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Determination of Some Haematological Parameters and Disease Resistance Capacity to *Aeromonas hydrophila* Infection in the Nile tilapia, *Oreochromis niloticus* L. Fed Dietary Supplementation of Ginger (*Zingiber Officinale*)

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ABSTRACT

Aquaculture is one of the fast-growing food industries in the world. However, this industry is hampered by diseases. The present study aimed to determine the effect of ginger powder on the haematology of the Nile tilapia, *Oreochromis niloticus*, and resistance to *A. hydrophila* infection. A completely randomized design was used for the experiment. A total of 300 healthy live experimental Nile tilapia individuals, with 20 ± 1.00g body weight and 11.06 ± 0.08cm body lengths, were randomly divided into five treatment glass aquariums. Each experiment was conducted in triplicates, representing four treatments (3, 5, 8 and 12 g ginger/kg diet) and one control (0.00 ginger/kg diet). After eight weeks of feeding trial, 0.2ml of *Aeromonas hydrophila* culture containing 1.0×10^7 CFU/ml (LD50) was given by intraperitoneal (IP) injection. Blood samples were collected before and after the bacterial infection of the fish for haematological analysis. The result showed that blood parameters of *Oreochromis niolticus* in all ginger concentrations were significantly higher compared to the control diet before infection ($P< 0.05$). *Oriochromis niloticus* fed a diet at a concentration of 5 g ginger/kg showed the highest red blood cell (RBC), haematocrit value, haemoglobin content, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC), lymphocytes and monocytes. Erythrocyte sedimentation rate (ESR), mean corpuscular volume (MCV), WBC, neutrophils and monocytes showed significant increment, while RBC, haemoglobin content, haematocrit value, MCH, MCHC, and lymphocytes decreased significantly after infection ($P< 0.05$). The results also revealed that the *Oriochromis niloticus* treated with 5, 8 and 12 g/kg of ginger showed a decrease in mortality rates, compared to the control diet after infection. The fish fed 5g/ kg of ginger had the highest survival rate (81.66%). In conclusion, 5 g/kg of ginger had positive effects on *Oriochromis niloticus* innate immunity system and prevented *Aeromonas hydrophila* infection. Therefore, the use of 5 g/kg dietary ginger powder in the diet of *Oriochromis niloticus* juvenile is recommended.

INTRODUCTION

Rapid population growth, urbanization and rise in income increase the demand for food **(FAO, 2019)**. Fish play a significant role in food security and good nutrition **(Beveridge *et al.*, 2013)**. Fish provided 17% of the global population's intake of animal protein **(FAO, 2014)**. In freshwater aquaculture, tilapias are the most farmed tropical fish species in the world after carp because of their suitability for aquaculture, good marketability and stable prices **(Waite *et al.*, 2014; Wang & Lu, 2016)**. The Nile tilapia, *Oriochromis niloticus*, can be easily cultivated in freshwater **(FAO, 2015)**. However, the production of aquaculture in general and tilapia culture, in particular, is hampered by diseases-causing microorganisms **(Van Hai, 2015)**.

Bacterial infection is one of the most significant threats to successful Nile tilapia production. *Aeromonas* spp. have been identified as the major causative bacteria threatening the Nile tilapia **(Baumgartner *et al.*, 2017)**. *Aeromonas* spp. are widespread in untreated and treated water. The occurrence of diseases by *Aeromonas* spp. is related to stress conditions such as overcrowding and poor water quality. *Aeromonas hydrophila*, one of *Aeromonas* spp. is a major fish pathogen known to infect a variety of fishes, predominantly present in freshwaters. *A. hydrophila* infection in *Oreochromis niloticus* has caused severe disease outbreaks causing 60% mortality **(Hardi *et al.*, 2017)**. This decline in fish production affects the protein supply of aquaculture and the economy of the world **(Nugroho *et al.*, 2017)**. Stress is frequently considered one of the factors contributing to disease outbreaks by these bacteria **(Laleh *et al.*, 2015)**. Skin redness, ulcers, swelling of tissues, haemorrhage and necrosis of the visceral organs are the major symptoms of the infected Nile tilapia by *A. hydrophila* **(Yardimci & Aydin, 2011)**.

Antibiotics have been used to prevent and treat bacterial diseases in fish. However, the use of antibiotics in aquaculture for disease control lead to problems with microbial resistance and unacceptable residues in aquaculture products and the environment. The resistant bacterial strains could hurt the therapy of fish diseases or human diseases, and the environment of fish farms increases the accumulation of chemicals in fish, which is not safe for a human being as the final consumer **(Heuer *et al.*, 2009)**. Therefore, there is a need to find an alternative to antibiotics to control bacterial diseases of fish including the Nile tilapia.

