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Research Article

# Effects of Adding Laurel (*Laurus nobilis*) Essential Oil to the Diet of Tilapia Fish on Growth and Intestinal Histology

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## ABSTRACT

The effects of adding laurel oil to the experimental diet on growth performance, biochemical compositions of fish and feeds, sand liver and intestine histology in Nile tilapia (*Oreochromis niloticus*) juveniles were evaluated. 180 fish (12±0.02 g) were used in the study. They were randomly placed in 12 tanks with a volume of 500 liters, with 15 fish per tank. The commercial laurel oil was added to the diets at 0, 0.3, 0.6, and 1.2%. The fish were fed with experimental diets twice a day as apparent satiation for 60 days. In the current study, weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR) and survival rates (SR) were statistically similar ($p>0.05$). While no difference was observed between protein and ash values in the biochemical analysis of fish, lipid values were found to be lower in the 0.3% and 0.6 supplemented groups compared to the control and 1.2% supplemented groups. In addition, there was no statistical difference in protein, lipid, and ash values in the biochemical composition of the feeds. In the study, essential oil components of *Laurus nobilis* oil such as Linalool, Elemene, Trans-Caryophyllene, Cis-α-Bisabolene, A-Terpinyl Acetate, Methyleugenol, β-Eudesmol were determined in low levels. The addition of 0.3% laurel oil to the diet did not cause histopathological findings, and it was found to improve liver and intestinal tissues. In conclusion, it is suggested that 0.3% laurel oil addition can be used as a feed additive in tilapia culture, especially considering the data obtained from growth and histological analyzes. Further studies are deserved need to examine the effects of laurel oil on immunity and resistance to various stress factors in other fish.

**Keywords:** Laurel oil, *Oreochromis niloticus, Laurus nobilis*, growth parameter,biochemical composition

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## INTRODUCTION

The number of fish caught in the world has reached the maximum level. Although aquaculture accounts for half of the total global production, it has grown rapidly over the past 30 years to meet people's food and nutrition security targets due to the increasing world population (Stratev et al., 2018). As aquaculture is an economical, quality, and healthy protein source, it has become a commercial sector that has an important role in meeting the global food demand and in contributing to the national economy in the future (Cottrell et al., 2021; Reverter et al., 2021). Due to these potentials, there are important expectations for the growth of aquaculture (Brugere et al., 2021). However, there are many challenges hindering the development of aquaculture (Reverter et al., 2021).

Aquatic organisms are under stress due to overstocking, deterioration of water quality, and malnutrition, weakening their immune systems and becoming more susceptible to diseases (Stratev et al., 2018; Dinardo et al., 2020; Lieke et al., 2020). This facilitates the emergence and spread of more virulent pathogens

in aquaculture systems (Reverter et al., 2021). In addition, inadequate hygiene facilitates the spread of pathogenic microorganisms to different regions by fish and equipment and leads to high mortality rates (Stratev et al., 2018; Lieke et al., 2020). Despite all efforts, economic losses from a disease outbreak in the aquaculture sector are estimated to be over $9.5 billion per year (Reverter et al., 2021).

The unconscious and continuous use of antibiotics for treatment and prevention of infectious diseases in aquaculture leads to the development of antibiotic-resistant bacteria, pollution of the aquatic environment, and accumulation of toxic residues in fish. This situation causes a global health problem among humans (Stratev et al., 2018; Reverter et al., 2021).Therefore, various alternative strategies such as the use of functional feed additives and vaccination have been suggested to prevent disease outbreaks and minimize the use of antibiotics in aquaculture (Stratev et al., 2018; Reverter et al., 2021). Studies in recent years have revealed that safer, easier to produce, biodegradable, and economical functional plant feed additives come to the fore (Awad & Awaad, 2017; Abdel-Latif et al., 2020; Dinardo et al., 2020; Hoseinifar et al., 2020).

The World Health Organization encourages the use of phytogenic substances (plantderivatives), their extracts, and phytochemicals (Kaur & Shah, 2017).

Although medicinal plants have been widely used to treat diseases in humans for thousands of years, studies of their effects on growth performance and protection of fish diseases have not yet reached the desired levels. Medicinal plants have an important potential in aquaculture, not only as a therapeutic tool but also in promoting growth and preventing stress and infectious diseases (Stratev et al., 2018; Hoseinifar et al., 2020; Dawood et al., 2020).

One of the plants with this potential is laurel, a medical and aromatic plant. *Laurus nobilis* is a tree belonging to the Lauraceae family, which is usually evergreen and always green in winter, and can reach 2-10 m in height. Being one of the richest countries of 600 medicinal and aromatic countries, Turkey is the largest supplier, accounting for 90% of the world's laurel production with a production of 32600 tons (Yilmaz & Deniz, 2017; Yilmaz & Ciftci, 2021).

Laurel leaves and their products are increasing every year depending on their use in many areas such as medicine, food, chemistry, and cosmetics (Yilmaz & Ciftci, 2021)). Dried leaves of laurel are used in many countries as a culinary seasoning, aromatic flavoring, and food preservative (Kara et al., 2020; Molina et al., 2020; Yilmaz & Ciftci, 2021). Soap, perfume, and body lotion are produced from the oil of laurel leaves (Yilmaz & Ciftci, 2021). In addition, to its use in the food, spice, and cosmetic sectors, it also meets the needs of many sectors with the essential oil (1-3%) obtained from its leaves and the fixed oil derived from its seeds (Yilmaz & Ciftci, 2021). It has been reported that non-toxic bay extracts act as a natural antibiotic in preventing food contamination by affecting the biofilm formation and virulence of Gram (+) and Gram (-) bacteria (Molina et al., 2020).

Laurel plant with its anthocyanin content in its fruit, is also used as a natural dye in the cosmetics, pharmaceutical, and food industries (Celik & Gul, 2020; Yilmaz & Ciftci, 2021). It is thought that the antioxidant, antiseptic, anti-inflammatory, anticonvulsant, antifungal, and immune-modulating properties in bay leaves are caused by 1.8 cineole and eugenol derivatives (Yilmaz & Deniz, 2017; Yilmaz & Ciftci, 2021). It has been reported that, in addition to methyl eugenol and 1.8-cineol, compounds such as camphor, β-caryophyllene, myrcene, eugenol, p-cymene, α-pinene, and β-pinene are found in essential oils obtained frombay leaves.

