RESEARCH PAPER

www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v16_1_20

Determination of Some Hematological Parameters and Antioxidant Capacity in Nile Tilapia *(Oreochromis Niloticus* Linnaeus, 1758) Fed Ginger (*Zingiber Officinale* Roscoe) to *Aeromonas hydrophila*

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Abstract

In this study, Nile tilapia (*Oreochromis niloticus*) fed with ginger (*Zingiber officinale*) at different ratios for 90 days in terms of their hematological, oxidative stress and growth parameters, mortality and RPS (relative percent survival) ratios against *Aeromonas hydrophila*, were investigated. The blood and tissue samples (liver, gill and gut) were taken from the 120 fish in the control and ginger groups (0.5%, 0.1%, 1.0%) infected with *A. hydrophila* for hematological parameters, oxidative stress indices (OSI). RBC indices, Hb and Hct values, differential count (D.C.) increased significantly in the 0.5% and 1.0% ginger groups. SOD and CAT enzymes activity were reduced in the liver, gill and gut tissues in the control group, these enzymes raised in the 0.5% and 1.0% ginger groups ($P<0.05$). No significant differences were determined amongs the experimental groups in terms of growth parameters. On the other hand, the rate of 61.6% RPS was the best indicator of inhibition effect of 1.0% ginger on pathogen. At higher doses of ginger, the increases in hematological and oxidative stress indices, and those in the rate of RPS were considered as the best results of antibacterial and antioxidant characteristic of ginger against the *A. hydrophila* infection in tilapia.

Keywords: *Zingiber officinale*, *Oreochromis niloticus*, Hematological parameters, Oxidative stress indices, Growth parameters.

Zencefille (*Zingiber officinale*) Beslenen Nil Tilapia (*Oreochromis niloticus*)'larında, *Aeromonas hydrophila*'ya Karşı, Bazı Hematolojik Parametrelerin ve Antioksidan Kapasitenin Belirlenmesi

Özet

Bu çalışmada, 90 gün boyunca farklı oranlarda zencefille (*Zingiber officinale*) beslenen Nil Tilapia (*Oreochromis niloticus*)'larında, *Aeromonas hydrophila*'ya karşı, hematolojik, oksidatif stres ve büyüme parametreleri, mortalite ve RPS (hayatta kalma yüzdesi) oranları incelenmiştir. *A.hydrophila* ile enfekte kontrol ve zencefil gruplarındaki (%0,1; %0,5; %1,0) 120 balıktan, hematolojik ve oksidatif stres endeksleri (OSE) için kan ve doku (karaciğer, solungaç ve barsak) örnekleri alınmıştır. RBC endeksleri, Hb ve Hct değerler, diferansiyel sayım (D.S.), %0,5 ve %1,0'lık zencefil gruplarında önemli olarak artmıştır. SOD ve CAT enzimleri aktiviteleri, kontrol grubundaki karaciğer, solungaç ve barsak dokularda azalmış, bu enzimler, %0,5 ve %1,0'lık zencefil gruplarında artmıştır ($P<0,05$). Büyüme parametreleri açısından deney grupları arasında önemli farklar kaydedilmemiştir ($P<0.05$). Diğer yandan, RPS'nin % 61,6 oranı, %1,0'lık zencefilin patojen üzerindeki baskılayıcı etkisinin en iyi göstergesi olmuştur. Zencefilin yüksek dozlarında, oksidatif stres enzimleri, hematolojik parametreler ve RPS oranındaki artışlar, tilapia'lardaki *A. hydrophila* enfeksiyonuna karşı zencefilin antibakteriyel ve antioksidant karakteristiklerinin en iyi sonuçları olarak düşünülmüştür.

