years to understand health status of fish due to many variables such as environment [24, 25], nutrition, and disease [26]. The hematological analyses carried out in the current study revealed no significant differences in any parameters ($P > 0.05$) (Table 3.). In the previous studies on fish consumed with diets containing plant-origin oil sources, it was reported that there was no difference in hematological parameters in accordance with the current study [19, 27]. The current and prior studies are considered to have no deleterious impact on carp hematological parameters since the digestibility and fatty acid profile of the oil source in the diets are appropriate for the species.

Dietary lipids comprise a variety of fatty acids with different chain lengths, unsaturation, and, as a result,

Table 2: Growth and feed utilization performance of fish fed with experimental diets.

	PSO0	PSO25	PSO50	PSO75	PSO100
Initial weight (g)	1.10 ± 0.01	1.10 ± 0.03	1.16 ± 0.07	1.14 ± 0.02	1.07 ± 0.01
Final weight (g)	6.16 ± 0.38^{c}	6.83 ± 0.25^{bc}	7.55 ± 0.17^{ab}	7.81 ± 0.33^{a}	7.33 ± 0.32^{ab}
RGR (%)	460.50 ± 40.50^{b}	520.40 ± 19.10^{ab}	548.90 ± 47.90^{ab}	584.70 ± 44.40^{a}	584.70 ± 34.90^{a}
SGR (% day^{-1})	1.91 ± 0.08^{b}	2.02 ± 0.03^{ab}	2.07 ± 0.08^{ab}	2.13 ± 0.07^{a}	2.13 ± 0.05^{a}
FCR	1.18 ± 0.09^{a}	1.04 ± 0.04^{ab}	0.94 ± 0.03^{b}	0.90 ± 0.04^{b}	0.96 ± 0.05^{b}

Data represent mean ± SD from three replicates for each group ($n = 3$). Values with different letters in the same row indicate significant differences between their groups ($P < 0.05$).

Table 3: Hematological parameters of common carp fed with experimental diets.

	PSO0	PSO25	PSO50	PSO75	PSO100
RBC ($10^6\,\mu L^{-1}$)	2.73 ± 0.19	2.60 ± 0.18	2.64 ± 0.20	2.71 ± 0.18	2.62 ± 0.17
Hb (mmol L^{-1})	11.36 ± 1.01	10.75 ± 0.77	10.73 ± 1.06	10.41 ± 1.02	10.48 ± 0.58
HCT (%)	29.70 ± 3.95	27.71 ± 1.76	29.65 ± 2.24	28.90 ± 3.12	30.33 ± 1.57
MCV (fL)	108.19 ± 7.40	106.58 ± 1.84	112.03 ± 4.26	106.52 ± 6.62	115.83 ± 5.85
MCH (pg cell^{-1})	41.51 ± 1.66	41.32 ± 0.94	40.48 ± 1.24	38.42 ± 2.36	40.01 ± 1.41
MCHC (g dL^{-1})	38.50 ± 2.71	38.78 ± 0.95	36.17 ± 1.77	36.15 ± 2.58	34.58 ± 1.41

Data represent mean ± SD from two replicates per tank total six fish for each group ($n = 6$). Values with different letters in the same row indicate significant differences between their groups ($P < 0.05$).

Table 4: Histopathological results of common carp liver fed with experimental diets.

mm	PSO0	PSO25	PSO50	PSO75	PSO100	P value
Hydropic/vacuolar degeneration	0.80 ± 1.09	1.40 ± 1.14	1.80 ± 0.83	$1.40 \pm 0.6\ 54$	0.80 ± 0.44	0.32
Lipidosis	0.20 ± 0.44	1.00 ± 0.70	0.60 ± 0.89	0.40 ± 0.54	0.60 ± 0.54	0.385
Congestion/Dilated sinusoids	0.80 ± 0.44	1.60 ± 0.54	0.80 ± 0.83	1.20 ± 0.83	0.80 ± 0.83	0.311

Data represent mean ± SD from two replicates per tank total six fish for each group ($n = 6$). Values with different letters in the same row indicate significant differences between their groups ($P < 0.05$).