Medicinal plants in aquaculture can be used as an alternative to antibiotics. Out of fifteen medicinal plants tested, ten (66.67%) species showed an antibacterial effect against *A. hydrophila* **(Chowdhuryl & Rahman, 2008)**. They can be given to fish through intraperitoneal injection to improve their health status of fish and protect against infectious diseases **(Bulfon *et al.*, 2015; Awad & Awad, 2017; Reverter *et al.*, 2017)**.

Medicinal Plant such as ginger (*Zingiber officinale*), garlic (*Allium sativum*), oyster mushroom (*pleurotus ostreatus*), oats (*Avena sativa*), beetroot (*Beta vulgaris*) and moringa (*Moringa oleifera*) among others have been used alternatives to antibiotics **(Bichi *et al.*, 2012; Bilen *et al.*, 2016; Devi *et al.*, 2016; Skariyachan *et al.*, 2016)**. They produce secondary metabolites **(Ravikumar *et al.*, 2012; Van Hai, 2015)**. Ginger (*Zingiber officinale*) for instance produces secondary metabolites such as alkaloids, steroids, flavonoids, gingerols, zingerone, carotenoids, vitamins and polyphenols, which have antibacterial, antifungal, anti-inflammatory and antioxidant effects on fish **(Stoner, 2013; Nile & Park, 2015)**. Ginger is also effective as an immunomodulatory agent in

fish and helps to reduce the loss in aquaculture owing to diseases **(Apines-Amar *et al*., 2012)**. Nenerthe;ess, ginger added to fish feed is often made through its ethanolic extract, which may lead to a reduction of some chemical compounds at the filtration stage. Thus, the objective of this study was to determine the effect of ginger powder on the haematology of Nile tilapia and resistance to *A. hydrophila* infection.

MATERIALS AND METHODS

Experimental fish and design

A completely randomized design (CRD) was used for the experiment. A total of fifteen experimental glass aquaria (65 × 40 × 50 cm) were used for the experiment. The water level was maintained at a volume of 40 litters throughout the study period. A total of 300 healthy live Nile tilapia of 20 ± 1.00 g body weight and 11.06 ± 0.08 cm lengths were taken from the Research Centre, Hawassa University, Ethiopia and randomly assigned to five treatment glass aquariums in the biology laboratory, Hawassa University. Each experiment was done in triplicates, representing four treatments and one control. Each replicate contained 20 fish. The fish were acclimatized before the experiment and fed commercial feed for 2 weeks three times daily until apparent satiation. At the end of two weeks, (after acclimatization) fish in each group were fed different concentrations of ginger three times at a level of 3% of body weight daily for 10 weeks (through experimental periods). Control diet fishmeal was free from ginger. Settled fish wastes of aquarium water were daily siphoned. Siphoned water was replaced by clean and aerated water from the storage tank. Water temperature (25.0 ± 2.0ºC), dissolved oxygen concentration (5.6 ± 0.15 mg l-1), and pH level (6.8 ± 0.2) were monitored once a week with a YSI 556(r) multi-probe system (YSI Environmental, Yellow Spring, OH, USA).

Preparation of feed

The fresh ginger (*Zingiber officinale*) rhizome used for the feeding trial was purchased from the local market in Hawassa, Ethiopia. It was dried under shade for one week. The dried ginger was ground to powder, homogenized, and then sieved using a hand sieve. Then different concentrations of ginger (3, 5, 8, and 12 g ginger/kg) and fish meal content were transformed into pellet form by a food grinder and stored at −3ºC before feeding.

Challenge test

A. hydrophila that had previously been isolated from the Nile tilapia of Lake Hawassa was grown in TSA at 28°C for 18 hrs. The bacteria suspension was adjusted to 1×10^7 CFU/ml in phosphate buffer saline using the McFarland scale. This concentration was obtained in a previous LD50 trial. In sum, three groups of 30 fish were infected with 0.2 ml *A. hydrophila* (1×10^4; $1\times10^{6,}$ and 1×10^7 CFU/ml), and mortality was recorded for 15 days. The LD50 was calculated according to **Plumb and Bowser (1983)**. At the end of the 8 week- feeding trial, fish were challenged with pathogenic *A. hydrophila.* A 0.2 ml of *A. hydrophila* culture containing 1.0×10^7 CFU/ml (LD50) was given by intraperitoneal (IP) injection, using a 21/gauge sterile needle **(Schaperclaus *et al*., 1992)**. Twenty-four hours after injection, fish were fed the same experimental diet as in the feeding trial for 15 days of the challenge period, and any clinical symptoms were recorded. The dead fish were daily removed, and mortality was confirmed by re-isolating

the microorganism from the internal organs of the dead fish. Survival rates of the Nile tilapia were computed in percentages as follows:

Survival rate (%) = Nf × 100/Ni

Where, Nf = Number of cultured fish alive at the end of the experiment, and

Ni = Number of cultured fish stocked at the beginning of the experiment

Blood collection and haematological examination

Blood samples were collected in the early morning hours before and after bacterial infection of the fish for haematological analysis. Three fish from each replicate were sampled and anesthetized with 50 mg $l^{-1}$of tricaine methanesulfonate (MS222, Sigma Chemical Co. St. Louis, MO, USA), and then blood samples were collected from the caudal peduncle with the use of a 5 ml syringe and needle which was treated with heparin to prevent clotting and transferred to sampling bottles, containing ethylene diamine tetra-acetic-acid (EDTA). After the collection, blood samples were taken to the Veterinary laboratory in Hawassa University, where the haematological analysis was carried out. Blood was analyzed with routine methods adopted in fish haematology **(Blaxhall & Daisley, 1973; Haghighi, 2010)**. The total erythrocyte count (RBC×10^6/µl) and total leukocyte count (WBC ×10^3/µl) were determined manually using a Neubauer's haemocytometer, with Hayem solution as a diluent. The haematocrit percentage was determined in duplicate by using microhaematocrit-heparinized capillary tubes of 75 µl volume and a microhaematocrit centrifuge at 15000 g for 5min **(Goldenfarb *et al.*, 1971)**. For the determination of haemoglobin concentrations Sahli's method was used. The values of haemoglobin were expressed as g/dilution **(Campbell, 2015)**. The values of red blood cell indices of mean corpuscular volume (MCV fl), mean corpuscular haemoglobin (MCH pg) and mean corpuscular haemoglobin concentration (MCHC g/dl) were calculated according to **Wintrobe (1993)**. WBC such as neutrophils, lymphocytes, and monocytes was performed by the diluent/dye direct method outlined by **Nat and Herrick (1952)** in a Neubauer chamber at a dilution of 1:100.

Statistical analysis

Data obtained were expressed as mean ± standard error of the mean. The results were analyzed with a one-way analysis of variance (ANOVA), using SPSS (Statistical Package for Social Science 2006, version 22). Differences at $P< 0.05$ were regarded as statistically significant. Data were presented as mean ± SE before and after the challenge test.

RESULTS

Clinical symptoms

After 36 hrs of infection with *A. hydrophila*, the Nile tilapia showed clinical symptoms such as exophthalmia, fin rot and swelling of tissues, redness of skin and haemorrhage (Fig. 1). The dead fish showed severe congestion in the abdominal area and a protruded vent due to the of fluid in peritoneal cavity.

