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Ameliorative Effect of Ginger on Hematology and Histopathology of Nile Tilapia (*Oreochromis niloticus*)

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SUMMARY

Ginger (*Zingiber officinale*) is a natural compound that helps an organism against illnesses, particularly during periods of stress. The aim of this experiment was to determine the immunostimulatory and histopathological effects of supplementing Nile tilapia diets with ginger rhizome. A total of 320 fish, with an average body weight between 20 and 24 g and a length of 10 cm, were split into four groups, T1, T2, T3, and T0, based on the amount of ginger supplemented (0.5, 1.0, and 1.5 g/l). Each group consisted of three replicates of fish. For a period of ninety days, the fish were fed thrice daily at 3% of their body weight. The outcomes validated that the addition of ginger rhizome exhibited a noteworthy immune-stimulatory impact, elevating WBC, hematocrit (Hct), and RBC levels in contrast to the control treatment. Similarly, fish treated with ginger showed a substantial rise in MCHC and platelets but a decrease in MCH and MCV. The electrolytes of blood Nile tilapia increased in response to varying dosages of ginger, but cholesterol, glucose, triglycerides, uric acid, urea, creatinine, LDL, and HDL decreased. Hepatocytes of fish given ginger, however, showed necrosis, sinusoidal channels, blood congestion, and a lack of vacuoles. It is reasonable to conclude that, as compared to the control, the fish's immunological state was significantly enhanced by adding ginger to their diet in place of antibiotics and other medications.

Keywords: Ginger, *Zingiber officinale*, Immunostimulatory, Histopathology, LDL, HDL

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INTRODUCTION

In intensive fish farming, nutrition is an essential component that depends on a number of variables, including feed availability. In addition, the fish's immunity and general well-being are greatly influenced by their nutritional state (Souza et al., 2020). Fish farmers are becoming increasingly aware of the range of natural food additives available for enhancing the growth and health of their fish (Hassanin et al.,

2014). Artificial additives can have an impact on fish immune systems and the environment's equilibrium (Dawood et al., 2018). Fish farmers are particularly interested in plant based natural additives due to their proven ability to boost fish growth, improve feed efficiency, enhance digestibility and optimize nutrient absorption. Furthermore, the antibacterial, antioxidant, and immunostimulant properties of natural additives are beneficial to fish health (de Souza et al., 2020).

The *Zingiberaceae* family includes the herbaceous and perennial ginger (*Zingiber officinale* Rosc.), which has its origins in China and India (Singh et al., 2005). Ginger essential oil derived from the rhizome, is widely utilized in food, cosmetic, and pharmaceutical industries for its antibacterial, anti-tumor, and anti-inflammatory properties, as well as for flavoring and condiments (Shakya, 2015). Ginger extracts have been shown in earlier research to have immunostimulant, digestive stimulant, antioxidant, and antimicrobial properties and useful in enhancing fish growth rate, protein and lipid metabolism (Mohammadi et al., 2020). *Oreochromis niloticus*, commonly known as Nile tilapia, is one of primary freshwater species farmed in aquaculture both globally (El-Sayed and Fitzsimmons, 2023) and in Pakistan. Notably, Nile tilapia offers several advantages for aquaculture production, including excellent feed conversion ratio, significant weight gain, adaptability to commercial feeds, and fillet that is widely appreciated by consumers (Teixeira et al., 2017). This study aimed to assess ginger's effect on the hematology and histology of the liver and intestine in juvenile Nile tilapia.

MATERIALS AND METHODS

EXPERIMENTAL SITE

The fish was bought from the Fish Farm located at the Department of Fisheries and Aquaculture, UVAS, Lahore, Pakistan. It was 10 cm long and typically weighed between 20 and 24 g. The fish were netted and transferred to iris aquariums equipped with heaters and aerators to control the water's temperature and oxygen content. The fish were kept in glass tanks for a week to acclimate before the experiment began.

EXPERIMENTAL DESIGN

A total of 320 Nile tilapia fingerlings were divided into four treatments groups based on the concentration of ginger supplementation such as T1 (0.5 g/L), T2 (1.0 g/L), T3 (1.5 g/L), and T0 (control). Each treatment was placed in glass tanks, containing 20 fish per tank, with a total capacity of 420 liters. Fish were fed commercially prepared feed three times daily at the rate of 3% of their body weight. Daily, 80% of water siphoned out along with fecal material using a suction pump and replaced with fresh water. The experiment lasted for 90 days.

