

## Morphological and Histological Investigation of the Effects of Garlic Extract on the Intestine of Goldfish (*Carassius auratus auratus*)

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### ABSTRACT

Garlic (*Allium sativum*) has been widely recognized for its therapeutic properties and potential use as a feed additive in aquaculture. This study aimed to investigate the morphological and histological effects of garlic extract on the intestinal health and growth performance of goldfish (*Carassius auratus auratus*). Goldfish were fed diets supplemented with 5 and 10 mg/g of garlic extract for three months. Results indicated significant improvements in both weight gain and intestinal villus length, particularly in the 10 mg/g group, compared to the control group. The control group showed a decline of 21% in the overall metric between the two sampling periods, with intermediate percentage increases during the individual sampling intervals. In contrast, the groups receiving garlic supplementation demonstrated significant increases. The 5 mg/g garlic group exhibited a 37% total increase, while the 10 mg/g garlic group showed the highest overall increase of 53%. These findings suggest that garlic extract can enhance digestive efficiency and overall growth in goldfish, presenting a promising natural alternative for growth promoters in ornamental fish species. Further research is recommended to explore its long-term effects and efficacy across different fish species.

**Keywords:** *Allium sativum*, Garlic Extract, Intestinal Histology

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### Sarımsak Ekstraktının Japon Balığının (*Carassius auratus auratus*) Bağırsağı Üzerindeki Etkilerinin Morfolojik ve Histolojik Olarak Araştırılması

#### ÖZ

Sarımsak (*Allium sativum*), terapötik özellikleri ve su ürünleri yetiştiriciliğinde yem katkı maddesi olarak potansiyel kullanımıyla geniş çapta tanınmıştır. Bu çalışma, sarımsak ekstraktının Japon balığının (*Carassius auratus auratus*) bağırsak sağlığı ve büyüme performansı üzerindeki morfolojik ve histolojik etkilerini araştırmayı amaçlamıştır. Japon balıkları, üç ay boyunca 5 ve 10 mg/g sarımsak ekstraktı ile zenginleştirilmiş diyetlerle beslenmiştir. Sonuçlar, özellikle 10 mg/g grubunda, kontrol grubuna kıyasla hem kilo alımında hem de bağırsak villus uzunluğunda anlamlı iyileşmeler olduğunu göstermiştir. Kontrol grubunda, iki örnekleme dönemi arasında toplamda %21'lik bir azalma gözlenirken, bireysel örnekleme aralıklarında orta derecede artışlar kaydedilmiştir. Buna karşılık, sarımsak takviyesi alan gruplar önemli artışlar sergilemiştir. 5 mg/g sarımsak grubunda toplam %37'lik bir artış gözlemlenirken, 10 mg/g sarımsak grubunda %53 ile en yüksek toplam artış tespit edilmiştir. Bu bulgular, sarımsak ekstraktının Japon balıklarında sindirim verimliliğini ve genel büyümeyi artırabileceğini, süs balığı türleri için umut vadeden doğal bir büyüme teşvik edici alternatif sunduğunu göstermektedir. Uzun vadeli etkilerini ve farklı balık türlerindeki etkinliğini araştırmak üzere daha fazla çalışmaya ihtiyaç duyulmaktadır.

**Anahtar Kelimeler:** *Allium sativum*, Bağırsak Histolojisi, Sarımsak Ekstraktı

To cite this article: Duman M, Mazi A, Oflaz A, Karu Ç, Güler S, Onguncan O, Ajmi N, Taşci G, Karatoprak YE, Biyyük F. Morphological and Histological Investigation of the Effects of Garlic Extract on the Intestine of Goldfish (*Carassius auratus auratus*). Kocatepe Vet J (2025) 18(4): 417-427