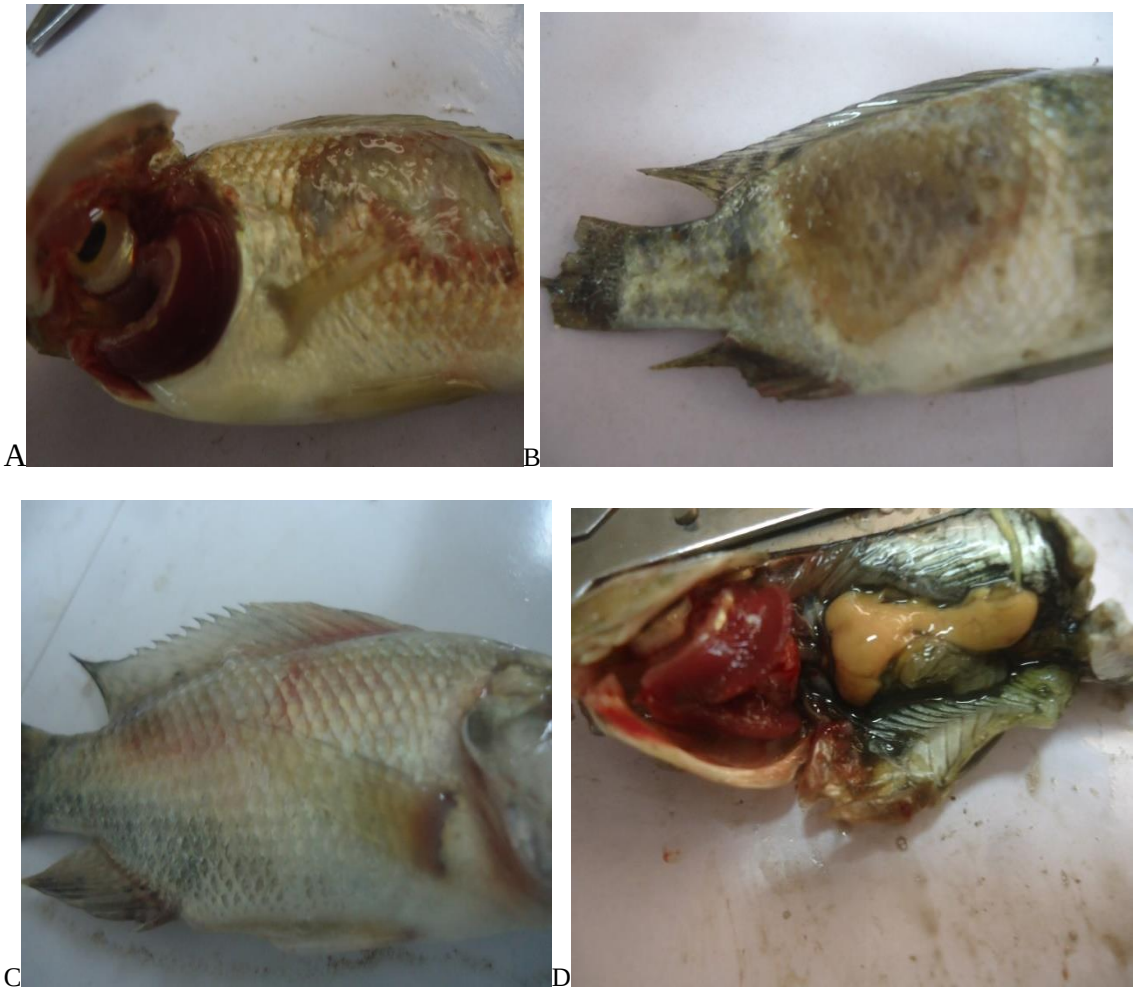

Fig. 1. Clinical symptoms of the Nile tilapia after infection with *A. hydrophila* displaying: **(A)** Exophthalmia; **(B)** Fin rot and swelling of tissues, and **(C)** Redness of skin (D)haemorrhage

Diseases resistant

Survival and mortality rate results of the Nile tilapia challenged with *A. hydrophila* are presented in Fig. (2). Results revealed that the addition of ginger to the *O. niloticus* diet enhances the body's health and resistance to infection with *A. hydrophila*. The fish in the treatment trial with 5 g/kg ginger had the highest survival rate (81.66%), compared to the other ginger concentration. The highest mortality was recorded in the control group (70%), followed by the 3 g *Z. officinale*/kg diet (66.7%) and 12 g *Z. officinale*/kg diet (55%).

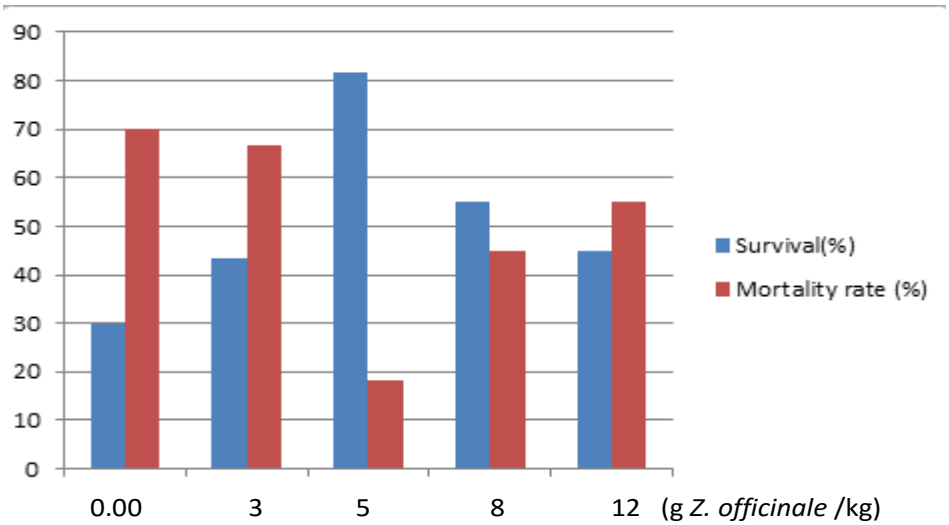

Fig. 2. Resistance of *Oreochromis niloticus* after challenged test with *Aeromonas hydrophila*

The effect of ginger powder on haematological parameters of *O. niloticus*

The haematological parameter results of *O. niloticus* are shown in Tables (1, 2). Blood parameters of *O. niolticus* in all ginger concentrations were significantly different ($P< 0.05$), except MCV, MCH and MCHC ($P> 0.05$) from the control diet before and after infection with *A. hydrophila.* The Nile tilapia *(O. niloticus)* fed a diet at a concentration of 5g of *Z. officiale*/kg feed had significantly the highest RBC, haematocrit, haemoglobin, WBC and lymphocytes value, while the ginger at a concentration of 12g of *Z. officiale*/kg feed recorded significantly the lowest WBC, lymphocytes, neutrophils and monocytes value before and after infection with *A. hydrophilus*. ESR and MCV showed increment, while MCH and MCHC decreased after infection of *O. niloticus* with *A. hydrophila* (Table 1)

Table 1. Mean values of haematological parameters; red blood cells (RBCs), haematocrit value, haemoglobin (Hb) content, erythrocyte sedimentation rate (ESR), mean corpuscle volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)] of Nile tilapia (*O. niloticus*) fed different concentrations of ginger (0.0, 3, 5, 8 and 12g of *Z. officinale*/kg feed).