Anahtar Kelimeler: *Zingiber officinale*, *Oreochromis niloticus*, Hematolojik parametreler, Oksidatif stres endeksleri, Büyüme parametreleri.

www.trjfas.org
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Introduction

In aquaculture, the irresponsible use of various chemotherapeutics, especially antibiotics used in the prevention and treatment of bacterial infection, inhibits the development and enhancement of the immune system and has also led to resistance of the bacteria and temporal and economic losses (Dügenci et al., 2003). Vitamins, chitin, chitosan, glucans, microorganisms, and herbal extracts used against the development of bacterial resistance are natural organic immunostimulants that are easy to find, cheap, environment friendly and have no health-threatening effects on humans and animals (Kumar et al., 2011).

Aeromonas hydrophila utilized to occur experimental infection belong to *Vibrionaceae* family and it is an oppurtunistic bacteria causing fish illness in fresh and salty waters, especially in waste waters. Motile *Aeromonas* species are bacteria which are present in the environments in which fish aquaculture is done especially in warm waters and it leads to mortality in significant rate. Stress is an important factor in occuring and distributing of the illness (Harikrishnan ve Balasundaram, 2005).

Ginger (*Zingiber officinale* Roscoe), one of the oldest known sources of herbal therapy, contains flavonoids, steroids, alkaloids, shogaols, gingerols, zingeron, vitamins, carotenoids, and polyphenols. Ginger also has antifungal, antiviral, antibacterial, anti-inflammatory, and antioxidant effects on many living creatures, including humans and fish and is a significant immunostimulant that degrades free radicals (Nya and Austin, 2009a; Otunola et al., 2010). Apines-Amar et al. (2012) reported that ginger and similar immunomodulators that had been applied before disease cause evident increased viability and decreased mortality. Khafagy et al. (2014) have reported that ginger provides significant additions to enforce the immune system on catfish in their study. In aquaculture systems, many studies were conducted on plant extracts, including green tea (*Camellia sinensis*), ginger (*Zingiber officinale*), rosemary (*Rosmarinus officinalis*), garlic (*Allium sativum*), nettle (*Viscum album*), and cumin (*Nigella sative*) against many fish pathogens, including *A. hydrophila*. According to the results obtained by applying mixtures of these plant extracts with feed, growth due to appetite, enhancement of the immune system, improvements in the hematological picture, decrease in mortality, inhibition of Gram (+) and Gram (-) bacteria, and increase in the antioxidant enzyme activation for defense in the body were determined (Yılmaz and Ergün, 2012; Dörücü et al., 2009; Abdel-Tawwab et al., 2010; El-Sayed et al., 2014; Khafagy et al., 2014).

In this study, the effects of powdered ginger, administered as a mixture with the feed, on the hematological profile, antioxidant capacity with growth parameters and mortality rates in tilapia against *Aeromonas hydrophila* were detected.

Materials and Methods

Experimental Animals and Design

In this study, healthy cultured 180 Nile tilapia (*Oreochromis niloticus)* provided from Cukurova University Fisheries Faculty, Freshwater Fish Research Station with initial body weights of 25.61±0.03 g and lengths of 12.40±0.09 cm were used. Fish were stocked in twelve, 3000 L cylindrical-conic tanks in flow-through freshwater system. The experimental fish were divided into four groups according to ginger-added doses such as 0.0 (control), 0.1%, 0.5%, and 1.0% in three replications.

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ISSN 1303-2712
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During a one-week adaptation period, the fish were fed with a control diet consisting of 2% of their body weights and with the experimental feed containing ginger extracts for 90 days (He et al., 2015). At the end of 90 days, the fish were undergone to a health examination, and the *A. hydrophila* pathogen was injected into all ginger treated groups. By the 5th day when disease symptoms were emerged in all the ginger groups, 120 fish in the experimental groups were examined for its hematological parameters and oxidative stress indices (OSI). On the other hand, 60 fish were left in the tanks in order to be observed for the intergroup mortality and RPS rates. During the study, 10 mL/L 40% phenoxyethanol (Sigma, USA) was applied to the fish for 4-5 minutes before the analyses (Velisek et al., 2007). Yellow Springs Instrument (YSI, Yellow Springs, OH, USA) mark oxygenmeter was used during the measurements. Water temperature (25.8±0.2°C) and oxygen levels (6.0±0.3 mg/L) in the tanks were constant during the experiment.