Submission: 17.03.2025 Accepted: 10.12.2025 Published Online: 12.12.2025

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## INTRODUCTION

The aquarium fish industry has significantly expanded from small glass bowls in our homes to large public aquariums, evolving into a global industry valued at an estimated 15–30 billion USD (Duman et al. 2024). This sector, involving over 120 countries, is led by Asia and developing nations, which produce approximately 60% of aquarium fish. The industry harbors rich biodiversity, encompassing about 5300 freshwater and 1800 marine fish species (Evers et al. 2019; Rhyne et al. 2012; Thusty et al. 2013). Feed additives are beneficial substances added to diets in small amounts to enhance the physiological and immunological status of fish, and their use as appropriate supplements has been recommended by many researchers (Adel et al. 2020; Ahmadifar et al. 2019; Dawood et al. 2018; El Basuini et al. 2020; Shekarabi et al. 2020). A wide variety of feed additives can be incorporated into diets not only to meet the basic nutritional requirements of the target species but also to improve growth performance, feed utilization, stress resistance, and overall health (Encarnação 2016). Balanced feeding that protects gut integrity and improves digestion can safeguard fish from diseases and enhance immunity (Dawood and Koshio 2020). Gut immunity primarily depends on the health of gut barriers, including epithelial cells, and on the gut-associated lymphoid tissues (GALT) developed under the influence of gut microbiota (Martin and Król 2017; Rombout et al. 2011). Garlic (*Allium sativum* L.), a plant-based feed additive, has been recognized for its broad therapeutic properties (D. Y. Lee et al. 2012). It is a widely used spice with documented positive effects on organisms (Abdel-Tawwab et al. 2020; Adineh et al. 2020; Vallejo Villalobos et al. 2008). Garlic contains various bioactive compounds such as allicin and other organosulfur compounds, phenols, polysaccharides, and saponins (Szychowski et al. 2018). These components confer antimicrobial, antioxidant, antihypertensive, anti-inflammatory, anticancer, cardiovascular protective, antidiabetic, and anti-obesity properties (Guo et al. 2012; Shang et al. 2019; Szychowski et al. 2018; Yun et al. 2014). Among medicinal plants, garlic has been demonstrated at the laboratory level to be one of the most effective in promoting animal health (Guo et al. 2012). This efficacy is attributed to its various therapeutic properties, particularly its broad-spectrum activity against pathogens and immunostimulatory effects (Guo et al. 2012; D. H. Lee et al. 2014). Furthermore, garlic is easily obtainable and cost-effective. In aquaculture, garlic exhibits antibacterial, antiparasitic, antioxidant, immunostimulatory, and growth-promoting activities (Bender-Bojalil and Bárcenas-Pozos 2013; D. Y. Lee et al. 2012). It can also serve as an alternative to many chemotherapeutic agents without leaving residues in the environment. However,

the commercial-scale use of garlic is still not a widespread practice. Juvenile ornamental fish exhibit comparatively weaker immunocompetence than larger conspecifics; accelerating early growth therefore serves a dual purpose—shortening the time to marketable size and improving disease resistance as immune function matures. We selected goldfish (*Carassius auratus auratus*) because they are among the most widely traded ornamental cyprinids, have well-established husbandry protocols, and constitute a relevant model for gut-focused nutritional interventions in the aquarium sector. Intestinal morphology is central to this goal, as villus architecture governs nutrient absorption efficiency, growth trajectory, and visible vitality/colouration prized by breeders and retailers. Garlic was chosen as the dietary additive because it is widely available and cost-effective, and its organosulfur-rich composition confers broad antimicrobial, antioxidant, and growth-promoting properties, making it an attractive and practical candidate for ornamental aquaculture. The present study investigates the effects of dietary garlic powder at different additive levels on live weight gain in *Carassius auratus*, supported by histological analysis of intestinal villus morphology and development.

## MATERIALS and METHODS

### Acquisition and Adaptation of Fish

Only clinically healthy goldfish were chosen at purchase. They showed no external lesions, deformities, ectoparasites, or signs of infectious or non-infectious diseases. During pre-transport inspection, the fish displayed normal behavior, fin position, ventilation rate, and swimming activity. Additionally, there were no abnormal behaviors or deaths during the acclimation period before their use in experiments. Goldfish (*Carassius auratus auratus*), aged between 0 and 2 months and originating from the same parental stock, were obtained from a single aquarium supplier. For transport, fish were transferred from the supplier to the laboratory in water-filled, covered transport containers sized to provide adequate water volume and oxygenation for their normal physiology. The transfer was completed within approximately 1 hour to minimize stress. Upon arrival, fish were gradually acclimated to the holding system to avoid thermal and osmotic shock, and they were monitored; no abnormal behavior or mortality was observed during this acclimation phase. Three experimental groups (mg/g as a control, 5 mg/g and 10 mg/g) were established, each containing 30 fish housed in separate aquaria. Prior to the start of the experiment, the fish were acclimated to the laboratory conditions for two weeks. During the experimental period, the fish were maintained in freshwater at an

average temperature of 25 °C, under standard hygienic conditions, with a minimum dissolved oxygen level of 4 ppm.

The required sample size for the study was determined using a priori power analysis. This analysis was performed using G\*Power 3.1.9.4 software, based on the statistical test “ANOVA: Repeated measures, between factors” within the “F tests” family. The parameters used in the power analysis included an effect size of  $f = 0.25$  (medium effect), significance level  $\alpha = 0.05$ , statistical power  $(1-\beta) = 0.80$ , 3 groups, 11 measurements, and a correlation among repeated measures of 0.5. Based on these parameters, the minimum required total sample size was calculated as 90 individuals, and this criterion was met by assigning 30 fish per group. As a result, the achieved power was calculated to be 0.81, indicating that the sample size was sufficient for reliable statistical evaluation.

All experimental procedures were conducted in accordance with ethical standards for animal welfare and were approved by the Local Ethics Committee (Approval No: 2022-17/0). All the animals were housed at the Aquatic Animal Disease Laboratory, located within the Faculty of Veterinary Medicine at Uludag University, Bursa.