Table 5: Histopathological changes in the liver and histomorphometric measurements of the intestines carp fish of fed with experimental diets.

mm	Parts of the intestine	PSO0	PSO25	PSO50	PSO75	PSO100
Villus width	Proximal loop	97.98 ± 12.17^{b}	126.03 ± 25.17^{a}	110.31 ± 19.60^{bc}	120.27 ± 23.21^{ab}	114.65 ± 22.45^{ab}
	Middle loop	97.36 ± 21.54^{b}	97.49 ± 20.60^{b}	107.31 ± 18.03^{ab}	112.85 ± 18.97^{a}	104.79 ± 20.13^{ab}
	Distal loop	99.23 ± 12.35^{b}	112.03 ± 16.80^{ab}	119.10 ± 20.06^{a}	119.46 ± 18.86^{a}	123.87 ± 23.13^{a}
Villus length	Proximal loop	221.36 ± 66.73^{c}	298.32 ± 57.88^{b}	325.04 ± 74.06^{ab}	309.07 ± 92.48^{b}	373.41 ± 87.76^{a}
	Middle loop	257.81 ± 47.59^{b}	343.51 ± 51.60^{a}	354.83 ± 101.02^{a}	361.38 ± 91.55^{a}	320.47 ± 54.50^{a}
	Distal loop	307.71 ± 38.24^{b}	332.85 ± 59.27^{b}	441.36 ± 121.93^{a}	441.57 ± 88.82^{a}	487.23 ± 88.93^{a}
Tunica muscularis thickness	Proximal loop	23.51 ± 6.70^{c}	34.84 ± 9.13^{b}	35.08 ± 5.93^{b}	35.69 ± 12.84^{b}	46.98 ± 12.75^{a}
	Middle loop	23.22 ± 8.63^{b}	25.61 ± 7.51^{ab}	24.60 ± 5.87^{b}	24.58 ± 6.99^{b}	30.83 ± 9.24^{a}
	Distal loop	22.05 ± 5.72^{b}	24.29 ± 6.78^{b}	23.44 ± 4.61^{b}	20.11 ± 4.56^{b}	36.21 ± 9.52^{a}

Data represent mean ± SD from two replicates per tank total six fish for each group ($n = 6$). Values with different letters in the same row indicate significant differences between their groups (($P < 0.05$)).

melting point and polarity [28]. Diets rich in fatty acids are frequently utilized in fish feeding to promote and accelerate development as well as provide physiologically useable energy. This limits the use of proteins as an energy source and promotes faster development [29]. Bile acids are the primary functional components of bile secretions that support lipid absorption in the intestines and maintain cholesterol homeostasis in the liver [30]. Bile acids are synthesized in the hepatopancreas and stored in the gallbladder in carp [31]. The width and length of the gallbladder were measured morphometrically in the current investigation, and it was discovered that the gallbladders of carps consumed the PO100 group diet, which were statistically larger than PO25 ($P < 0.05$). The current study's findings revealed that using poppy seed oil as the primary oil source in carp diets improved bile secretion.

The liver and intestine are the two most vital organs in nutrition digestion and absorption. As a result, it is critical to investigate these organs in nutrition research [32]. The liver regulates lipid metabolism, including both the synthesis and degradation of fatty acids [33]. The dietary oil supply and acid profile abnormalities could change the functioning and

Table 6: Morphometric measurement of spleen, gallbladder, and intestinal parts of carp fed with experimental diets.