Due to the large number of plants grown in Turkey and especially endemic species, the medical evaluation of species has gained importance in recent years (Kara et al., 2020). Laurel (*Laurus nobilis*) is also a plant grown in Turkey and used medicinally (Karik et al., 2015; Kivrak et al., 2017; Celik & Gul, 2020). Apart from its beneficial pharmacological properties, the usage area of bay leaf essential oil is gradually expanding due to its many advantages such as thermal stability, and non-phototoxic effect (Yilmaz & Deniz, 2017). However, there are very limited studies on the use of laurel in aquaculture (Cagiltay et al., 2011; Turan et al., 2016; Dernekbasi et al., 2017). Although these medicinal plants have been tested as an immune mechanism in trout (Bilen & Bilen, 2012). Until now, there is no study to assess the effects of this plant oil on growth performance in tilapia. This is the first research to investigate the growth, biochemical composition, and histological effects of laurel oil use in tilapia (*Oreochromis niloticus*) which is the second most produced fish group worldwide after carp, which makes a very important contribution to global food security.

## MATERIALS AND METHODS

### Experimental diet and experimental design

The present study was carried out at Iskenderun Technical University Fisheries Application and Research Unit. Tilapia (*Oreochromis niloticus*) fingerlings used in the current study were provided by the Aquaculture Unit of Faculty. Fish were adapted for a week before the study. During the adaptation period, the fish were fed with the control diet as *ad libitum* 3 times a day. Trout feed supplied from a commercial enterprise was used as control feed (Table 1). 180 fish (12.0 ± 0.02 g) were randomly distributed to 12 tanks with a 500-liter capacity, 15 fish per tank. In the study, a semi-open recirculating system supported by air stones was used. The experiment was performed in a Completely Randomized Design with three replications. The fish were fed with experimental diets twice a day at 9:00 am and 17:30 pm as apparent satiation. During the experiment, the water temperature varied between 19-24 °C, and the pH was 7.5-8.5.

Natural photoperiod application was applied (12 D: 12L). The dissolved oxygen level in the tanks was measured as approximately 5 mg $L^{-1}$. The commercial *Laurus nobilis* oil used in the study was supplied from laurel leaves in Hatay.

Experimental feeds were prepared by mixing 0% (Control), 0.3%, 0.6% and 1.2% laurel oil with 8 ml distilled water for 100 g feed. To ensure a homogeneous mixture of laurel, oil was added to the

feed, Alphi 1 (Hexagon Product Development Pvt. Ltd. India) was mixed in a multi- dimensional mixer at 80 revolutions for 10 minutes. Before adding the laurel oil, and distilled water mixture to the feed, the continuity of homogeneity between oil and water was ensured by continuously mixing them. After mixing, the pellets were dried for a certain period and then kept at 4°C until use.

**Table 1.** Ingredients (%) and chemical composition (%) of the control diets.

| | |
|---|---|
| Moisture - %Max | 10 |
| Crude Protein - % (Min.) | 48 |
| Crude Oil - % (Min.) | 18 |
| Crude ash -% (Max.) | 10 |
| Celulose -% (Max.) | 2 |
| Gross Energy- (Kcal/kg.) (Min.) | 4800 |
| Digestable Energy-(Kcal/kg.) (Min.) | 4300 |
| Metabolic Energy (Kcal/kg. (Min.) | 4000 |
| OMEGA-3 (Gr./kg.) (Min.) | 10 |
| OMEGA-6 (Gr./kg.)W3/W6 | 3 |
| Ca % (Min./max. | 2.5 |

Raw materials:Fish Meal, Fish Oil, Soybean meal, Soybean oil, Chicken meal, Wheat Gluten, Corn Gluten, Vitamin and Mineral.

### Growth parameters

Fish were weighed and sampled after 24 hours of fasting. The growth performance parameters of *Oreochromis niloticus* were performed at 20-day intervals using the following formulas (Yazici et al., 2021).

(WG, g) = (final weight (fw) − initial weight (iw)),

(SGR, % day−1 ) = (ln fw − ln iw) / times (days) × 100,

(FCR, %)= [feed intake (g) / weight gain (g) and

(SR, %) = (final number of fish / initial number of fish) * 100 were calculated.

WG: Weight gain; SGR: Spesific Growth Rate; FCR: Feed Conversion Ratio; SR: Survival Rate

### Biochemical composition of fish and experimental diet

After the trial was completed, it was carried out according to the AOAC (1997) procedures to determine the crude protein content of the control and treatment group fish and the experimental feed. Bligh & Dyer (1959) method was used to evaluate the crude lipid content, and the method defined by Vollenweider et al. (2011). It was used to determine the raw ash amount. Body proximate analysis of tilapias and experimental diets were applied in triplicate.

### Fatty acid contents and Essential oil components of *Laurus nobilis* oil

Fatty acid methyl esters (FAMEs) of *Laurus nobilis* oil were prepared using the protocol reported by Parry et al., 2005.

Essential oil components of laurel oil were determined with Gas Chromatography equipped with a 5% Phenyl Polysylphenylene siloxane column (0.25 mm diameter*60 m long column with 0.25 µm thickness. A Flow rate of helium was used as carrier gas was 1mL min -1. MS transfer line, ionization, injection, and column temperatures were 250 °C, 220 °C, 220 °C, and 50 °C, respectively. Column temperature increased 3°C per minute from 50 °C to 220 °C. Each compound was determined with the X Calibur Program and Mas Spectra (Kocer et al., 2022).