#### **Application of Garlic Extract to Fish and Monitoring of Growth Factors**

The water-soluble garlic extract (99% purity) used in the study was obtained from the Animal Nutrition and Nutritional Diseases at Bursa Uludag University, Faculty of Veterinary Medicine. The fish were fed commercial goldfish feed supplemented with garlic powder at doses of 0, 5, and 10 mg/g, constituting 8–10% of their body weight. The dose levels of 5 and 10 mg/g were selected based on preliminary trials and previous literature suggesting physiological efficacy without inducing toxicity in similar fish species (Dadgar et al. 2019). To ensure adherence to the garlic powder, the feed was coated with fish oil at 10 ml/100 g, dried, and then used for feeding. The control group received only feed with fish oil (0 mg/g garlic powder). Daily records were kept of feed intake rates, feeding behavior, and the amount of uneaten feed and fecal waste. The aquaria were cleaned daily using a siphon to maintain water quality. The fish were fed the garlic-supplemented diets for two months, and every 30 days, eight fish were sampled to measure growth rates. Parameters such as length, width, weight, coloration, general activity within the aquarium, and feeding behavior were assessed to determine the impact of garlic extract on growth (Saha and Bandyopadhyay 2017).

#### **Relationship between Growth Performance and Feed Cost**

To evaluate growth performance, parameters such as weight gain, feed intake, and feed conversion ratio

(FCR) were calculated based on standard formulas (Sotoudeh and Yeganeh 2017; Lugert et al. 2014). Feed cost efficiency was determined by calculating the cost of consumed feed relative to the weight gained, using the market price of commercial goldfish feed. The calculations were performed using the following formulas:

Weight Gain (*WG*):

$$WG = (W_{final} - W_{initial}) \times T$$

Where:

- $W_{final}$ : Average final weight of the fish (g)
- $W_{initial}$ : Average initial weight of the fish (g)
- $T$ : Experiment duration (days)

Feed Intake (*FI*):

$$FI = (F_d \times N) \times T$$

Where:

- $F_d$ : Daily feed quantity (g)
- $N$ : Number of fish per group
- $T$ : Experiment duration (days)

Feed Conversion Ratio (*FCR*):

$$FCR = \frac{F \times T}{LIW \times T}$$

Where:

- $F$ : Total feed consumed (g)
- $LIW$ : Average weight gain of the fish (g)
- $T$ : Experiment duration (days)

Feed Cost Calculation (*FCC*):

$$FCC = FCR \times P$$

Where:

- $FCC$ : Cost per gram of weight gained (currency unit)
- $FCR$ : Feed conversion ratio
- $P$ : Cost per gram of feed (currency unit)

#### **Histological Monitoring of Effectiveness of Garlic in Fish**

At 30-day intervals, eight fish from each experimental group were collected for histological examination. The fish were euthanized using clove oil anesthesia (Catalog Number: 3502–20006, 96.37% eugenol) (Fernandes et al. 2016), and tissue samples were collected from the intestines. Tissue samples were fixed in a 10% formaldehyde solution (Isolab; Cat. No. 923.12.2500) and then subjected to routine histologic procedures (Agbebi et al. 2013). Standard histological processing involved dehydration in a graded ethanol series (Merck; Cat. No. 100983), clearing in xylene (Merck; Cat. No. 108297.2500), and embedding in paraffin (Merck; Cat. No. 107164). Sections were cut

and stained using Crossman's modified Mallory's triple stain (For Hematoxylin: Merck; Cat. No. 105174; For Acid Fuchsin: Merck; Cat. No. F8129; For Anilin Blue: Merck; Cat. No. B8563) for microscopic examination. The photographic imaging of the preparations was performed using a Nikon Eclipse 80i light microscope (Nikon Corp., Tokyo, Japan) and NIS-Elements imaging software (Nikon Corp., Tokyo, Japan). Histological changes in tissue samples were compared with those of the control group (0 mg/g) to observe histological differences and measure tissue parameters suggested by Al-Salahy and Mahmoud, (2003).

### Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics software (Version 20; IBM Corp., Armonk, NY, USA). The normality of the data was assessed using the Shapiro-Wilk test. Since the assumptions of normal distribution and homogeneity of variances were not met, non-parametric tests were preferred. The Friedman test was used to evaluate temporal changes within control and treatment groups throughout the experimental period. At the end of the trial, intergroup comparisons of fish weights were conducted using the Kruskal-Wallis H test. When significant differences were detected, pairwise comparisons were performed using the Dunn-Bonferroni post hoc test. A significance level of adjusted  $p < 0.05$  was considered statistically significant. The results obtained at each sampling time point are presented as mean  $\pm$  standard deviation (mean  $\pm$  SD).

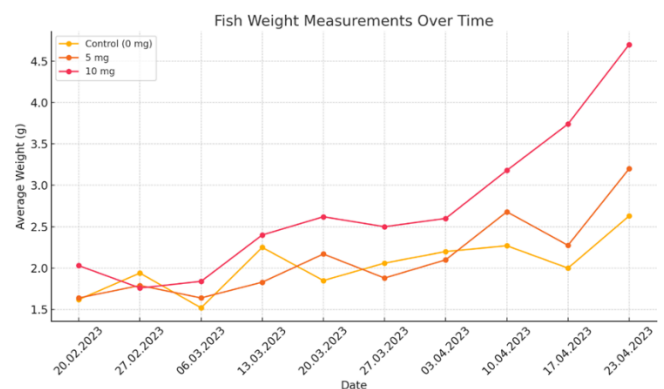
## RESULTS

### Effects of Garlic Extract on Growth Parameters

Initial body weights of the fish ranged from 1.62 to 2.03 g. Following the acclimation period, daily, weekly, and total feed consumption were calculated for the control group (0 mg/g garlic) and for the experimental groups fed diets supplemented with 5 mg/g and 10 mg/g garlic extract. Throughout the trial, the growth performance of the fish was monitored weekly, and individual body weights were recorded. Garlic extract supplementation was found to have statistically significant effects on fish growth (adj.  $p < 0.05$ ).