Variable	Experimental diet	Before infection	After infection
RBC (10^6/mm^3)	0.0 g *Z. officinale*/kg feed (control)	3.0 ± 0.26^{bB}	1.27 ± 0.06^{aA}
	3 g *Z. officinale*/kg feed	3.04 ± 0.00^{cB}	1.43 ± 0.1^{bA}
	5 g *Z. officinale*/kg feed	3.87 ± 0.15^{eB}	3.55 ± 0.11^{eA}
	8 g *Z. officinale*/kg feed	3.11 ± 0.06^{dB}	2.77 ± 0.12^{dA}
	12 g *Z. officinale*/kg feed	2.92 ± 0.05^{aB}	2.10 ± 0.93^{cA}
Haematocrit (%)	0.0 g *Z. officinale*/kg feed (control)	36.71 ± 0.22^{bB}	20.52 ± 0.53^{aA}
	3 g *Z. officinale*/kg feed	37.68 ± 0.16^{cB}	21.77 ± 0.77^{cA}
	5 g *Z. officinale*/kg feed	40.70 ± 0.2^{eB}	37.59 ± 0.21^{eA}

Table 1. continued

Variable	Experimental diet	Before infection	After infection
	8 g *Z. officinale*/kg feed	38.14 ± 0.08^{dB}	31.57 ± 0.06^{dA}
	12 g *Z. officinale*/kg feed	36.15 ± 0.08^{aB}	20.96 ± 0.56^{bA}
Haemoglobin (g/dl)	0.0 g *Z. officinale*/kg feed (control)	7.59 ± 0.17^{bB}	3.69 ± 0.21^{bA}
	3 g *Z. officinale*/kg feed	7.64 ± 0.2^{cB}	4.21 ± 0.49^{cA}
	5 g *Z. officinale*/kg feed	9.12 ± 0.1^{eB}	7.29 ± 0.53^{eA}
	8 g *Z. officinale*/kg feed	8.05 ± 0.03^{dB}	5.66 ± 0.11^{dA}
	12 g *Z. officinale*/kg feed	7.50 ± 0.04^{aB}	3.58 ± 0.07^{aA}
ESR (mm/hr)	0.0 g *Z. officinale*/kg feed (control)	5.61 ± 0.12^{dA}	8.68 ± 0.05^{dB}
	3 g *Z. officinale*/kg feed	5.19 ± 0.07^{bA}	8.72 ± 0.13^{eB}
	5 g *Z. officinale*/kg feed	4.68 ± 0.08^{aA}	5.55 ± 0.22^{aB}
	8 g *Z. officinale*/kg feed	$5.\ 85\pm0.09^{eA}$	7.33 ± 0.10^{bB}
	12 g *Z. officinale*/kg feed	$5.\ 41\pm0.12^{cA}$	8.65 ± 0.21^{cB}
MCV(fl)	0.0 g *Z. officinale*/kg feed (control)	121.18 ± 0.98^{cA}	133.44 ± 7.41^{aA}
	3 g *Z. officinale*/kg feed	123.69 ± 1.15^{dA}	130.48 ± 4.39^{aA}
	5 g *Z. officinale*/kg feed	105.1 ± 4.52^{aA}	119.26 ± 1.13^{aA}
	8 g *Z. officinale*/kg feed	117 ± 8.26^{bA}	121.14 ± 4.67^{aA}
	12 g *Z. officinale*/kg feed	123.85 ± 2.18^{eA}	134.29 ± 13.05^{aA}
MCH(pg)	0.0 g *Z. officinale*/kg feed (control)	22.97 ± 1.67^{aA}	$25.06\pm0.78a^{A}$
	3 g *Z. officinale*/kg feed	24.25 ± 0.47^{dA}	25.09 ± 0.81^{aA}
	5 g *Z. officinale*/kg feed	25.52 ± 1.14^{eA}	23.54 ± 0.67^{aA}
	8 g *Z. officinale*/kg feed	23.14 ± 0.64^{cA}	25.91 ± 0.59^{aB}
	12 g *Z. officinale*/kg feed	23.79 ± 0.61^{bA}	25.69 ± 0.55^{aA}
MCHC (g/dl)	0.0 g *Z. officinale*/kg feed (control)	20.68 ± 0.57^{bB}	16.84 ± 0.05^{aA}
	3 g *Z. officinale*/kg feed	20.30 ± 0.46^{aB}	18.93 ± 0.64^{bA}
	5 g *Z. officinale*/kg feed	22.41 ± 0.34^{eA}	21.36 ± 0.52^{eA}
	8 g *Z. officinale*/kg feed	21.11 ± 0.08^{dB}	19.04 ± 0.57^{cA}
	12 g *Z. officinale*/kg feed	20.74 ± 0.13^{cA}	19.46 ± 0.62^{dA}