Preparation of Ginger Extracts and Fish Diet

Powdered ginger was bought from Plant Products Marketing. Powdered ginger materials were softened for 2 h with the help of a rotating water bath at 90 °C. After filtration, extracts were lyophilized. Ginger was mixed with distilled water and sprayed on the basal diet provided by the Pınar Feed Factory (Dügenci et al., 2003). Experimental diets were prepared in 0.0%; 0.1%; 0.5%, and 1.0% concentrations, respectively**.** The diets were dried in the air and, subsequently, moisture, ash, crude protein, and crude fat analyses were performed (Bligh and Dryer, 1959).

Bacterial Strain and Challenge Study

The *A. hydrophila* was strain (Eastern Fish Disease Laboratory, Leetown, WV, USA) that was used in this assay was stored at -80 °C in a glycerol solution. This strain, which was kept at -80 °C, was inoculated on tryptone soy agar (TSA) (Sigma Chemical Co., Germany), and bacterial culture was left to grow for 24 h at 27 °C on TSA medium. Later, this strain was inoculated in tryptone soy broth (TSB) (Sigma Chemical Co., Germany). Bacterial culture was again left to grow for another 24 h at 27 °C on TSB medium, and finally the culture broth was centrifuged and washed with serum physiologic. Bacterial density in serum physiologic was counted by the agar plate-spread method as inoculated with serial dilution on TSA (Güven and Demirel Zorba, 2011). In *in vitro* analysis, for prepared bacteria dilution done bacterial cfu amount, as a result it was calculated (between 30 and 300 number of bacterial colonies). Then, the bacterial suspension was stored at 4 °C until use. *A. hydrophila* was applied at 100 µL at a concentration of 1.89×10^{9} cfu/mL^{-1}, Fish put life at risk without the need to determine LD 50. And the bacterial suspension in serum physiologic was inoculated intraperitoneally by using a 1 mL insulin syringe in all experimental groups after 90 days. All the specimens treated were observed for their response against the injected bacterial strain. Clinical observations were performed in the control and ginger groups from 48 hours following the application.

Disease resistance

A. hydrophila was cultured, harvested and challenged as described above. Mortalities were recorded from 60 fish in the experimental groups (0.0% (control), 0.5%, 0.1%, 1.0% ginger groups) daily

www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v16_1_20

during 15 days after infections. Then, *A. hydrophila* were reisolated from the liver, gill and gut of all dead fish. Relative percent survival (RPS) was calculated by using formula of Ellis (1988),

$$\text{RPS} = 1 - \frac{(\text{Percent mortality in treated group})}{(\text{Percent mortality in control group})} \times 100$$

Growth Parameters

In all experimental fish samples, the initial body weight (IBW), final body weight (FBW), and lengths were measured using a ruler and a balance (0.001 g sensitivity). Specific growth rates (SGR) and condition factor (CF) were calculated as follows:

$$\text{SGR} = 100^{\times} \frac{\ln[\text{FBW(g)}] - \ln\ [\text{IBW (g)}]}{\text{time interval (days)}}$$

$$\text{CF} = \frac{\text{Weight (g)}}{[\text{Length (cm)}]3}{}^{\times} 100 \qquad \text{(Laird and Needham, 1988)}$$

Hematological Analyses

Blood samples were taken from caudal veins and stored in tubes containing EDTA at 4 °C until the period of analyses (Blaxhall and Daisley, 1973). Erythrocyte (RBC) and leukocyte (WBC) analyses were performed in Thoma microslides using Natt-Herrick solution (Natt and Herrich, 1952). Cyanmethemoglobin and microhematocrit methods were used to determine hemoglobin (Hb) and hematocrit (Hct). Erythrocyte indices were calculated as:

MCV (Mean Corpuscular Volume) (μ^3) = (Hct) (%) ÷ RBC (10^6/mm^3) × 10

MCH (Mean Corpuscular Hemoglobin) (pg) = Hb (g/100 mL) ÷ RBC (10^6/mm^3) × 10

MCHC (Mean Corpuscular Hemoglobin Concentration) = Hb (g/100 mL) ÷ (Hct) (%) × 100 (Stolen et al., 1994).