On February 20, 2023, the initial mean weights were recorded as 1.62 g for the control group, 1.64 g for the 5 mg group, and 2.03 g for the 10 mg group. Although some fluctuations were observed among the groups during the trial period, the growth performance was markedly higher in the group fed with 10 mg/g garlic extract. At the final sampling on April 23, 2023, mean weights were measured as 2.63 g in the control group, 3.20 g in the 5 mg group, and 4.70 g in the 10 mg group. Accordingly, the total weight gain was

calculated as 25.2% for the control group, 95.1% for the 5 mg group, and 131.5% for the 10 mg group. Growth performance data were analyzed using the Friedman test, and post-hoc pairwise comparisons were conducted to determine the statistical significance of differences between groups. The results showed a significant difference between the control and 5 mg groups ( $Z = -3.639$ ;  $p < 0.001$ ; adj.  $p = 0.001$ ), between the control and 10 mg groups ( $Z = -18.022$ ;  $p < 0.001$ ; adj.  $p < 0.001$ ), and also between the 5 mg and 10 mg groups ( $Z = -14.383$ ;  $p < 0.001$ ; adj.  $p < 0.001$ ). The mean rank values were calculated as 1.44 for the control group, 1.72 for the 5 mg group, and 2.84 for the 10 mg group. These findings indicate that garlic extract supplementation positively influences fish growth and that this effect becomes more pronounced with increasing doses. The highest growth performance was observed in the group fed with 10 mg/g garlic extract, which gained approximately twice as much weight as the control group (Figure 1-4, Table 1).



**Figure 1:** Fish weight measurements for two months. Control: no garlic additive; 5 mg: garlic additive 5 mg/g; 10 mg: garlic additive 10 mg/g in fish diet.

### Feed Conversion Ratio (FCR) and Feed Cost

Feed conversion ratio (FCR) improved significantly with garlic supplementation. The FCR was highest in the control group at 10.45, while the 5 mg and 10 mg groups recorded FCRs of 6.43 and 3.95, respectively (Table 2). Feed cost analysis revealed significant savings associated with garlic supplementation. Based on a standard feed price of 500 Turkish lira (TL) per kilogram, the feed cost per 1 gram of weight gain was calculated as 5.22 TL for the control group, 3.21 TL for the 5 mg group, and 1.97 TL for the 10 mg group (Table 2). These results demonstrate that adding 10 mg of garlic extract to the feed not only enhanced growth but also reduced feed costs significantly, with the cost for the 10 mg group being approximately 2.6 times lower than that of the control group (Figure 1, Table 3).

### Histological Monitoring of Garlic Efficacy in Fish

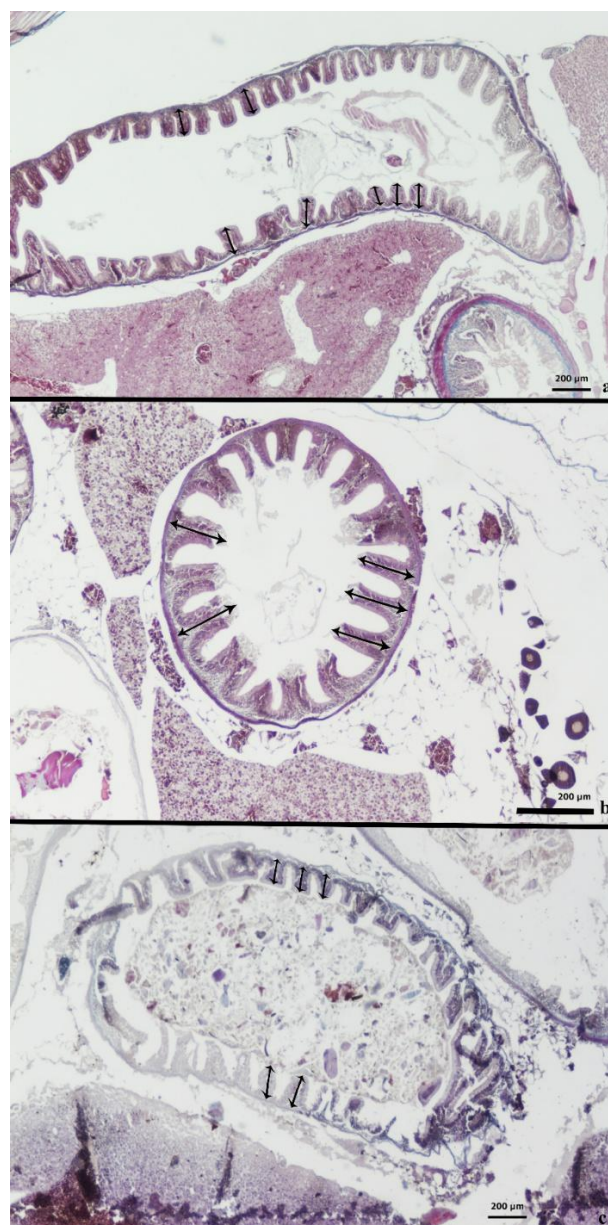
All tissue samples collected during the experiment were fixed in 10% formaldehyde solution.