mm	PSO0	PSO25	PSO50	PSO75	PSO100
Spleen width	2.82 ± 0.40	3.29 ± 1.51	3.11 ± 0.70	3.50 ± 0.85	3.71 ± 0.54
Spleen length	7.97 ± 2.48^{b}	9.36 ± 2.58^{ab}	9.50 ± 2.07^{ab}	10.45 ± 1.85^{ab}	11.47 ± 2.02^{a}
Gall bladder width	2.58 ± 0.22^{ab}	2.46 ± 0.30^{b}	2.80 ± 0.42^{ab}	2.53 ± 0.30^{ab}	3.19 ± 0.71^{a}
Gall bladder length	6.58 ± 1.12^{ab}	5.94 ± 1.24^{b}	6.75 ± 0.92^{ab}	6.76 ± 0.84^{ab}	7.85 ± 1.00^{a}
Intestinal swelling width	3.25 ± 0.62	3.85 ± 0.44	3.63 ± 0.64	3.24 ± 0.56	4.13 ± 0.72
Intestinal swelling length	19.37 ± 1.67	20.15 ± 2.62	21.08 ± 2.51	20.61 ± 2.08	22.31 ± 2.39
Proximal loop ascendens limb width	2.25 ± 0.41	2.28 ± 0.24	2.66 ± 0.42	2.47 ± 0.35	2.41 ± 0.39
Proximal loop ascendens limb length	11.08 ± 2.56	11.76 ± 1.35	11.66 ± 1.96	10.45 ± 2.13	11.49 ± 2.02
Proximal loop descendens limb width	1.89 ± 0.37	1.99 ± 0.23	2.24 ± 0.44	2.29 ± 0.30	2.21 ± 0.40
Proximal loop descendens limb length	9.70 ± 1.59	10.62 ± 1.57	9.63 ± 1.06	10.03 ± 2.05	9.63 ± 1.91
Middle loop ascendens limb width	1.35 ± 0.25^{c}	1.52 ± 0.16^{bc}	1.97 ± 0.35^{ab}	1.85 ± 0.32^{abc}	2.13 ± 0.50^{a}
Middle loop ascendens limb length	9.41 ± 1.22	10.62 ± 1.57	9.63 ± 1.06	10.03 ± 2.05	9.63 ± 1.91
Middle loop descendens limb width	1.88 ± 0.35	1.68 ± 0.27	2.38 ± 0.88	1.98 ± 0.71	2.06 ± 0.41
Middle loop descendens limb length	13.80 ± 2.63	16.62 ± 2.71	15.33 ± 2.31	14.87 ± 1.90	14.91 ± 3.00
Distal loop ascendens limb width	2.09 ± 0.64	2.15 ± 0.23	2.53 ± 0.58	2.36 ± 0.69	2.40 ± 0.35
Distal loop ascendens limb length	10.02 ± 2.33	11.29 ± 1.30	10.82 ± 1.28	11.49 ± 2.61	10.68 ± 1.64
Distal loop descendens limb width	1.85 ± 0.49^{b}	2.04 ± 0.32^{ab}	2.77 ± 0.64^{a}	2.18 ± 0.43^{ab}	2.46 ± 0.60^{ab}
Distal loop descendens limb length	14.98 ± 1.69^{b}	18.57 ± 1.17^{ab}	16.69 ± 2.46^{ab}	16.41 ± 3.61^{ab}	19.30 ± 2.78^{a}

structure of this organ [32]. When lipid or energy levels in the diet increase, hepatic cells' ability to oxidize fatty acids rises or protein synthesis is hindered, resulting in histopathological alterations such as lipid droplets or vacuolization in the fish liver [34, 35]. The source of oil in the diet and the quantity of total oil in the formulation have a direct impact on fish liver health. The livers of herbivorous carp species fed with a source of oil blended from fish, corn, and pork oils included in the diet at various proportions showed significant variations at the end of the feeding trial. It was reported that, in the study with the isonitrogenous diet, although swollen hepatocyte clusters filled with fat droplets were observed in hepatocytes of fish fed with the high-fat diet, they were of very small volumes compared to the normal part of the liver [36]. Hepatocytes of fish fed with high-fat diets were observed to have large vacuoles holding lipids and glycogen in research looking at the impact of dietary fat on carp liver health [37]. In a previous study, examining the differences in liver cells of sea bream fed diets containing different proportions of fat sources, it was found that the amount of fat in the feed may cause swollen hepatocyte foci with irregular nuclei in the liver tissues of the fish [38]. As it is understood from previous studies, dietary fat and the origin of the fat used in the diet directly affect liver health. In the current study of hydropic-vacuolar degeneration, lipidosis, congestion, and dilatation in hepatocytes, no significant difference ($P > 0.05$) was found between control and poppy seed oil-fed fish groups. However, hydropic/vacuolar degeneration and severe fatty deposits in hepatocytes were seen in PO25 and PO50 group carps, but not in PO100 group carps. In light of these findings, it is projected that using more than 75% or even 100% poppy oil in carp fish feed formulations will have a good influence on liver histopathology.