Blood smears were also prepared, dried in air and stained with May-Grünwald and Giemsa stains to identify the WBC type as lymphocyte, monocyte, neutrophil, and eosinophil (Fujimaki and Isoda, 1990).

Measurement of Oxidative Stress Indices

After the emergence of the symptoms following the injections, homogenates were prepared for SOD, CAT, and MDA analyses from the liver, gills, and gut tissues and the measurements were performed with spectrophotometric methods. MDA level in the tissue samples was measured with the TBA and expressed as nmoles/mg protein. The protein concentration of the tissue samples was measured with a Spectronic-UV 120 spectrophotometer with the method of Lowry (Lowry et al., 1951). CAT activity was determined by measuring the decrease in the hydrogen peroxide concentration at 230 nm by the method of Beutler (Beutler, 1975) and expressed as U/mg protein. SOD activity was measured in the tissue samples according to the method described by Fridovich (Fridovich, 1995) and expressed as U/mg protein.

Statistical Analyses

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One-way analyses of variance was applied using SPSS statistics 17.0 software (Erol, 2010). The data were expressed as the mean± standard deviations (mean±SD). For all statistical tests, $P<0.05$ was considered statistically significant.

Results

The results of moisture, ash, crude fat, and crude protein analyses for ginger and basal feed (control) groups are presented in Table 1. According to the feed analyses results, there were no significant differences among the experimental groups ($P>0.05$) (Table 1). On the other hand, there were no statistical differences in IBW (Initial Body Weight), FBW (Final Body Weight), and SGR (Specific Growth Rate) values of tilapia between the control and the ginger groups ($P>0.05$) (Table 2).

Clinical and Necropsy Findings of Fish

In the clinical examinations of the control fish, lethargy and less feed acceptability were determined at the 48th hour. On the other hand, in the 0.1 and 0.5% ginger groups hyperemic areas in the fin base, erosions in the caudal fin and fin tips, and discoloration of skin were observed from the 5th day. No symptoms were observed in the 1.0% ginger group in necropsy examinations, whereas erosions in the gill filaments; mucus accumulation, enlarged liver, gall bladder, and intestines, color change, liquid accumulation and hyperemic areas in kidney tissues were observed in the necropsy examination of the control group (Figure 1).

Hematological Parameters

In our study, significant increases in RBC, Hct, Hb and RBC indices were observed in the groups containing higher ratios of ginger compared to the control group (Table 3) ($P<0.05$). On the other hand, WBC and percentage share of lymphocytes, monocytes, neutrophil and eosinophil cells, which form the first step of the body defense, and basic elements of the non-specific immune system, increased significantly with the increasing effect of ginger ($P<0.05$) (Table 4). Blood smears from the experimental groups did not reveal any swelling, shrinkage or other deformations in blood cells.

Oxidative Stress Indices (OSI)

In our study, SOD and CAT enzyme levels were significantly increased in all tissues with increased ratios of ginger ($P<0.05$). MDA values did not change significantly in gill tissue ($P>0.05$), whereas MDA values gradually decreased in liver and gut tissues in increased ratios of ginger ($P<0.05$) (Table 5). In our research, 1.0% ginger concentration increased SOD and CAT levels in all tissues while decreasing MDA levels in liver and gut tissues.