Pre-treatment villus length was quantitatively established, with a mean baseline value of 153 $\mu$ m across groups. Histological examination during the first month revealed that the average villus lengths were 185.40  $\mu$ m in the control group, 196.28  $\mu$ m in the 5 mg group, and 309.54  $\mu$ m in the 10 mg group. In addition to villus length, villus diameters were also measured. Mean villus diameters were recorded as 15.2  $\mu$ m in the control group, 22.6  $\mu$ m in the 5 mg/g group, and 28.4  $\mu$ m in the 10 mg/g group. These values were included in the statistical analysis using the same methodology applied for villus length. These measurements indicate a considerable increase compared to the pre-experimental values, where villi were too small to be measured. By the end of the first month, villus lengths in the 5 mg group had increased by approximately 6% compared to the control group, while the 10 mg group showed a 58% increase compared to the 5 mg group and a 67% increase compared to the control group.

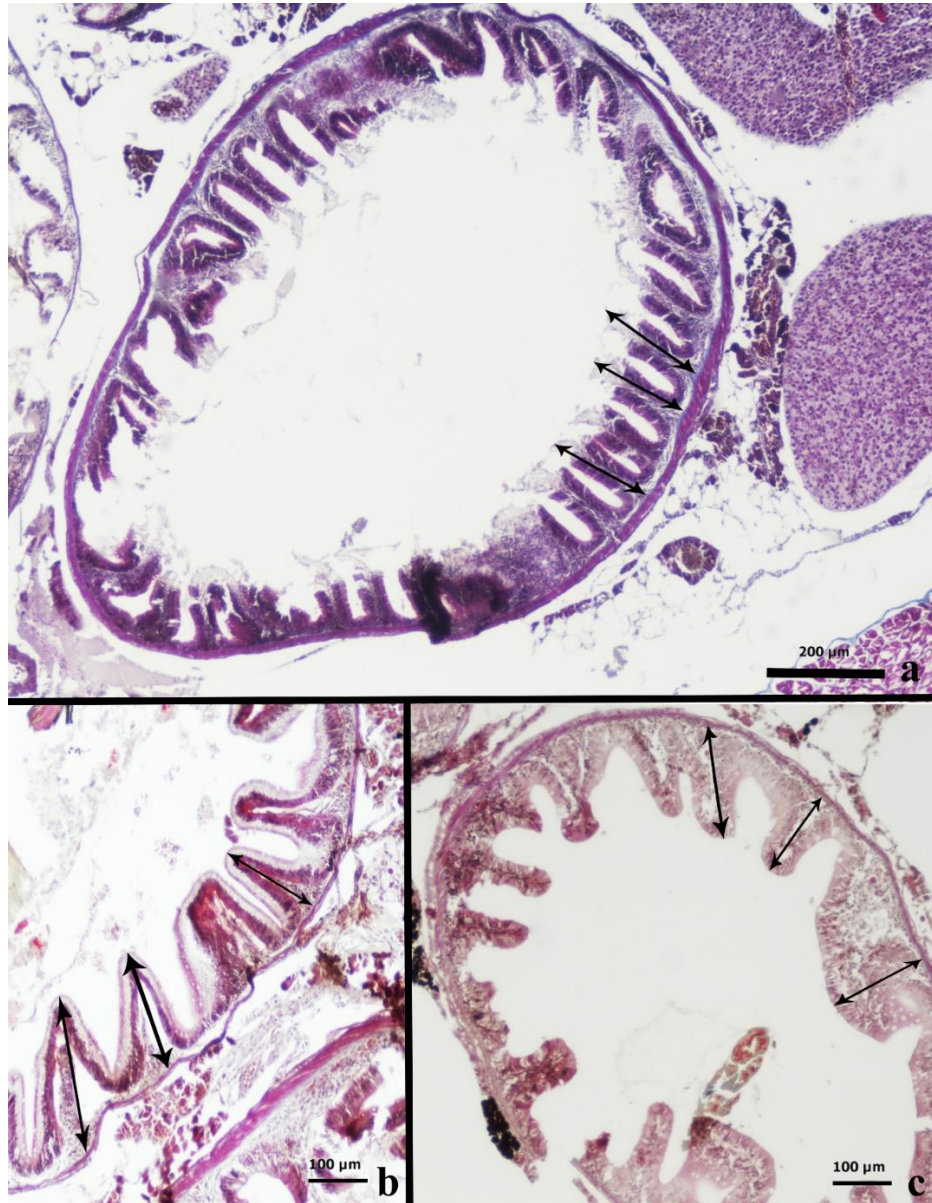
At the end of the third month (second sampling), villus length in the 5 mg group had increased by 84% relative to the control group, while the 10 mg group showed increases of 76% compared to the 5 mg group and 223% compared to the control group. Moreover, between the first and second sampling, villus length decreased by 21% in the control group, whereas increases of 37% and 53% were observed in the 5 mg and 10 mg groups, respectively.