GINGER RHIZOME'S EXTRACTION METHOD

The ginger rhizomes were sourced from the local market, cleaned for 24 hours, and dried at 50°C. They were then ground into powder, and 80% ethanol was added in a 1:3 (W:V) ratio. The mixture was agitated at 120rpm using a magnetic stirrer and allowed to rest at room temperature for 72 hours. It was filtered using Büchner funnel and Whatman paper to eliminate impurities, resulting in an alcoholic extract. The

alcohol was removed with a rotary evaporator maintained at 60°C (El-Refai et al., 2018).

HEMATOLOGICAL ANALYSIS

Blood was collected from caudal vein of the fish using sterile syringe and transferred to 2.0 ml microtubes containing EDTA anticoagulants. Samples were collected early in the morning for hematological analysis. Two blood smears were created and subsequently stained with a rapid dye. Total cell count was performed in a Neubauer chamber using a 1:100 diluent/dye direct technique (Natt and Herrick, 1952). Following the total nucleated cell count, a differential count of thrombocytes and leukocytes was carried out. Packed cell volume was assessed by microhematocrit method (Jain, 1986). The following tests were performed on blood: hematocrit value (WHITBY and BRITTON, 1963), hemoglobin content (Van Kampen and Zijlstra, 1961), and erythrocyte count (Shokr and Mohamed, 2019). The mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) was calculated using appropriate formulae (Bain et al., 2016).

Plasma was separated by centrifuging at 3000 rpm for 15 minutes and stored at -20°C. the Biuret method was utilized to measure protein concentration (Wootton, 1964). A Boehring Mannheium kit was used to determine glucose concentration (Trinder, 1969). The calorimetrical method was applied to measure triglycerides, cholesterol, and total lipids, using a kit supplied by Takara Bio Inc. Japan (Knight et al., 1972). Additionally, the evaluation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), ALP, uric acid, creatinine, and electrolytes conducted using kits provided by Takara Bio Inc. Japan (Reitman and Frankel, 1957). An ELISA Kit used to establish the protocol for Immunoglobulin (IgM) assay.

HISTOMORPHOMETRY ANALYSIS

The samples of liver and intestine (~100 mg) were preserved in 70% alcohol after being fixed in 10% formalin for a whole day. The samples underwent ethanol series dehydration. Subsequently, they were fixed using paraffin blocks at 60˚C and cleared in xylol. Sections measuring 5µm in thickness were then stained with periodic acid-Schiff (PAS) and Harris hematoxylin and eosin (HHE) (Prophet, 1992). After staining, the slides were mounted in Entellan® medium and examined with a Nikon Scope binocular biological microscope equipped with a camera. For each tissue, ten microphotographs per replication were taken into consideration, for a total of 200 micrographs per treatment. The application Motic Images Plus 3.0 was used to analyze and quantify the microphotographed materials.

STATISTICAL ANALYSIS

Data was subjected to ANOVA and LSD using statistical software SAS 9.1.Duncan's multiple range tests applied to determine the differences among treatments (Db, 1955) at $P < 0.05$. The data was presented as mean ± SD.

RESULTS AND DISCUSSION

IMMUNOSTIMULATORY EFFECTS OF GINGER

According to Table 1, fish fed diets containing various doses of ginger (0.5, 1.0, and 1.5g/l) experienced significant increases in hemoglobin content and erythrocyte count. These findings align with previous studies (Hassanin et al., 2014; Şahan et al., 2016; Shokr and Mohamed, 2019). When included in fish diets at appropriate levels, ginger has been shown to enhance non-specific immunity and improved health outcomes, such as a reducing mortality rates (Hassanin et al., 2014). The improved survival rates can be attributed to the bioactive compounds found in ginger, such as saponins, tannins, flavonoids, and polyphenols, which boost the immune system and help protect fish from infections (Talpur et al., 2013). Table 2 shows that RBC indices, including MCV and MCH in Nile tilapia fed diets with different levels of ginger displayed non-significant reductions, while MCHC showed a significant increase. The high ginger treatment suggesting that the fish's immune system was strengthened. Similar results found when fish were fed various amounts (3, 6, 9, 12, and 15 gm/kg) of ginger compared to control group (Şahan et al., 2016). Table 3 indicates that treated groups had significantly higher counts of thrombocytes. Basophils, eosinophils, lymphocytes, monocytes, and WBCs in Nile tilapia compared to control group. All treatments groups demonstrated significant increase in total plasma protein relative to control, while total plasma lipid as exhibited the opposite trend as shown in table 4. These results consistent with earlier findings (Şahan et al., 2016).