Mean ± standard deviation followed by different superscript letters (a, b, c, d) in the same column in each treatment trial is significantly different at $P< 0.05$. Capital letters (A, B) in the same row indicated significant difference at $P< 0.05$ before and after infection of the Nile tilapia with *A. hydrophila.*

WBCs, lymphocytes, neutrophils and monocytes of *O. niloticus* were significantly higher than those of the control group before infection of *O. niloticus* with *A. hydrophila* and their results are presented in Table (2). WBC was significantly different in all treatments both before and after infection ($P< 0.05$). WBC was dominated by lymphocytes (23.18 ± 0.16-27.73 ± 0.15 10^3/µl), followed by neutrophils (5.22 ± 0.47-7.06 ± 0.23 10^3/µl) and monocytes (4.26 ± 0.16- 6.88 ± 0.08 10^3/µl) after infection of *O. niloticus* with *A. hydrophila* (Fig. 3 & Table 2).

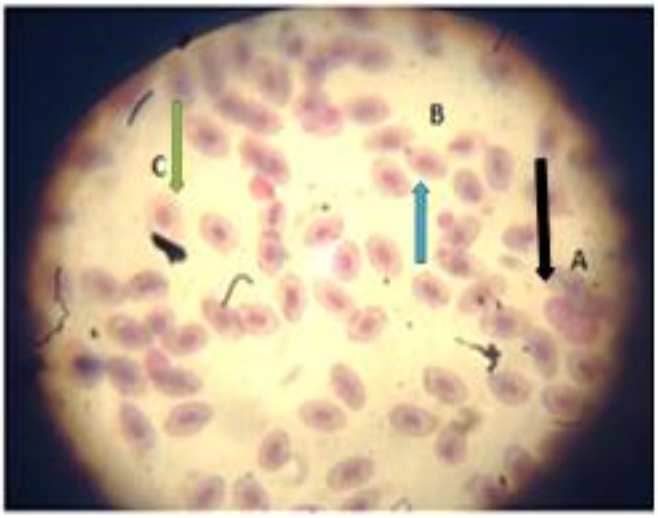

Fig. 3. Different types of white blood cell (a) lymphocyte, (b) neutrophils and (c) monocyte

Table 2. Mean values of haematological parameters [white blood cells (WBCs), lymphocytes, neutrophils and monocytes] of Nile the tilapia (*O. niloticus*) fed different concentrations of ginger (0.0, 3, 5, 8 and 12g of *Z. officinale* /kg of feed)

Variable	Experimental diet	Before infection	After infection
WBC(10^3/mm3)	0.0 g *Z. officinale*/kg feed (control)	33.72 ± 0.23^{aA}	44.37 ± 0.12^{bB}
	3 g *Z. officinale*/kg feed	40.30 ± 0.12^{cA}	45.91 ± 0.75^{dB}
	5 g *Z. officinale*/kg feed	43.38 ± 0.13^{eA}	45.51 ± 0.12^{cB}
	8 g *Z. officinale*/kg feed	41.37 ± 0.09^{dA}	53.49 ± 0.06^{eB}
	12 g *Z. officinale*/kg feed	38.75 ± 0.07^{bA}	44.29 ± 0.30^{aB}
Lymphocyte (10^3/µl)	0.0 g *Z. officinale*/kg feed (control)	27.23 ± 017^{bB}	23.34 ± 0.90^{bA}
	3 g *Z. officinale*/kg feed	27.63 ± 0.09^{cB}	24.56 ± 0.23^{cA}
	5 g *Z. officinale*/kg feed	30.19 ± 0.13^{eB}	27.73 ± 0.15^{eA}
	8 g *Z. officinale*/kg feed	28.37 ± 0.04^{dB}	25.21 ± 0.48^{dA}
	12 g *Z. officinale*/kg feed	25.09 ± 0.06^{aB}	23.18 ± 0.16^{aA}