Data on intestinal villus length were analyzed using the Friedman test, and post-hoc pairwise comparisons were conducted to evaluate statistical significance between groups. The analysis revealed significant differences between the control and 5 mg group ( $Z = -2.828$ ;  $p = 0.005$ ; adj.  $p = 0.014$ ), between the control and 10 mg group ( $Z = -5.657$ ;  $p < 0.001$ ; adj.  $p < 0.001$ ), and between the 5 mg and 10 mg groups ( $Z = -2.828$ ;  $p = 0.005$ ; adj.  $p = 0.014$ ).

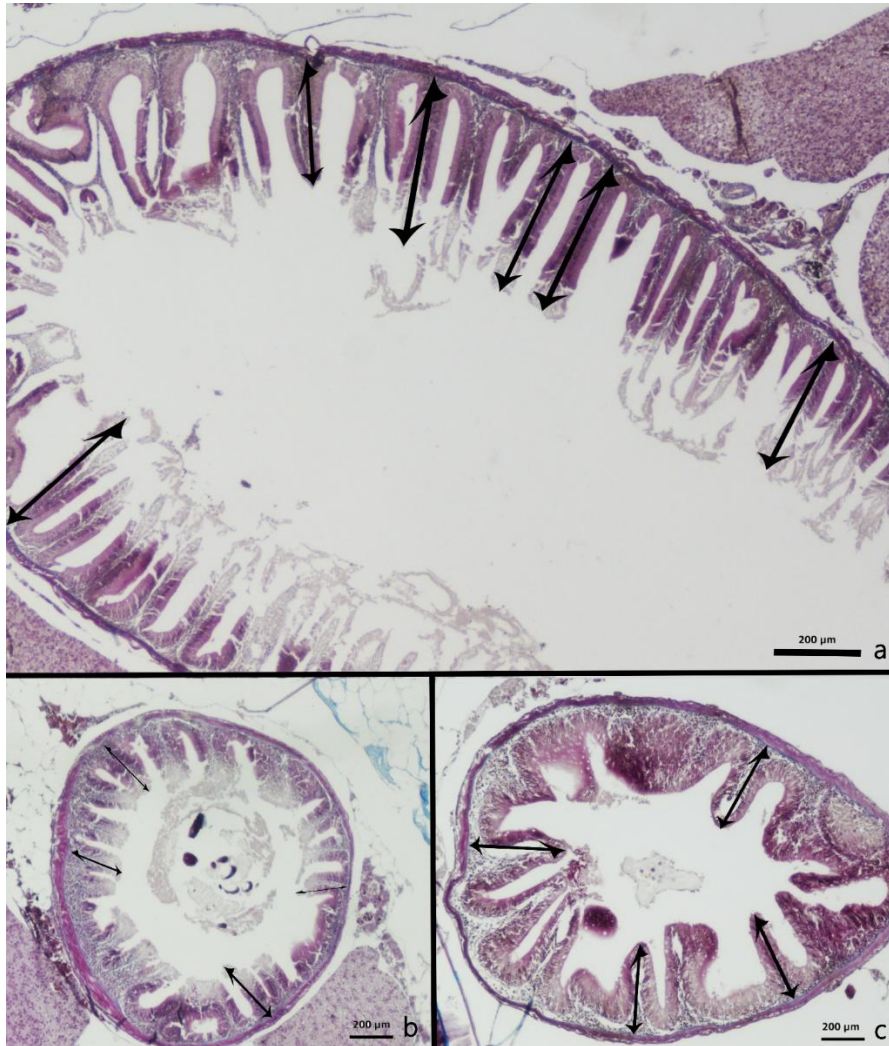
The mean rank scores were 1.00 (control), 2.00 (5 mg/g), and 3.00 (10 mg/g). These results demonstrate a statistically significant, dose-dependent improvement in intestinal morphology and histology, characterized by the elongation and broadening of villi, which increases the absorptive surface area, enhances nutrient absorption and digestion, and supports faster growth—especially at a dose of 10 mg/g.



**Figure 2:** Villus lengths of the anterior and posterior intestines in the control group treated with 0 mg/g of garlic extract (a (x4), c (x4): histological images of anterior intestinal tissue; b (x10): histological images of posterior intestinal tissue). Arrows indicate the specific regions where villus length measurements were taken. Measurements were taken from both anterior and posterior regions, and average values represent the combined mean of both segments. Histological images (a, b, c) indicate specific regions where measurements were obtained.



**Figure 3:** The villus lengths of the anterior and posterior intestines in the group treated with 5 mg/g of garlic extract (a (x4), c (x10): histological images of anterior intestinal tissue; b (x10): histological images of posterior intestinal tissue). Arrows indicate the specific regions where villus length measurements were taken. Measurements were taken from both anterior and posterior regions, and average values represent the combined mean of both segments. Histological images (a, b, c) indicate specific regions where measurements were obtained.