Table 1: The hematological parameters, including RBCs, HCT, Hb, and Platelets, of Nile tilapia treated with various doses of ginger (0.5, 1.0, and 1.5 g/L).

Parameters	T0	T1	T2	T3
RBCs ($\times 10^6/\mu L$)	1.6±0.3	1.8±0.4*	2.0±0.4**	2.2±0.3***
HCT (%)	26.3±2.4	29.1±1.5*	30.1±1.4**	31.3±2.3**
Hb (g/dL)	7.8±2.4	8.5±1.5*	8.9±1.5*	9.4±1.5**
Platelets $10^3 mm^{-3}$	290±2.4	291±1.2**	295±1.2***	296±1.2 ***

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control.

Table 2: The blood indices, including MCV, MCH, and MCHC, of Nile tilapia exposed to different doses of ginger (0.5, 1.0, and 1.5 g/L).

Parameters	T0	T1	T2	T3
Mean cell (or corpuscular) volume (MCV)fL10^{-15}	19.3±1.1	17.0±2.2**	18.1±1.2*	18.6±3.4
Mean cell hemoglobin (MCH) pg 10^{-12}	31.3±1.5	29.7±2.7*	29.5±1.5**	30.4±2.7*
Mean cell hemoglobin concentration (MCHC)g/dL	26.2±1.1	29.9±2.4***	28.5±1.5**	27.4±2.7*

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control.

Fish treated with ginger exhibited a significant reduction in triglycerides, cholesterol, LDL and HDL compared to the control group, as shown in table 5. Similar outcomes were observed across various dosages of supplementation (3, 6, 9, 12, and 15 gm/kg) of ginger (Şahan et al., 2016). The administration of ginger led to notable increase in plasma levels of IgM, calcium (Ca), sodium (Na), and potassium K as indicated in 6. Additionally, several studies reported that fish receiving ginger showed significantly higher total IgM levels, which enhanced their immunostimulation (Apines-Amar et al., 2012; Talpur et al., 2013; Hassanin et al., 2014; Masoud and Rohani, 2014; Şahan et al., 2016). According to tables 7 and 8, the ginger supplementation resulted in severe hypoglycemia throughout the trial, along with reduction in white muscle glycogen and depletion of liver glycogen. The data further demonstrate that ginger administration resulted in substantial decrease in total lipids in both liver and white muscle.

Table 3: The average values of WBCs, neutrophils, lymphocytes, eosinophils, basophils, monocytes, and thrombocytes in Nile tilapia treated with various doses of ginger (0.5, 1.0, and 1.5 g/L).

Parameters	T0	T1	T2	T3
Total leuk./µL	5643±143	5666±133*	5676±141*	5675±210*
Seg. Neutro./µL)	1851±155	1868±211*	1976±401**	1987±211**
Lymphocytes/µL)	2441±132	2642±213*	2754±222**	2767±217**
Eosinophyls/µL)	133±12	143±17*	153±14**	158±22***
Basophyils/µL)	211±31	222±21*	239±24**	246±21***
Monocytes/µL)	1547±211	1511±110*	1521±127*	1522±121*
Thromb/µL)	34215±2233	35548±1501*	35947±3017*	36160±4421**

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control.

Table 4: The impact of different doses of ginger (0.5, 1.0, and 1.5 g/L) on serum total protein, lipids profile, and glucose levels of Nile tilapia.

Parameters	T0	T1	T2	T3
Glucose (mg/dl)	100±1.3	100±1.8	99±1.2*	98±1.5**
Total protein (g/dl)	6.5±0.2	6.8±0.1	7.3±0.2*	7.4±0.6**
Total lipid	4.5±0.1	4.2±0.3	4.1±0.5*	4.0±01**
Cholesterol (mg/dl)	190±10	189±12	185±14**	172±16***
Triglycerides (mg/dl)	3.8±0.2	3.7±0.2	3.5±0.3*	3.5±0.1*
HDL (mg/dl)	3.5±0.5	2.8±0.2*	3.0±0.1*	2.7±04*
LDL (mg.dl)	1.5±0.1	1.4±0.2*	1.3±0.1**	1.3±0.2**

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control.

HISTOMORPHOMETRY OF LIVER AND INTESTINE

In treatment group T0, hepatocytes displayed spherical nuclei of varying sizes, with some binucleate polyhedral cells showing scattered chromatin and well-defined nucleoli, as illustrated in Figure 1. Certain hepatocytes from fish given a

concentration of 1.5 g/l exhibited a lack of vacuoles, along with signs of necrosis, sinusoidal channel formation, and blood congestion (Figure 2).