Histomorphometric and micromorphometric examination of the digestive system may differ depending on the diet of the fish, and studies have even repeatedly demonstrated changes in fish feed that have an influence on the morphometry of the digestive tract of fish in a short period of time [5]. The carps lack a specialized stomach [39, 40] and the intestines are anatomically segmented into intestinal swelling, proximal loop (ascending and descending limb), middle loop (ascending and descending limb), and distal loop (ascending and descending limb) [41]. In the current study, morphometric measurements of the intestinal segments were carried out, and the width of the ascendens limb of middle loop and the width and length of the descendens limb of the distal loop increased significantly in the groups to which poppy oil was added; the highest increase was observed in the PO100 group ($P < 0.05$). The lengthening and expansion of the intestines are linked to an increase in the surface area of the gastrointestinal system, which is an important function for nutritional absorption. The increased surface area in the intestinal tract might be interpreted as a greater number of folds and villus in the intestines, resulting in more nutritional absorption [42]. The length of the intestinal folds is a helpful metric for monitoring fish intestine metabolism and is thus frequently utilized [43, 44]. Valladao et al. [45] reported that the Nile tilapia fed with diets enriched with essential oils extracted from plant sources were found to develop longer intestinal villus. Regarding the intestinal morphometry of common carp (*Cyprinus carpio* L.), it was determined that there was a significant improvement in villus height, villus width (at the tip and crypt/villus junction), and crypt depth in a dose-dependent manner in fish groups fed with thyme essential oil [46]. The intestinal villus length of carp fish fed with diets used poppy seed oil was assessed in the study, and it was discovered that the villus length of the proximal, middle, and distal loops of the PO100 group carp fish increased when compared to the other study groups ($P < 0.05$). Villus width increased statistically substantially in the proximal, middle, and distal loops in the poppy oil-supplemented groups compared to the control group ($P < 0.05$). Tunica muscularis thickness increased in the proximal, middle, and distal lobes in the poppy seed oil-used groups compared to the control group,

with the PO100 group showing significant growth ($P < 0.05$). These data suggest that poppy oil improves nutrient absorption in the intestine by increasing the length and width of the intestinal villus and the thickness of the tunica muscularis. This was also consistent with the growth results acquired in this investigation.

5. Conclusion

In the light of the analyzes and measurements made within the scope of the research, it has been proved that the use of poppy seed oil instead of fish oil in the feed formulation in the feeding of carp fish, which is the most widely cultivated fish species in the world, improves the digestive system of carp fish and positively affects the growth performance and has a strengthening effect on the health of common carp. In addition, the results clearly showed that the use of only poppy seed oil as the main lipid source in carp diets increased growth without any detectable negative effect on the fish.

Data Availability

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

OSK and ÜA conceptualized and administrated the study; OSK, ÜA, HSE, and RT feeding process and animal manipulations; BD and FT micromorphometric and histopathologic examinations; OSK, ÜA, HSE, and RT carried out laboratory analyses; OSK, UA, AT, and KG carried out statistical analyses; ÜA, BD, FT, and RT wrote and prepared the original draft; OSK, HSE, AT, and KG wrote, reviewed, and edited the original draft. All authors have read and agreed to the published version of the manuscript.

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