### Histological examination

At the end of the 60th day of the study, the digestive tracts, and livers of 5 fish were taken randomly from each treatment group for histological examination and fixed with 10% PBF, (phosphate buffered formaldehyde). Following fixation, samples of the tissue were covered with embedding material and then placed in paraffin blocks. Tissue sample sections of 4-5 µm thickness were examined under a light microscope after staining with the hematoxylin-eosin (H&E) staining method (Yazici et al., 2021).

### Statistical analysis

Data on the Nile tilapia of *Oreochromis niloticus* growth performance parameters of different levels of laurel oil were subjected to One-way ANOVA. Normality and homogeneity were tested by using Kolmogorov Smirnov and Levene tests, respectively. All statistical analyses were done by using SPSS 17.0 software according to Duncan's New Multiple Range Test to identify the 5% level of significance of variance among the treatment groups's means. All experimental data were expressed as mean ± standard deviation (SD).

## RESULTS AND DISCUSSIONS

### Growth performance

In this study, the effects on growth performance were investigated, body composition, and histology of Nile tilapia (*Oreochromis niloticus*) of *Laurus nobilis* oil supplemented with feeds. In this study, the addition of laurel oil to the feeds did not affect the growth parameters of tilapias such as WG, SGR, FCR, and SR ($p>0.05$). Ning et al. (2021) reported that when Genetically Improved Farmed Tilapia (GIFT) added 300 mg kg-1 Blend Essential Oil (EO) to tilapia feeds, did not cause any change in growth parameters such as WG, SGR, FCR, and SR in parallel with our study (Table 2). On the other hand, studies on different essential oils and fish species obtained variable results in growth performance. According to Ghafarifarsani et al. (2022) reported that no change was observed in SGR in carp fed with 1-2% Thyme EO added feed, and their results were consistent with our results. In contrast, they suggested that 1-2% Thyme EO addition to the subject caused an increase in WG, SR, and a decrease in FCR. Yousefi et al. (2022) found that WG, SGR, and SR values were higher than the control, while the FCR values were found to be lower than the control in the study they carried out with Thyme EO and Immunogen prebiotic added feeds in trout. Like our study, Abdel Latif et al. (2021), when they added Origano EO (1-2%) to carp feeds, they did not detect any change in FCR and SR values, but they reported an increase in WG and SGR, unlike our study. Heluy et al. (2020) obtained variable results at different rates in their study with Origano EO on trout. WG value was highest and FCR was lowest in fish fed with 0.75 g kg-1

OEO, while WG was lowest and FCR was highest in 1.5% supplemented groups. No changes were observed in SGR and SR in parallel with our current study.

**Table 2.** Growth performance of Nile tilapia fed with different *Laurus nobilis* oil level for 60 days. Data expressed as mean ± standard deviation (SD).

| Parameter | Control | 0.3% | 0.6% | 1.2% |
|---|---|---|---|---|
| IW (g) | 12.77±0.18[a] | 12.75±0.14[a] | 12.77±0.18[a] | 12.75±0.22[a] |
| FW (g) | 35.86±1.94[a] | 32.28±0.41[a] | 30.07±1.57[a] | 33.37±2.43[a] |
| WG (g) | 23.08±1.88[a] | 19.53±0.54[a] | 17.30±1.40[a] | 20.62±2.65[a] |
| FCR | 1.01±0.11[a] | 1.14±0.02[a] | 1.38±0.17[a] | 1.05±0.19[a] |
| SGR (%) | 0.84±0.05[a] | 0.75±0.02[a] | 0.66±0.09[a] | 0.90±0.16[a] |
| SR (%) | 100 | 100 | 100 | 100 |

Lines assigned with the same letter show no significant difference (p>0.05).

### Body composition

The fish body composition is important in terms of reflecting the nutritional status and health of the generally farmed species (Hoang et al., 2019). It is also important for consumers who prefer to consume and buy fish with lower lipids and higher protein content (Safavi et al., 2019). Recently, the effects of some essential oils on body composition have been investigated. For instance, it has been reported that the addition of Origanum EO (Heluy et al., 2020) and Blend EO (Ning et al. 2021) to tilapia feeds, and Thyme EO to carp feeds (Abdel-Latif et al., 2021; Ghafarifarsani et al., 2022) did not affect body composition. In this study, in line with the studies mentioned, the addition of laurel oil did not affect the biochemical composition of the fish except for lipids (Table 3). The addition of 0.3% and 0.6% laurel oil was found to be lower than both the control group and the 1.2% group. Lipids are well-known as one important dietary component, which tend to show greater fluctuations than other carcass components (Peng et al., 2008). Rasoarahona et al. (2005) revealed that the reasons for the differences observed in body lipid levels of tilapia species may be related to species, season, age, sexual maturity, geographical origin, and diet composition. It has been suggested that optimum dietary lipid content is important, as low, and excess dietary lipids can have adverse effects on fish growth, feed consumption, health, and immunity (Rahimnejad et al., 2015).

**Table 3.** Body composition of Nie tilapia fed diet supplemented with different proportions of *Laurus nobilis* levels. Data expressed as mean ± SD.

| Biochemical compositions of fish | | | |
|---|---|---|---|
| **Treatment groups** | **Protein** | **Lipid** | **Ash** |
| **Control** | 76.63±0.09 | 4.71±0.26[a] | 4.26±0.46 |
| **0.3%** | 69.30±3.93 | 3.49±0.21[b] | 4.38±0.79 |
| **0.6%** | 74.08±1.67 | 3.61±0.37[b] | 3.61±0.29 |
| **1.2%** | 68.43±5.47 | 4.26±0.06[a] | 3.40±0.21 |

Values in a row with different superscripts indicate significant difference (p<0.05)

Table 4 shows the biochemical composition of the feed.