**Figure 4:** Villus lengths of the anterior and posterior intestines in the group treated with 10 mg/g of garlic extract (a (x10): histological images of anterior intestinal tissue; b (x10), c (x10): histological images of posterior intestinal tissue). Arrows indicate the sample areas where villus length measurements were taken. Measurements were taken from both anterior and posterior regions, and average values represent the combined mean of both segments. Histological images (a, b, c) indicate specific regions where measurements were obtained.

**Table 1:** Average weight measurements (mean  $\pm$  SD) of fish during the two-month feeding period

<b>Date</b>	<b>20.02.2023</b>		
Garlic dose	Control (0 mg)	5 mg	10 mg
Average weights (mean $\pm$ SD)	1.62 $\pm$ 0.11 g	1.64 $\pm$ 0.09 g	2.03 $\pm$ 0.12 g
<b>Date</b>	<b>27.02.2023</b>		
Garlic dose	Control (0 mg)	5 mg	10 mg
Average weights (mean $\pm$ SD)	1.94 $\pm$ 0.15 g	1.79 $\pm$ 0.16 g	1.76 $\pm$ 0.1 g
<b>Date</b>	<b>6.03.2023</b>		
Garlic dose	Control (0 mg)	5 mg	10 mg
Average weights (mean $\pm$ SD)	1.52 $\pm$ 0.16 g	1.64 $\pm$ 0.12 g	1.84 $\pm$ 0.12 g
<b>Date</b>	<b>13.03.2023</b>		
Garlic dose	Control (0 mg)	5 mg	10 mg
Average weights (mean $\pm$ SD)	2.25 $\pm$ 0.12 g	1.83 $\pm$ 0.16 g	2.4 $\pm$ 0.14 g
<b>Date</b>	<b>20.03.2023 (1st sampling)</b>		
Garlic dose	Control (0 mg)	5 mg	10 mg
Average weights (mean $\pm$ SD)	1.85 $\pm$ 0.16 g	2.17 $\pm$ 0.13 g	2.62 $\pm$ 0.18 g
<b>Date</b>	<b>27.03.2023</b>		
Garlic dose	Control (0 mg)	5 mg	10 mg
Average weights (mean $\pm$ SD)	2.06 $\pm$ 0.18 g	1.88 $\pm$ 0.15 g	2.5 $\pm$ 0.13 g
<b>Date</b>	<b>27.03.2023</b>		
Garlic dose	Control (0 mg)	5 mg	10 mg
Average weights (mean $\pm$ SD)	2.2 $\pm$ 0.14 g	2.1 $\pm$ 0.15 g	2.6 $\pm$ 0.16 g
<b>Date</b>	<b>3.04.2023</b>		
Garlic dose	Control (0 mg)	5 mg	10 mg
Average weights (mean $\pm$ SD)	2.27 $\pm$ 0.16 g	2.68 $\pm$ 0.15 g	3.18 $\pm$ 0.19 g
<b>Date</b>	<b>10.04.2023</b>		
Garlic dose	Control (0 mg)	5 mg	10 mg
Average weights (mean $\pm$ SD)	2 $\pm$ 0.15 g	2.275 $\pm$ 0.11 g	3.74 $\pm$ 0.19 g
<b>Date</b>	<b>17.04.2023</b>		
Garlic dose	Control (0 mg)	5 mg	10 mg
Average weights (mean $\pm$ SD)	2.1 $\pm$ 0.14 g	2.5 $\pm$ 0.14 g	4.3 $\pm$ 0.13 g
<b>Date</b>	<b>23.04.2023 (2nd sampling)</b>		
Garlic dose	Control (0 mg)	5 mg	10 mg
Average weights (mean $\pm$ SD)	2.63 $\pm$ 0.15 g	3.2 $\pm$ 0.12 g	4.7 $\pm$ 0.14 g

**Table 2:** Feed conversion ratio (FCR) and feed cost per 1g weight gain across experimental groups

Experimental group	FCR	Feed cost (for 1g live weight gain, Turkish lira)
Control (0 mg garlic extract)	10.45	5.22 TL
5 mg garlic extract	6.43	3.21 TL
10 mg garlic extract	3.95	1.97 TL

**Table 3:** Intestinal villus morphometric measurement (mean  $\pm$  SD) in fish groups treated with garlic.

Garlic Applied Group	Measurement (1st Sampling 20.03.2023)	Increase (%)	Measurement (2nd Sampling 23.04.2023)	Increase (%)	Total Change from the 1st Sampling to the 2nd Sampling (%)
Control (0 mg/g)	185.4 $\pm$ 2.36 $\mu$ m	6%	146.3825 $\pm$ 1.95 $\mu$ m	84%	-21%
5 mg/g	196.28 $\pm$ 1.29 $\mu$ m		268.96 $\pm$ 1.86 $\mu$ m	37%	
10 mg/g	309.535 $\pm$ 1.84 $\mu$ m	58%	473.205 $\pm$ 3.00 $\mu$ m	76%	53%

## DISCUSSION

The results of this study provide robust evidence for the positive effects of garlic supplementation on the growth performance and intestinal morphology of *the goldfish* (*Carassius auratus*). Our findings align with previous research, such as studies by Lee (2012) and Büyükdeveci et al. (2018), which highlighted significant growth improvements in fish-fed garlic-supplemented diets. In this study, garlic supplementation resulted in significant improvements in growth performance, with the highest growth observed in the 10 mg/g garlic group, leading to a total weight gain increase of 153% by the end of the experiment.