Table 2. continued…

Variable	Experimental diet	Before infection	After infection
Neutrophil(10^3/µl)	0.0 g *Z. officinale*/kg feed (control)	3.44 ± 0.08^{cA}	5.21 ± 0.08^{bB}
	3 g *Z. officinale*/kg feed	3.33 ± 0.07^{bA}	5.48 ± 0.05^{cB}
	5 g *Z. officinale*/kg feed	4.54 ± 0.09^{eA}	7.06 ± 0.23^{eB}
	8 g *Z. officinale*/kg feed	4.22 ± 0.10^{dA}	5.81 ± 0.14^{dB}
	12 g *Z. officinale*/kg feed	3.30 ± 0.12^{aA}	5.22 ± 0.47^{aB}
Monocytes(10^3/µl)	0.0 g *Z. officinale*/kg feed (control)	1.38 ± 0.03^{aA}	4.69 ± 0.21^{aB}
	3 g *Z. officinale*/kg feed	2.86 ± 0.05^{bA}	5.58 ± 0.05^{dB}
	5 g *Z. officinale*/kg feed	3.61 ± 0.04^{dA}	6.88 ± 0.08^{cB}
	8 g *Z. officinale*/kg feed	3.05 ± 0.05^{cA}	5.66 ± 0.21^{eB}
	12 g *Z. officinale*/kg feed	2.85 ± 0.06^{bA}	4.26 ± 0.16^{bB}

Mean ± standard deviation followed by different superscript letters (a, b, c, d, e) in the same column in each treatment or prevention trial showed a significant difference at $P< 0.05$. Capital letters (A, B) in the same raw indicated significant difference at $P< 0.05$ before and after infection of the Nile tilapia with *A. hydrophila*

DISCUSSION

Medicinal plants have been used for diseases resistance in fish due to the active phytochemicals such as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids or essential oils **(Reverter *et al.*, 2014)**. In the present study, the effect of *Z. officinale* powder on the haematological parameters of the Nile tilapia and its resistance to *A. hydrophila* infection was determined. It was observed that all concentrations of the ginger-supplemented diet exhibited significantly higher values of RBC, compared to fish fed the control diet. The study of **Ferri-Lagneau *et al*. (2012)** demonstrated that ginger extract stimulated haematopoiesis in fish, which could explain the present result. Ginger also contains antioxidant that protects the RBC against haemolysis by free radicals **(Mohammed *et al.*, 2020)**. The result also showed that the RBC of the Nile tilapia infected with *A. hydrophila* in the control diet and all ginger-supplemented diets decreased significantly ($P< 0.05$). Similar to the present study, **Talpur *et al*. (2013)** and **Hardi *et al*., (2016)** reported that the RBC was decreased in tilapia infected with *A. hydrophila*. Decreased RBC counts indicate that erythrocytes are being affected or destroyed by the infection. The changes in fish haematology are expected for fish infected with diseases **(Hrube & Smith, 2010)**.

Haematocrit value (or packed cell volume) is the simplest measure of erythrocyte content in blood as a percentage of erythrocytes in blood volume. In the current study, the percentage of haematocrit was 36.71 ± 0.22 % (0.00 g/kg), 37.68 ± 0.16 % (3 g/kg), 40.70 ± 0.2 % (5 g/kg), 38.14 ± 0.08 % (8 g/kg) and 36.15 ± 0.08% (12 g/kg) before infection of the Nile tilapia with *A. hydrophila*, and 20.52 ± 0.53% (0.00 g/kg), 21.77 ± 0.77 % (3 g/kg), 37.59 ± 0.21% (5 g/kg), 31.57 ± 0.06 % (8 g/kg) and 20.96 ± 0.56 % (12

g/kg) after infection, respectively. This indicates that the percentage of haematocrit value significantly declined after the Nile tilapia was infected with *A. hydrophila* ($P< 0.05$). This shows that haematocrit value was affected by bacterial infection and developed an anaemic state. In this context, **Brum *et al.* (2017)** described the anaemia of the Nile tilapia fed diets supplemented with 10 g/kg of ginger essential oil.