**Table 4.** Biochemical composition of Commercial diet supplemented with different proportions of *Laurus nobilis* levels. Data expressed as mean ± SD.

| Biochemical compositions of feed | | | |
|---|---|---|---|
| **Treatment groups** | **Protein** | **Lipid** | **Ash** |
| **Control** | 59.62±0.57 | 19.31±0.69[a] | 6.94±0.98 |
| **0.3%** | 59.99±0.20 | 21.14±0.16[b] | 7.01±0.13 |
| **0.6%** | 59.11±0.39 | 21.83±1.17[ab] | 6.86±0.18 |
| **1.2%** | 59.12±0.41 | 22.60±1.18[ab] | 7.16±0.22 |

Values in a row with different superscripts indicate significant difference (p<0.05)

### Fatty Acid Contents and Essential Oil Components of *Laurus nobilis*

Fatty acid values of *Laurus nobilis* were given in Table 5. Saturated and unsaturated fatty acids of *Laurus nobilis* values were 36.2% and 62.89%, respectively. The highest values of saturated and unsaturated fatty acids were observed in 18.34% (Palmitic Acid-C16:0) and 35.72% (Oleic Acid-C18:1n9), respectively. The level of Lauric acid (C12:0) from saturated fatty acids was 15.95%. The amount of linoleic acid (C18:2n6) obtained in the study was 25.04%.

**Table 5.** Fatty Acids of *Laurus nobilis* oil (%).

| Fatty Acids | % |
|---|---|
| C10:0 | 0.31 |
| C12:0 | 15.95 |
| C13:0 | 0.01 |
| C14:0 | 0.75 |
| C15:0 | 0.02 |
| C16:0 | 18.34 |
| C17:0 | 0.04 |
| C18:0 | 0.78 |
| **Saturated Fatty Acids** | 36.2 |
| C16:1n7 | 0.6 |
| C16:2n4 | 0.01 |
| C18:1n9 | 35.72 |
| C18:2n6 | 25.04 |
| C18:3n3 | 1.06 |
| C20:1n9 | 0.42 |
| C20:2n6 | 0.01 |
| C20:4n6 | 0.03 |
| **Unsaturated Fatty Acids** | 62.89 |
| **Total Fatty Acids** | 99.09 |

Table 6 shows the essential oil components of *Laurus nobilis*. In the study, essential oil components of *Laurus nobilis* oil such as Linalool, Elemene, Trans-Caryophyllene, Cis-α- Bisabolene, A-Terpinyl Acetate, Methyleugenol, β-Eudesmol were determined in low levels.

**Table 6.** Essential oil components of *Laurus nobilis* oil (%)

| Chemical Components | % |
|---|---|
| Linalool | 0.01 |
| Elemene | 0.2 |
| Trans-Caryophyllene | 0.06 |
| Cis-α-Bisabolene | 0.06 |
| A-Terpinyl Acetate | 0.16 |
| Methyleugenol | 0.01 |
| β-Eudesmol | 0.02 |

### Fatty acid and essential oil components

In the current study, lauric acid, palmitic acid, oleic acid, and linoleic acid levels were obtained at 15.95%, 18.34%, 35.72%, and 25.04%, respectively. Marzouki et al. (2008) found that the most common fatty acids of the *Lycium barbarum* (whole berry oil) were lauric acid (27.6%), Oleic acid (27.1%), Linoleic acid (21.4%), and Palmitic acid (17.1%). The study results of Ayanoglu et al. (2018) revealed that the major fatty acids of the *Laurus nobilis* species were lauric acid, oleic acid, palmitic acid, and linoleic acid. Yilmaz & Deniz (2018) showed that the most plentiful fatty acids for fleshy parts of *Laurus nobilis* berries were oleic acid (27.06% - 48.93%) and linoleic acid (29.18%-34.39%) from unsaturated fatty acids and palmitic acid (20.70%-33.07%) from saturated fatty acids. Petkova et al. (2019) indicated that the main fatty acids (FAs) in the lipid fractions were oleic, palmitic, and linoleic. The results obtained from fatty acid levels in the study are consistent with the literature.

Previous studies have shown that around 270 essential oil components can be found in bay leaves. Among them, 1,8-cineol (22-66.90%), sabinene (4.5–12.7%), α-terpinyl acetate (4.09-22.60%), α-pinene (2.2–15.9%), linalool (0.9-26.9%), α-terpineol (0.9%–12.0%), β-pinene (1.9%) –15.3%, terpineol-4 (0.9–4.1%) constitute the major part (Santos et al., 2014; Karik et al., 2015; Kivrak et al., 2017; Fidan et al., 2019; Stefanova et al., 2020; Kizak et al., 2020). Laurel oils have a variety of pharmacological effects, including antimicrobial, cytotoxic, and immune-modulating. The properties and compositions of the essential oil vary according to the harvested region, altitude, sun exposure time, and harvest conditions. The results in this study were found at lower levels than those reported in the literature.

### Histological Examination

The effects of adding laurel oil at various rates on tilapia liver tissue are shown in Fig. 1, 2, 3. The liver, which is a primary organ in the metabolizing of nutrients, can reflect deteriorations or effects in nutritional status with changes in its histological structure (Caballero et al., 2004). In this study, large fat vacuoles were observed around the exocrine pancreas in tilapia fish fed with 0.6% and 1.2% laurel oil. In parallel with the present study, dense vacuoles have been reported to develop around the exocrine pancreas of the liver of tilapia fish fed with menthol oil added to the diets of tilapia fish kept at high stocking density (Dawood et al., 2020).

The nuclei of hepatocyte cells were localized at the cell margin. In addition, the presence of dense zygmogen granules was detected between the acinar cells located in the exocrine part of the pancreas (Fig. 1a, b). It was observed that the nuclei of hepatocyte cells, in which lipid vacuoles decreased in the liver tissue of fish fed with 0.3% laurel oil, were more centrally located related to the control group. Zygmogen granules were also found in the acinar cells of the pancreatic tissue (Fig. 2a, b). Although the number of lipid vacuoles increased in fish fed 0.6% laurel oil compared to the 0.3% group, the nuclei of hepatocyte cells were found to be centrally located. While the formation of large fat vacuoles was observed on and around the outer surface of the exocrine pancreas, the presence of zygmogen granules in the exocrine pancreas was not detected (Fig. 3a, b). Fish fed with 1.2% laurel oil had an increase in fat vacuoles in the liver, as well as swelling of large blood vessels, and large fat vacuoles increased significantly in exocrine tissue (Fig. 4a, b).