Table 5: The effect of ginger supplementation on the liver and kidney of Nile tilapia.

Parameters	T0	T1	T2	T3
AST (u/l)	37±0.2	36±0.4	35±0.2*	33±0.3**
ALT (u/l)	20±0.1	19±0.3	18±02*	17±0.4**
ALP (u/l)	25±0.5	25±0.6	22±0.4**	23±0.2**
Uric acid (mg/dl)	16±0.2	15±0.3*	12±0.1*	10±0.2**
Creatinine (mg/dl)	0.35±0.2	0.33±0.1*	0.30±0.02**	0.30±0.02**
Urea (mg/l)	30.8±5.2	27.5±3**	27.6±5**	24.6±4***

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control.

Table 6: **The average values of serum electrolytes and IgM in Nile tilapia treated with different doses of ginger (0.5, 1.0, and 1.5 g/L).**

Parameters	T0	T1	T2	T3
Ca (nmol/L)	12.2±1	12.5±2*	12.7±1**	12.9±1**
Na (nmol/L)	150±4	155±3*	157±2**	158±4**
K (nmol/L)	5.1±0.2	5.7±0.3*	5.8±0.4*	6.1±0.3**
IgM value (µg/ml)	26±0.6	29±0.2*	31±0.3**	32±0.1***

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control.

Table 7: Alteration in liver composition of Nile tilapia under the influence of different doses of ginger (0.5, 1.0, and 1.5 g/L).

Parameters	T0	T1	T2	T3
Glycogen (mg/g dry wt)	115±7.2	121±8.5*	120±7.4**	126±11**
Total lipid (mg/g dry wt)	71±5.5	71±2	70±4	69±4*
Triglycerides (mg/g dry wt)	26±2.4	25±3.1	23±1*	22±2**
Cholesterol (mg/g dry wt)	4.5±0.1	3.5±0.2*	3.7±0.1*	3.6±0.1*
TFAA (µg/g dry wt)	485±52	486±50	487±51*	490±50**

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control.

Table 8: The average values of muscle fats in Nile tilapia treated with various levels of ginger (0.5, 1.0, and 1.5 g/L).

Parameters	T0	T1	T2	T3
Glycogen (mg/g dry wt)	12±1.2	13±1*	13±0.1**	13.7±1.1**
Total lipid (mg/g dry wt)	8.7±0.5	8.3±0.2	8±0.4*	7.7±0.3**
Triglycerides (mg/g dry wt)	35±3	34±3	30±1*	29±2**
Cholesterol (mg/g dry wt)	4.2±0.1	3.6±0.3*	3.4±0.2*	3.2±0.1*
TFAA (µg/g dry wt)	440±22	438±20	430±41*	384±30**

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control.

The intestinal morphology of fish, particularly the surface area of their villi, significantly impacts nutrient absorption and overall health (Brum et al., 2017). Fish in this study that were administered ginger concentrations above 1.0 g/l had smaller villi in both height and width, potentially hindering their ability to absorb nutrients and electrolytes (Adeoye et al., 2016).n contrast, fish supplemented with 0.5 ml showed a healthy digestive tract and high absorption efficiency (Mohammadi et al., 2020), as well as intact mucosal integrity, which contributed to increased villi size (Carvalho et al., 2011).