Specifically, the control group (0 mg/g garlic) exhibited a modest weight gain over the study period, with an initial average weight of 1.62 g, which increased to 2.63 g by the second sampling. This represents a growth rate of approximately 62%. In contrast, the 5 mg/g garlic group exhibited more substantial growth, with an initial average weight of 1.64 g, which increased to 3.20 g by the second sampling, corresponding to a 95% growth rate. The most pronounced growth was observed in the 10 mg/g garlic group, where the initial average weight of 2.03 g rose to 4.70 g, reflecting a remarkable growth rate of 131%. These differences highlight the dose-dependent effect of garlic on growth performance, with higher doses of garlic resulting in more substantial increases in live weight gain. Lee et al. (2012) detected greater weight gain and specific growth rates in juvenile sterlet sturgeon fed a diet containing 0.5% garlic extract compared to a control group. Similarly, Büyükdeveci et al. (2018) investigated the effects of diets containing garlic extract on rainbow trout (*Oncorhynchus mykiss*) and found that weight gain and specific growth rates improved significantly in fish-fed garlic-supplemented diets. A literature review indicates that studies on the effects of garlic as a feed additive are more limited in ornamental aquarium fish than in farmed fish and often lack evaluations of intestinal development and morphology.

The present study demonstrated significant enhancements in intestinal villus morphology in goldfish (*Carassius auratus*) fed diets supplemented with garlic, highlighting the potential of garlic as an effective feed additive. Histological evaluations showed that garlic supplementation improved villus diameter, mucosal layer density, and overall structural integrity of the intestinal tissues compared to the control group. Fish in the control group exhibited an average villus diameter of 15.2  $\mu\text{m}$ , accompanied by microvillus loss and a reduction in mucosal density. However, fish in the 5 mg/g garlic group showed villus diameters averaging 22.6  $\mu\text{m}$ , while the 10 mg/g garlic group displayed the most substantial improvements, with villus diameters reaching 28.4  $\mu\text{m}$ . This increase in villus dimensions likely enhanced nutrient absorption efficiency, contributing to the observed growth improvements.

Garlic's beneficial effects on the intestinal systems of fish have been supported by previous studies. Agbebi et al. (2013) demonstrated that garlic inclusion rates of up to 30% in compounded feed significantly improved feed utilization and growth performance in fish without causing adverse effects on tissues. However, other studies (Al-Salahy and Mahmoud 2003) indicated that repeated high doses of garlic could lead to histological changes in organs, suggesting that the dose must be carefully optimized to balance benefits and avoid potential harm.

In addition to garlic's direct effects, studies combining garlic with other supplements have shown synergistic benefits. For instance, Abdel-Latif et al. (2024) explored the use of yeast and garlic supplementation in Nile tilapia (*Oreochromis niloticus*) and found improvements in growth, immune responses, and resistance against *Aeromonas sobria*. This combination also reduced fish mortalities from bacterial infections and minimized antibiotic use, highlighting the potential of such integrative feeding strategies in aquaculture.

Unlike previous studies that primarily focus on commercial aquaculture species, the present research provides one of the few detailed investigations into the intestinal histomorphology of ornamental fish, particularly goldfish, following garlic supplementation. Another noteworthy study investigated the effects of garlic nanoparticles on Nile tilapia, revealing that a dose of 600 mg/g of nano garlic provided the best physiological state and performance (Abdel-Latif et al. 2024). While various studies have documented the efficacy of garlic additives in different fish species, the optimal safe dose for ornamental aquarium fish remains largely unexplored.

## CONCLUSION

Our study addresses this gap, providing evidence that dietary inclusion of 10% garlic additive significantly improved growth in goldfish without causing any pathological changes in tissues or organs. The growth rate of the experimental fish increased by an impressive 223%, providing preliminary evidence for a potentially safe and effective garlic dose in ornamental fish species. These findings underscore the potential of garlic as a natural, cost-effective feed additive that enhances both growth performance and intestinal health in goldfish. Further research is recommended to explore its long-term effects and applicability across other ornamental fish species and aquaculture practices. These results may be of particular interest to ornamental fish breeders and aquarium professionals seeking natural dietary supplements to enhance fish health and growth without resorting to synthetic additives.

**Conflict of interest:** The authors have no conflicts of interest to report.

**Authors' Contributions:** MD, AM, AO and ÇK contributed to the project idea, design and execution of the study. NA and GT contributed to the acquisition of data. SG and OO created the histopathological images and resulted the analyses. All authors drafted, wrote and reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

**Ethical Approval:** The experimental procedures were conducted in accordance with ethical guidelines. Approval was obtained from the Local Ethics Committee (decision number: 2022-17/0).

**Acknowledgement:** This research was supported by TUBITAK (1919B012210176) and approved by the Local Ethics Commission decision number with 2022-17/0.

**Explanation:** This research article was prepared based on the results obtained from the project conducted within the scope of TUBITAK 2209-A - Support Program for University Students' Research Projects.

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