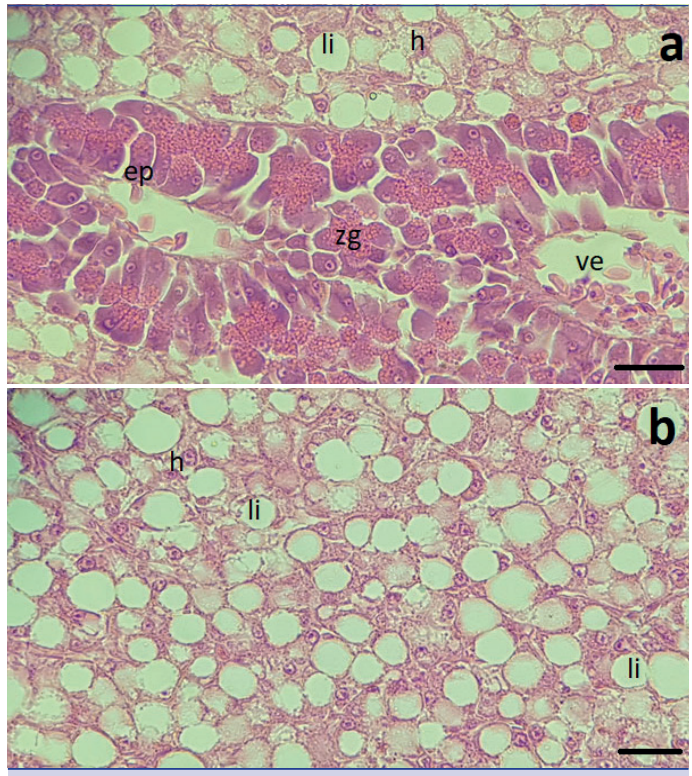


**Figure 1.** Liver tissue of fish in the control group bar: 15μm (H&E). zg: zymogen granules, ep: exocrine pancreas, h: hepatocytes, li: lipid vacuoles, ve: vein

In our study, vacuolization was observed in hepatocyte cells with the addition of laurel oil. Similar to our study, it was observed in tambaqui fry-fed 1.0 mL kg $^{-1}$ginger EO (Chung et al., 2021) and rainbow trout fed with *Origanum onites* essential oil (Yigit et al., 2017). However, in the tilapias examined, no pathological picture such as necrosis was found in hepatocyte cells reported by these researchers.

### Intestinal tissue

The effects of laurel oil on the foregut (Fig. 5a, b, c, d) midgut (Fig. 6a, b, c, d) and hindgut (Fig. 7a, b, c, d) have been shown.

It was determined that the nuclei of enterocyte cells in the foregut tissue of the control group were centrally located and the number of

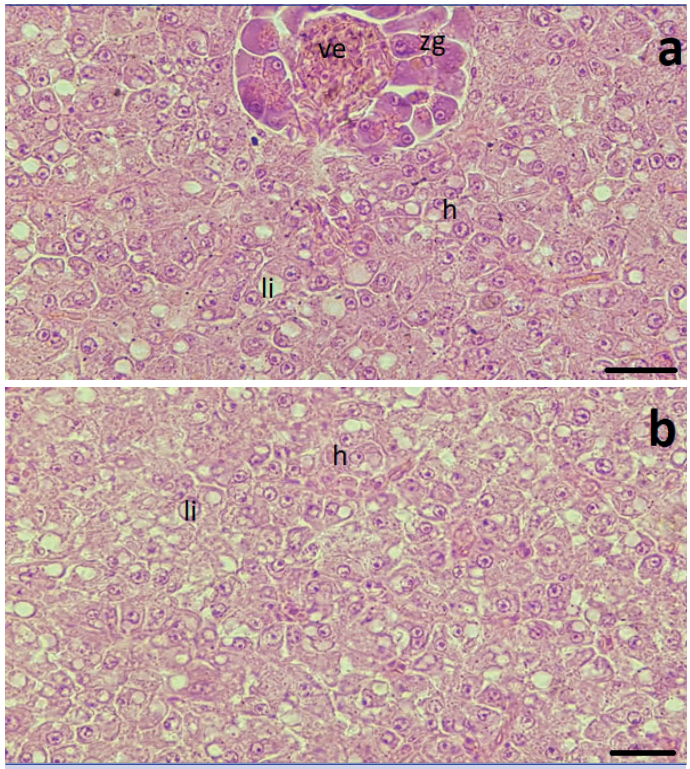


**Figure 2**. Liver tissue of tilapia fish fed with 0.3% laurel oil supplement bar: 20µm. zg: zymogen granules, h: hepatocytes, li: lipid vacuoles, ve: vein

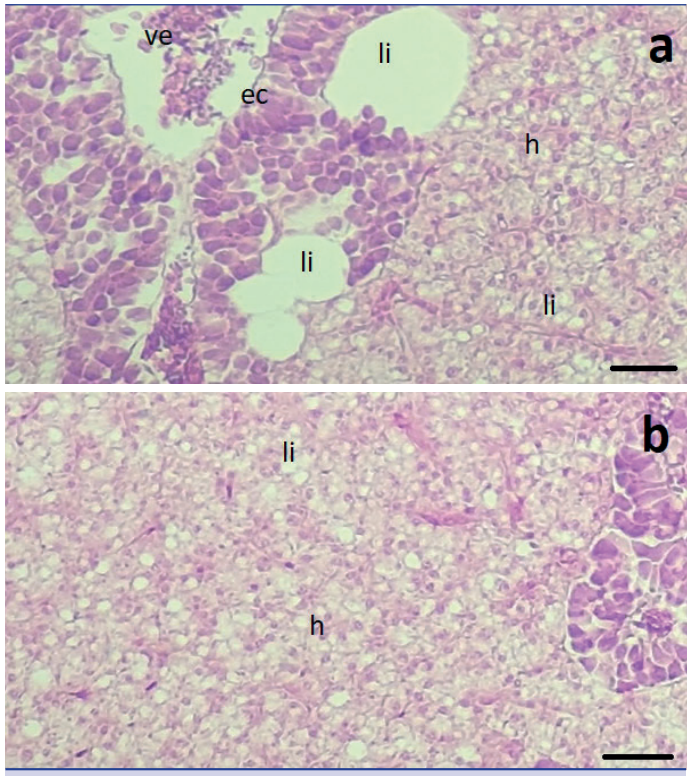


**Figure 3**. Liver tissue of tilapia fish fed with 0.6% laurel oil supplement bar: 20µm (H&E). zg: zymogen granules, ec: exocrine pancreas, h: hepatocytes, li: lipid vacuoles, ve: vein