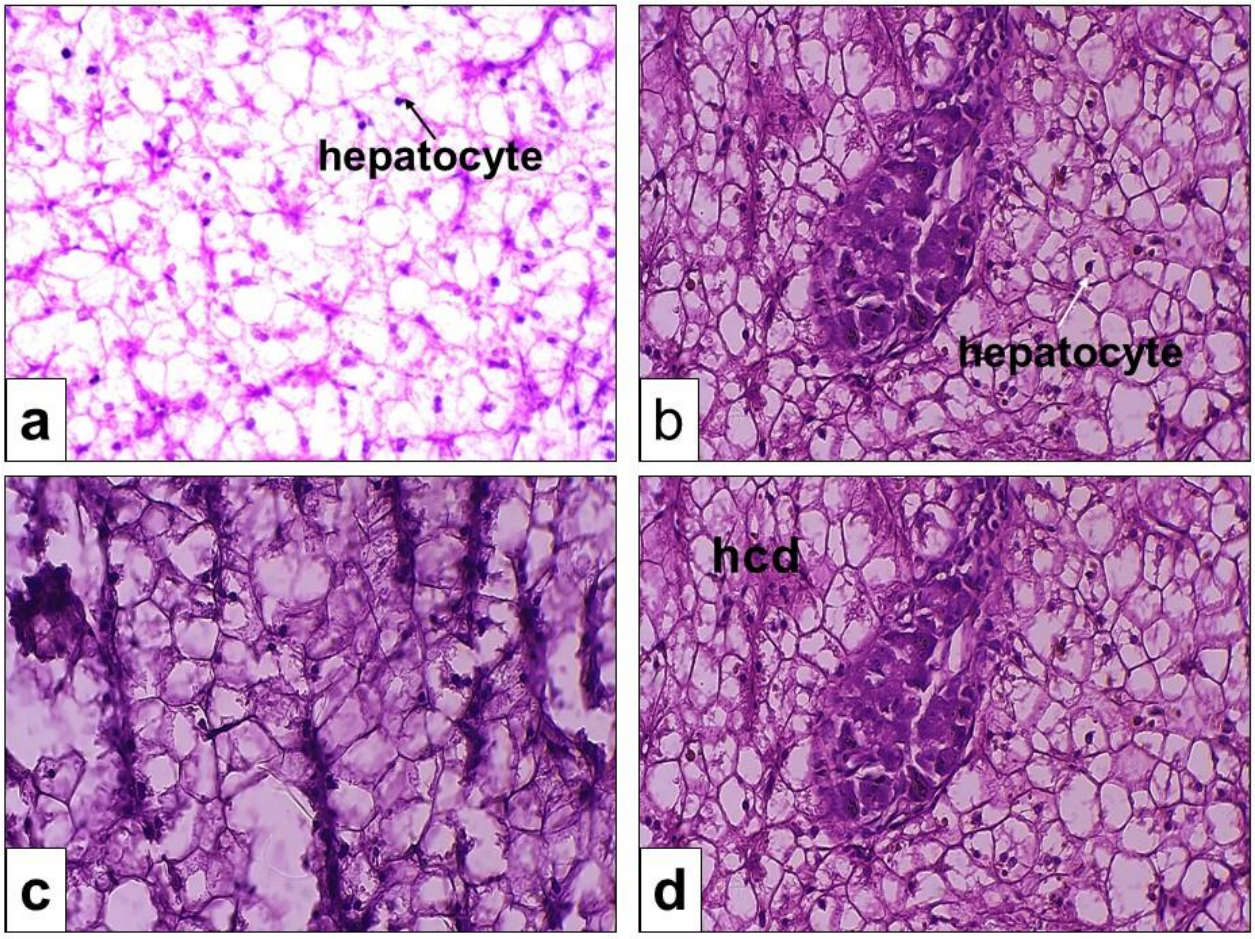

Figure 1: Histological alterations in Nile tilapia liver cells fed with varying Zingiber officinale concentrations (400× magnification, HE and PAS stains): **a**) Control (0.0 g/l): Polyhedral cells with centrally located nuclei, dispersed chromatin, prominent nucleoli, glycogen vacuoles, and visible sinusoidal channels. **b**) 0.5 g/l: Hepatopancreas area, nuclei with dispersed chromatin, discontinuous cell membranes, stellate cells, and hepatocytes with pyknotic nuclei. **c**) 1.0 g/l: Hepatocytes with thin cytoplasmic vacuoles, enlarged cells with displaced nuclei, and blood cells between hepatocyte cords. **d**) 1.5 g/l: Some hepatocytes with central nuclei, signs of necrosis, and altered sinusoidal channels.

Furthermore, the addition of 250 mg/kg o*f Melaleuca alternifolia* essential oil to the diet of Nile tilapia enhances the height of intestinal villi (Valladão et al., 2017).

The administration of 1.0 or 2.0 ml/kg essential oil from *Aloysia triphylla* resulted an increased in numbers of intestinal folds in silver catfish (Zeppenfeld et al., 2016). Nevertheless, morphometric characteristics of the intestine did not change in Nile tilapia fed diets containing ginger oil (Brum et al., 2018).

The gut plays a crucial role in both the immune system and the absorption of nutrients and digestion, ultimately affecting an animal's production (Nicholson et al., 2012) and contributing to improved overall health and productivity (Vallejos-Vidal et al., 2016).