Disease resistance

RPS (Relative Percent Survival), survival and mortality ratios were calculated for all experimental groups and shown in Table 6. The low mortality of 46.7% and 33.3% were recorded in fish treated with 0.5 and 1% with ginger supplementation diets to provide maximum protection. However, cumulative mortality was high in fish

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ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v16_1_20

treated with 0.0% (control) and 0.1% of ginger supplementation feeds of 86.7% and 73% mortality for 15 days. (Table 6)

Discussion

Ginger has been known to be an important immunomodulator, high antioxidant activity, and gastrointestinal effects. In previous studies performed, ginger is safety used for human, animal, fish, environmental health, antihepatotoxic, an appetizer, and it has an important effect on growth (Sivaram et al., 2004). El-Sayed et al.(2014) determined important increases in growth performances at a ratio of 5% of the body weights in tilapia fed with ginger (1%) four times a day. He et al. (2015) reported that feeding with 2% of the body weight ratio was suggested in Tilapia (*O. niloticus*) in terms of liver enzymes and health indicator hematological parameters compared to a 6% feeding ratio. Daily feeding ratios applied during our study were selected after considering the studies on healthy fish (El-Sayed et al., 2014; He et al., 2015; Talpur et al., 2013), and no problem was encountered regarding the appetite and feed acceptibility of the fish. However, it was found that ginger, which is reported to have appetizing, growth-stimulating effects on fish species, had no significant effects on the growth parameters in feeding with 2% of the body weight of the fish in our study.

In profilactive studies on fish, it has been reported that immunostimulants enhanced the general defense system and decreased the mortality against pathogens and increased the viability rate (Khafagy et al., 2014; Nya and Austin, 2009b; Maqsood et al., 2011). The results in our study on the effects of 1.0% ginger, an important antibacterial agent, were compatible with those obtained in various studies (Nya and Austin, 2009a; El-Sayed et al., 2014; Talpur et al., 2013). Hematological parameters are used to provide information about the health and physiological status of fish, feeding conditions and water quality in which they live (Fazio et al., 2013). WBC, RBC, Hct, and Hb values are particularly recommended on a routine basis to monitor the health of the stock in fish farms. In the previous study on the effects of herbal immunostimulants on hematology and the immune system, it has been reported that plants bioactive substances caused an increase in blood cells counts, and this triggered the immune system and enchanced a natural defense in distinct fish species (Nya and Austin, 2009a; Talpur et al., 2013; Ajeel and Faragi, 2013; Haghighi and Rohani, 2013).

In our study, significant increases have been reported in the leukocyte cells and their indices that are both natural defense cells and in the RBC, Hb and Hct, especially in 1.0% ginger group. These elevations determined in the hematological parameters support the findings obtained by the investigators concerning the subject.

In long-term feeding studies conducted with ginger, it has been reported that hematological parameters were stimulated for the prevention of disease, and consequently interrelated positive effects were observed amongs the parameters (Ajeel and Faragi, 2013; Aly et al., 2008). In a study on *Oncorhynchus mykiss*, it was reported that WBC counts increased, phagocytic cells were activated, and the fish became more resistant to infectious diseases at the end of a 12-weeks feeding period with ginger (Haghighi and Rohani, 2013). Following a similar feeding period in our study, positive increases were determined in hematological parameters which support the health and naturel defense of the fish. The increases in lymphocytes, monocytes, neutrophils, and eosinophils, which are the basic elements of the defense system, showed the effect of ginger in body defenses and this was also confirmed in the other studies. The 0.5% and 1.0% ratios of ginger not only increased the percentages of lymphocyte, monocyte, neutrophil, and eosinophil in *O. niloticus* but also decreased the mortality,