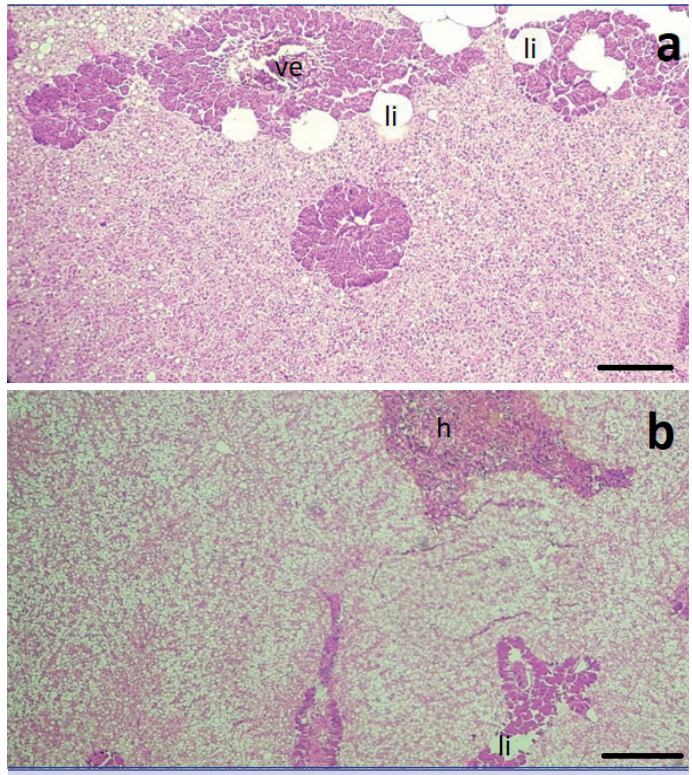


**Figure 4**. Liver tissue of tilapia fish fed with 1.2% laurel oil supplement bar: 100µm (H&E). zg: zymogen granules, h: hepatocytes, li: lipid vacuoles, ve: vein

goblet cells was low (Fig. 5a). It was observed that goblet cells in the intestinal tissue of fish fed 0.3% laurel oil was localized towards the ends of the villi (Fig. 5b). Fish fed with 0.6% laurel oil showed an increase in the number of goblets cells as well as thickening and branching formations in the villi compared to the control group (Fig. 5c). In fish fed 1.2% laurel oil, on the other hand, branching of the villi and enlargementof the lamina propria were observed (Fig. 5d).

EOs given at appropriate rates provide improvement in intestinal histology, while other rates either causes histological disorders or shows no effect (Valladao et al., 2017; Brum et al., 2018).In this study, it was determined that the surface area increased with the thickening of the villi rather than the longitudinal growth in the hindgut of the fish fed with laurel oil.

Branching was detected in the midgut villi of the fish belonging to the control group (Fig. 6a). In the midgut tissue of fish fed 0.3% laurel oil, it was observed that these branches increased, the lamina propria expanded, and there was a serious increment in the number of goblet cells (Fig. 6b). However, it was determined that the short villi enlarged and the lamina propria thickened in the midgut of the fish fed with 0.6% laurel oil (Fig. 6c). It was noted that this thickening was much wider in fish fed 1.2% laurel oil, and there were also thickenings in the mucosal layer of the midgut tissue (Fig. 6d).

Goblet cells play a very important role in protecting intestinal barriers against pathogenic microorganisms by producing mucus and antimicrobial substances. They also stimulate intestinal local immunity by secreting chemokines and cytokines (Knoop