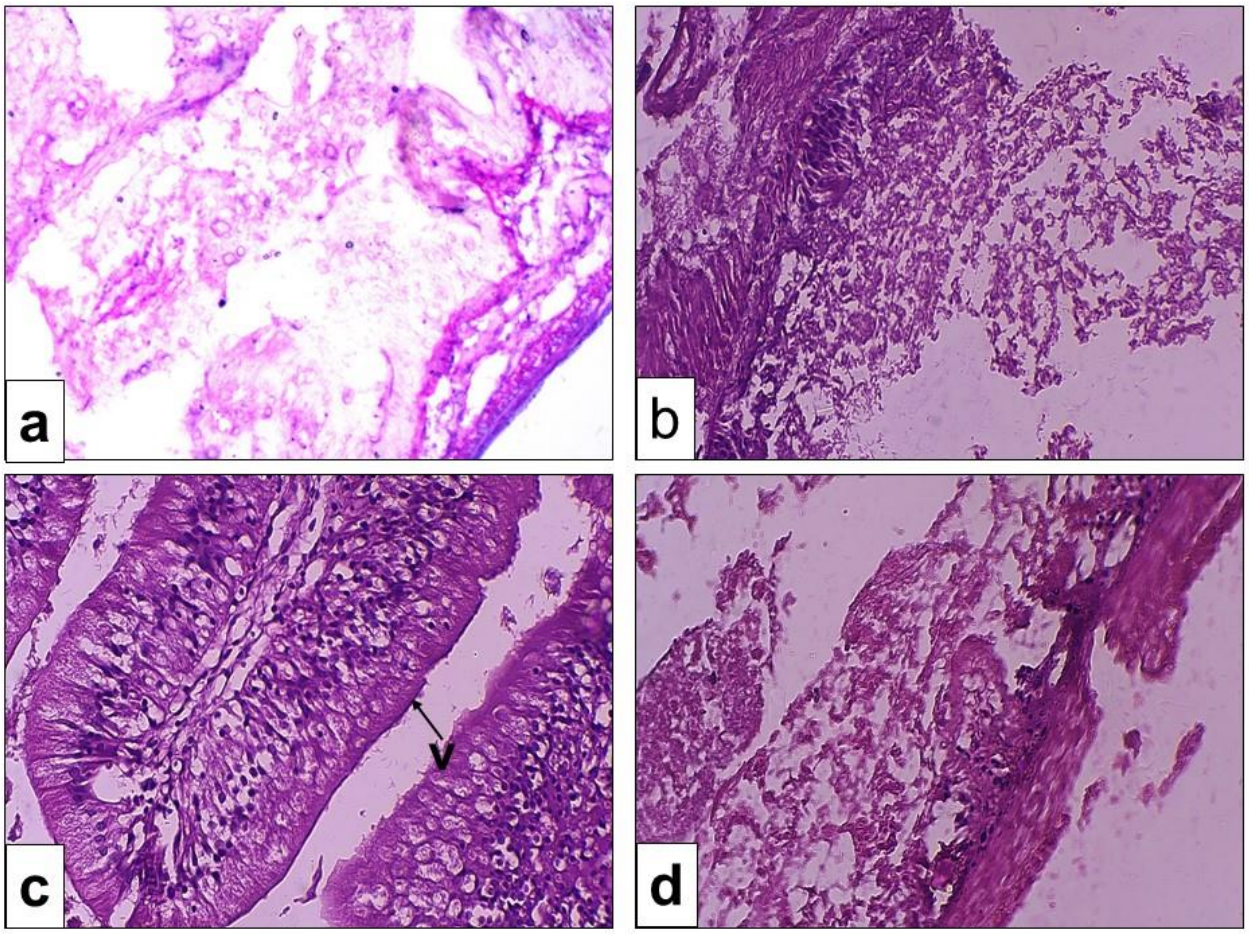

Figure 2: Histological alterations in the intestinal villi of Nile tilapia fed with different concentrations of Zingiber officinale (400× magnification, HE and PAS stains): **a**) 0.0 g/l: Normal intestinal villi structure. **b**) 0.5 g/l: Alterations in the interior of intestinal villi. **c**) 1.0 g/l: Changes in enterocytes. **d**) 1.5 g/l: Presence of brush border and goblet cells.

CONCLUSION

This study indicates that ginger could strengthen the immune system of Nile tilapia when incorporated as a dietary supplement. Ginger's biological properties support its role as an immunostimulant, showing positive effects in preventing disease infections. Incorporating ginger into fish diets as a substitute for antibiotics and other treatments significantly improves the immunological health of Nile tilapia.

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