www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v16_1_20

and these were regarded as the best indicators for coping with the pathogen in our study. Various studies pointed out that ginger is a strong antioxidant which has protective, regenerative, and function-enhancing features by maintaining the degradation of the free radicals (Mallikarjuna et al., 2008; Lebda et al., 2012). Live organisms affected by pathogens and various stress factors are exposed to free radicals caused by oxidative stress, and the living organisms are protected from these free radicals as a result of the activities of the antioxidant system. The most important indicators of these activities are the increase in the SOD and CAT enzyme levels (He et al., 2015; Livingstone, 2001; Ritola et al., 2002). Amongs antioxidant enzymes, SOD is considered as the first line of defense against oxygen toxicity due to its inhibitory effects on oxyradical formation. The dismutation of the superoxide anion radical is catalyzed by SOD into water and hydrogen peroxide after which SOD is detoxified by CAT (Manno et al., 1985). Secombes and Oliver (1997) reported that SOD reached higher levels against free radicals formed as a result of defense mechanisms including respiratory burst, during bactericidal activity. Malondialdehyde (MDA), which is one of the end products of lipid peroxidation caused by oxygen free radicals, is an important oxidative stress indicator which shows the degree of lipid peroxidation. On the other hand, the increase in MDA levels is one of the important indicators of the damage occurred in the body at the cellular level (Yagi, 1984). Lebda et al. (2012) reported that phenolic compounds of ginger (gingerols, shogaols, volatile oils, flavonoids, and phenolic ketone derivatives) promote antioxidant activity against free radicals and inhibit lipid peroxidation. In our study, high MDA values obtained in the lowest ginger dose (0.1%) and the control group are also the indicators of the increase in free radicals in these groups. In a similar study, it has been reported that MDA levels decreased and antioxidant enzymes activity (SOD and CAT) increased in tilapia in groups in which garlic, an herbal immunostimulator, was used intensively (Metwally, 2009). Our study results show that antioxidant enzymes (SOD and CAT) increased their activity probably therefore MDA levels decreased in the experimental groups with increased ginger levels. It was emphasized that this may be associated with various bioactive substances (shagol, gingerols, zingeron, etc.) which are found in the composition of ginger and they provide an antioxidant property to the ginger.

In our study, important results were obtained regarding the effects of ginger on *A. hydrophila*, a pathogen that causes high mortality and serious economic losses in many freshwater fish in the aquaculture industry. At higher doses of ginger (0.5% and 1.0%), the increase observed in the ratios of RPS against *A. hydrophila* and increase determined in OSI values are the best evidence of the fact that the health of tilapia is stimulated and consequently it provided defense against the pathogen by inhibiting it. This study examined the health effects of feeding fish with ginger-added diet in aquaculture systems, resistance formation against diseases, antibacterial and antioxidant properties that would cope with the oxidative stress and provided a perspective on this subject.

Acknowledgement

This research was supported by the Academic Research Projects Unit of Cukurova University (Project Number: SÜF-2010-YL-21).

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www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v16_1_20

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www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v16_1_20

Table 1- The content of nutrients in experimental diets

Experimental Diets		Moisture (%)	Ash (%)	Crude Protein (%)	Crude Fat (%)
Basic Diet	0.0 (Control)	8.13±0.04	8.13±0.06	43.75±0.02	21.36±1.74
Ginger (%)	0.1	8.09±0.08	8.09±0.02	43.85±1.59	21.06±1.73
	0.5	8.38±0.03	8.38±1.24	44.90±0.42	22.38±1.24
	1.0	8.95±1.00	8.95±0.09	43.64±0.32	22.05±1.56

The results were given as mean ± S.D**.**

Table 2- Growth parameters of Nile Tilapia fed with ginger at different ratios. IBW: Initial Body Weight; FBW: Final Body Weight; SGR: Specific Growth Rate; CF: Condition Factor.

Experimental Diets		IBW g	FBW g	SGR % day^{-1}	CF g/cm^3
Basic Diet	0.0 (Control)	25.61±0.09	77.65±3.49	57.82±3.51	1.80±1
Ginger (%)	0.1	25.57±0.05	77.84±3.84	58.08±3.93	1.82±0.65
	0.5	25.68±0.04	76.28±4.13	56.32±3.83	1.83±0.10
	1.0	25.53±0.08	74.40±3.30	54.38±3.61	1.86±0.69

The results were given as mean ± S.D**.**

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www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v16_1_20

Table 3- Hematological profiles of infected Nile Tilapia fed with different levels of ginger