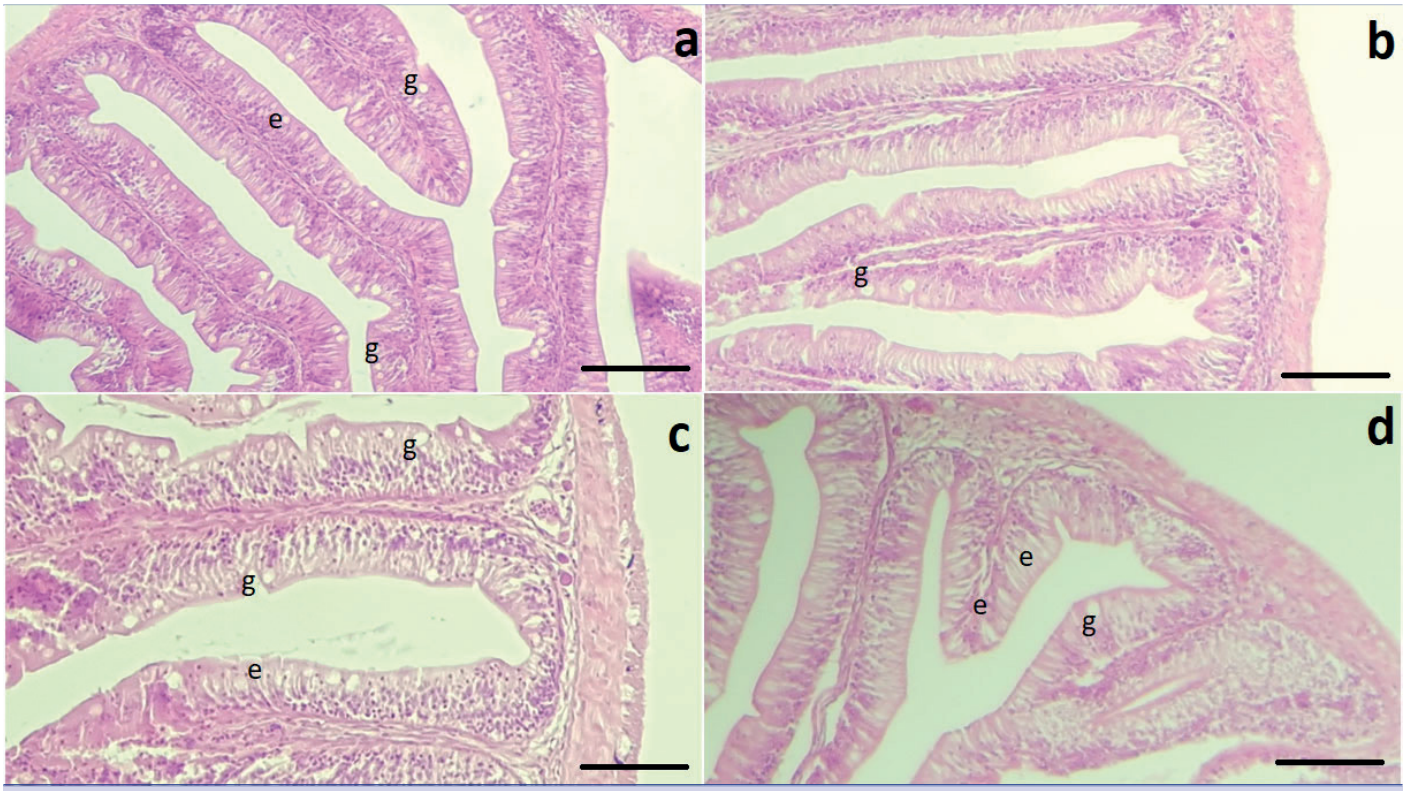


**Figure 5**. Foregut histology of fish fed control group (a), 0.3% (b), 0.6% (c), and 1.2% (d) with laurel oil (a,b, and d bar: 100µm, c bar 60µm) (H&E). e: enterocyte, g: goblet cells

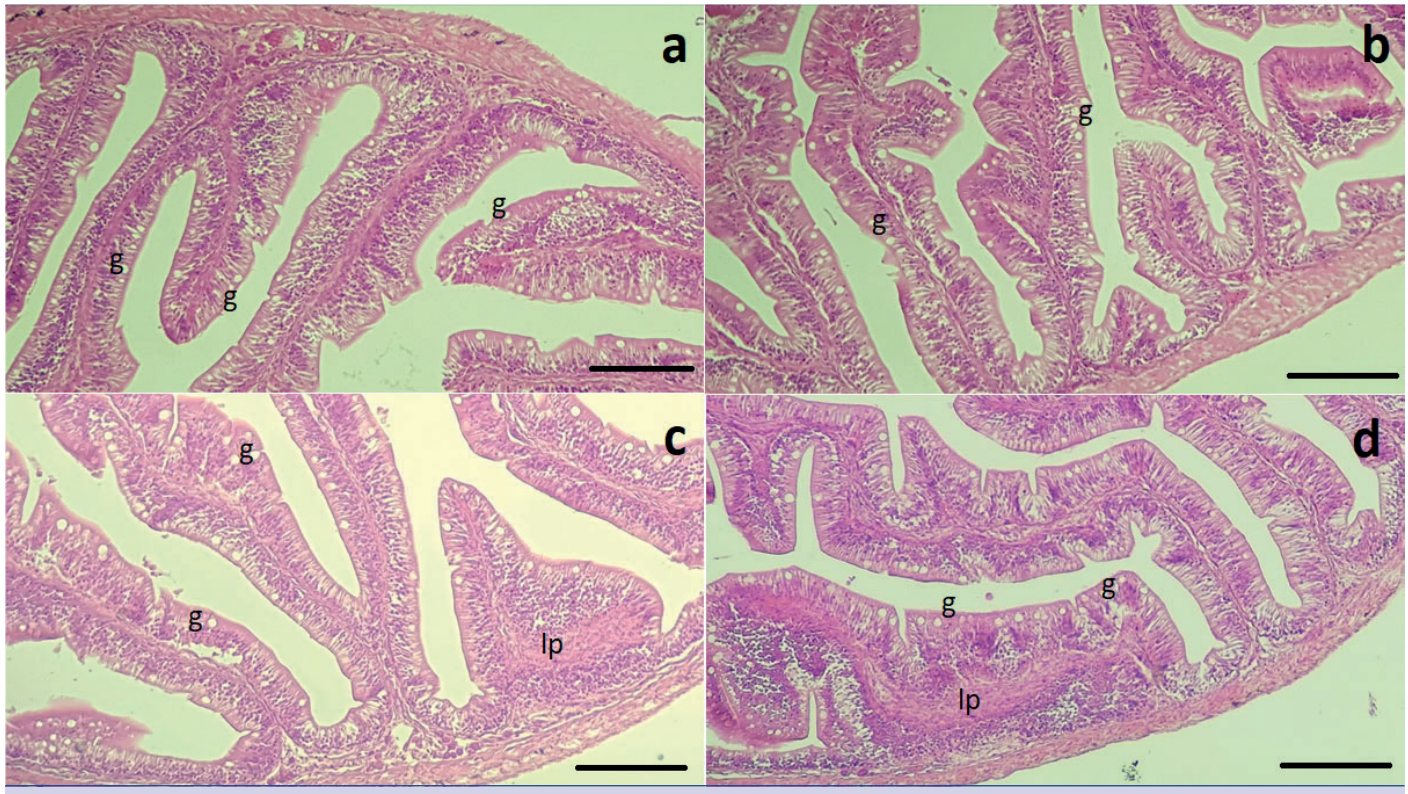


**Figure 6**. Histological sections of midgut tissues of tilapia fish fed with control (a), 0.3% (b), 0.6% (c), and 1.2% (d) laurel oil diet. (bar 100 µm) (H&E). g: goblet cells, lp: lamina propria