Experimental Diets		RBC (10^6/mm^3)	Hb (g/dL)	Hct (%)	RBC Indices		
					MCV (μ^3)	MCH (Pg)	MCHC (%)
Basic Diet	0.0 (Control)	1.62±0.22^a	5.79±1.00^a	32.16±3.31^a	186.39±3.39^a	35.71±2.65^a	19.12±2.60^a
Ginger (%)	0.1	1.98±0.33^{ab}	7.09±1.15^{ab}	36.97±4.04^b	188.72±3.93^a	5.78±2.60^a	19.37±2.79^a
	0.5	2.13±0.27^b	8.84±1.10^b	40.41±3.72^c	203.72±3.82^b	41.52±2.76^b	22.39±2.37^b
	1.0	2.00±0.24^b	8.89±1.02^b	40.04±2.14^c	200.20±3.12^b	43.45±2.64^b	22.85±2.63^b

Data are represented as mean ± SD. In the same column with different letters values are significantly different ($P<0.05$).

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ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v16_1_20

Table 4- Leukocyte profiles of infected Nile Tilapia fed with different levels of ginger

Experimental Diets		WBC (10^3/mm^3)	WBC Indices			
			Lymphocyte (%)	Monocyte (%)	Neutrophil (%)	Eosinophil (%)
Basic Diet	0.0 (Contr.)	15.72±2.51^a	41.62±2.06^a	42.32±3.74^a	1.71±0.24^a	0.00±0.00^a
Ginger (%)	0.1	16.22±5.79^a	50.39±3.04^b	44.76±4.21^a	1.85±0.26^a	0.54±0.18^b
	0.5	22.22±4.50^b	53.05±4.70^b	46.17±3.11^{ab}	3.00±0.84^b	0.94±0.37^c
	1.0	22.27±6.47^b	53.39±3.40^b	57.40±7.02^c	4.10±1.90^b	1.68±0.69^d

Data are represented as mean ± SD. In the same column with different letters values are significantly different (P<0.05).

Table 5- Oxidative Stres Biomarkers in liver, gill and gut tissues of infected Nile Tilapia fed with different levels of ginger.

	Ginger (%)	MDA (nmoles/mg protein)	CAT (U/mg protein)	SOD (U/mg protein)
Liver	0.0 (Control)	0.19±0.05^c	169.75 ±10.84^a	12.76±0.41^a
	0.1	0.15±0.06^c	206.52±11.82^b	18.98±1.83^a
	0.5	0.9±0.01^b	268.61±12.84^c	23.42±0.55^b
	1.0	0.06±0.00^a	270.21±56.70^c	29.09±2.73^b
Gill	0.0 (Control)	0.05±0.00	55.96±1.91^a	3.50± 0.01^a
	0.1	0.03±0.00	108.16±7.89^b	8.35±0.70^b
	0.5	0.02±0.00	117.34±10.33^c	12.33±1.97^{bc}
	1.0	0.03±0.00	124.34±14.54^c	19.49±0.98^c
Gut	0.0 (Control)	0.23±0.00^c	69.27±4.68^a	21.60±1.40^a
	0.1	0.21±0.04^c	98.23±4.39^b	27.99±2.00^{ab}
	0.5	0.12±0.01^b	100.86±11.76^b	33.72±2.31^b
	1.0	0.08±0.00^a	201.50±61.00^c	58.16±12.40^c

Values are represented as mean ± SD. In the same column with different letters values are significantly different (P<0.05).

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www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v16_1_20

Table 6- Mortality Rate, Survival and Relative Percentage Survival (RPS) of Nile Tilapia fed with ginger at different ratios.

Experimental	Diets	Number of observed fish	Mortality (%)	Survival	RPS (%)
Basic Diet	0.0 (Control)	15	13 (86.7%)[a]	2	0
Ginger (%)	0.1	15	11(73%)[a]	4	15.8
	0.5	15	7 (46.7%)[b]	8	46
	1.0	15	5 (33.3%)[b]	10	61.6

In the same column with different letters values are significantly different (P<0.05).

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www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences DOI: 10.4194/1303-2712-v16_1_20

Figure 1-General view of fin, gill, liver, and abdomen in control group of Nile tilapia infected with *A. hydrophila*.