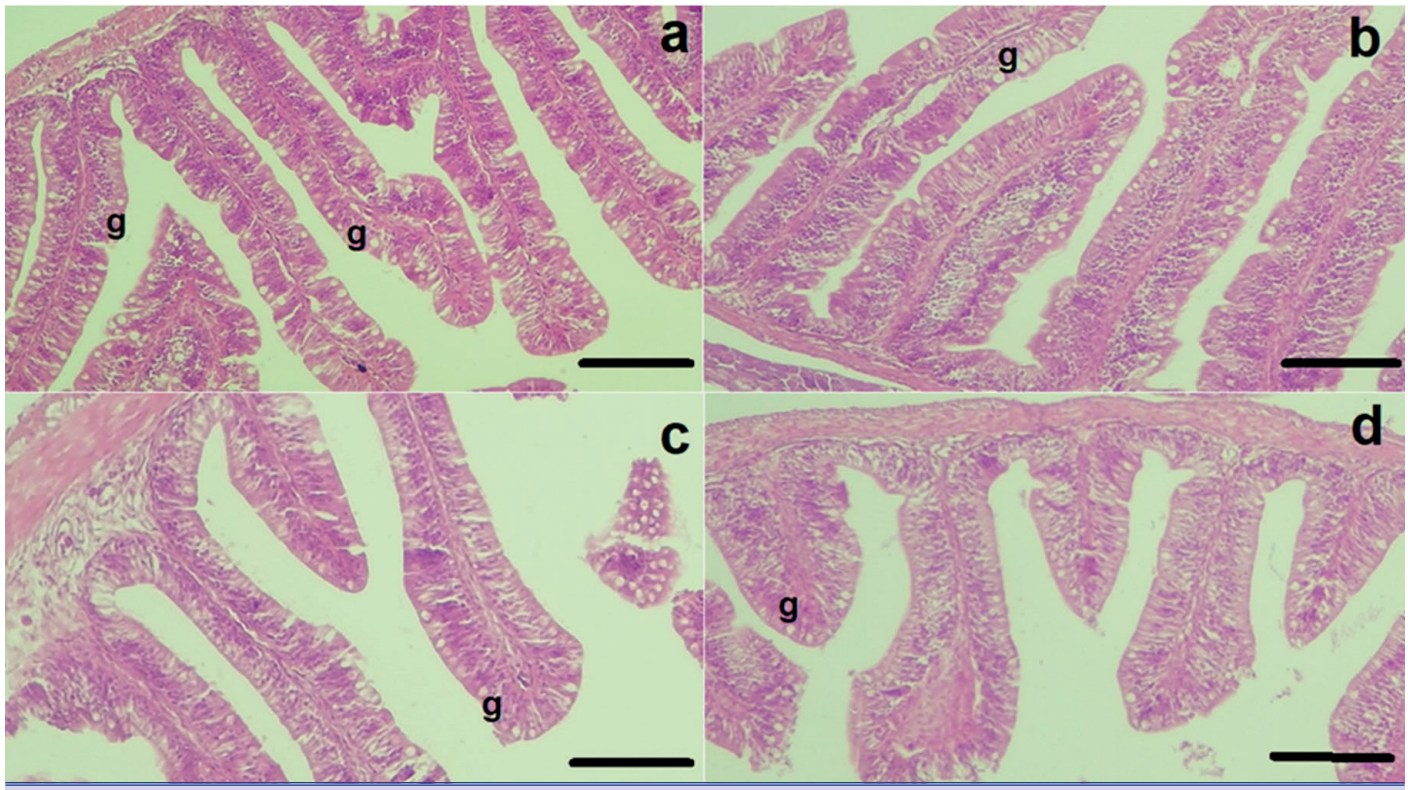


**Figure 7**. Histological sections of hindgut tissues of tilapia fish fed with control (a), 0.3% (b), 0.6% (c), and 1.2% (d) laurel oil diet (bar 100 µm).

& Newberry, 2018; Dawood, 2021). In the present study, an increase in the number of goblet cells was observed in the midgut of tilapia fed with laurel oil-added feeds. In contrast, Chung et al. (2021) reported that essential oils obtained from ginger reduced the height of villi in the intestines and reduced goblet cells. In addition, it is suggested that 0.6% and 1.2% laurel oil in the midgut, which is important for digestion, causes cell infiltration in the lamina propria, which may cause a decrease in the absorption of nutrients by reducing the contact between villi and nutrients, as reported by other researchers (Valladao et al., 2017; Chung et al., 2021).

Branching was detected in the hindgut villi of the fish of the fed control diet (Fig. 7a). It was determined that these branches were decreased, and the villi enlarged in the hindgut tissue of fish fed with 0.3% laurel oil (Fig. 7b). Thickening of the lamina propria and a decrease in the number of goblet cells were detected in the hindgut of the fish fed with 0.6% laurel oil (Fig. 7c). It was noted that the villi shortened and thickened and there was a serious decrease in the number of goblet cells in fish fed 1.2% laurel oil (Fig. 7d).

Intestinal anatomy and histology are very important for nutritional evaluation and immunological functions in fish health. Therefore, histological examination of the gut can be considered a potential indicator of the overall health of the fish and the efficacy of feed additives (Yigit et al., 2017; Abdel-Latif et al., 2021; Acar et al., 2021).

## CONCLUSION

As a result of the study, the addition of laurel oil to the feed did not show negative results in growth performance. In addition, unlike other groups, histopathological findings were not found in the intestines and livers of fish fed 0.3% bay oil. In addition, it has shown positive effects such as branching of villi in the intestines, expanding the food use surface area in the midgut, increasing goblet cells, and reducing the fat vacuoles in the liver. Therefore, it is recommended to use 0.3% laurel oil as an additive to feeds. More experiments should be designed to determine the efficacy of laurel oils in different fish species farmed as well.

**Conflict of Interest:** The authors declare that there are no conflicts of interest.

**Ethical Approval:** Animal care and experiments were carried out considering national and /or international guidelines.

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