Haemoglobin content is directly related to the oxygen-binding capacity of the blood. Therefore, it is crucial for the survival of fish. The results of the current study indicate that haemoglobin contents increase significantly in ginger-treated Nile tilapia, compared to the control diet; these findings coincide with the results of **Haghighi and Rohani (2013)**, **Chelladuria *et al.* (2014)** and **Kanani *et al.* (2014)**. Ginger showed a significant difference in haemoglobin before and after infection of the fish ($P< 0.05$; Table 2). There was a significant decrease in haemoglobin after infection of fish with *A. hydrophila*. This may be due to an increased rate of breakdown of RBC by pathogenic bacteria and/or a reduction in the rate of formation of RBC **(Ayotunde *et al.*, 2011)**. According to **Lie *et al.* (1989)**, the haemoglobin content decreases due to RBC swelling and poor haemoglobin mobilization of the spleen and other haematopoiesis organs. In the present study, the significant reduction of haemoglobin after infection of fish could be the result of severe anaemia. The anaemic response could be a result of destruction of intestinal cells involved in the production of vitamin B12 used in the production of the haemoglobin portion of the red cells **(Gardner & Yevich, 1970)**, haemodilution **(Sampath *et al.*, 1993)** or the disruption in erythrocyte production **(Omoregie, 1995)**, . The present result disagrees with the result of **Brum *et al.* (2017)** who reported no significant effect of ginger oil on the haemoglobin of the Nile tilapia.

The erythrocyte sedimentation rate (ESR) is a common haematology test that may indicate and monitor an increase in inflammatory activity within the body caused by one or more conditions such as autoimmune disease, infections, or tumours. The current study showed that the ESR of *O. niloticus* was significantly increased after the fish were infected with *A. hydrophila*. **Blaxhall and Daisley (1973)** stated the values are usually raised with increased tissue destruction as in acute infection and heavy metal poisoning among others. In the current study, the maximum value of MCV was recorded with 12 g/kg ginger supplemented diet. The results also showed that the application of powdered ginger with varying concentrations significantly increased MCV and MCH ($P< 0.05$), while significantly decreasing the value of MCHC after infection of the Nile tilapia with *A. hydrophila*, except 5 g/kg and 12 g/kg ginger- supplemented diet. Similar to this study, **Haniffa and Mydeen (2010)** demonstrated that, catfish (*Silurus asotus*) exhibited a decrease in MCHC during *A. hydrophila* infection. But contrarily to the present study, **Stanley *et al.* (2017)** reported no significant difference ($P> 0.05$) for MCV, MCH and MCHC at varying concentrations of powdered ginger. Significant increases or decreases in red cell indices may indicate macrocytic or microcytic anaemia. In the current study, microcytic anaemia was observed.

WBC plays a crucial role in the protection of diseases caused by pathogenic organisms **(Harikrishnan & Balasundaram, 2005)**. WBC counts in the Nile tilapia fed different concentrations of ginger powder were significantly higher, compared to the control group ($P< 0.05$). Similar to the present study, **Haghighi and Mostafa (2013)** reported that, a fish fed ginger-supplemented diet showed a significant immune-stimulatory effect and increased RBC, haematocrit value and WBC values when

compared to the control ($P< 0.05$). An increase in WBC was believed to be caused by the migration of white blood cells from the spleen to the blood circulation **(Puisford *et al.*, 1994)**. According to **Sutili *et al.* (2018)**, feeding fish with ginger-supplemented diets produces immunomodulatory effects. The current results revealed that WBC increased significantly in the Nile tilapia after infection with *A. hydrophila* ($P< 0.05$). This result agrees with those of a previous study, which recorded that the WBC increased to tackle the infection in tilapia infected with *A. hydrophila* **(Hardi *et al.*, 2016)**.

Three types of WBC; namely, neutrophils, lymphocytes and monocytes were identified in the circulating blood of the Nile tilapia. The value of neutrophils was significantly increased in the ginger-supplemented diet, compared to the control diet ($P< 0.05$). The maximum value of neutrophils 7.06 ± 0.23 10^3/µl was recorded at the concentration of 5g of *Z. official*/kg diet after infecting the Nile tilapia with *A. hydrophila*. These results are in line with the results of a previous study, which found that fish treated with immunostimulants usually show enhanced phagocytic cell activities **(Sakai, 1999)**. The results are supported by **Talpur *et al.* (2013)** who reported a beneficial effect of ginger, improving the immunity system of fish. The lymphocyte was the most common leukocyte observed in the current study. The number of lymphocytes in fish injected with *A. hydrophila* was significantly lower than those which were not infected ($P< 0.05$). Decreases in lymphocytes after infection of the Nile tilapia with *A. hydrophila* were associated with re-trafficking of cells to lymphoid tissues, which consequently leads to clearance of these cells from the bloodstream **(Harris & Bird, 2000)**. Blood monocytes contribute to tissue-resident macrophage populations during inflammatory conditions and the depletion of resident macrophages in their environment **(Hashimoto *et al.*, 2013)**. In the current study, the number of monocytes was significantly increased in the Nile tilapia fed ginger- supplemented diet after infection with *A. hydrophila* ($P<0.05$); this may be due to intensification of the cell defence mechanism **(Tavares-Dias & Faustino, 1998)**. In addition, it was observed that the monocyte value of the Nile tilapia fed 5g/ kg of ginger was higher than samples fed 8 and 12g/ kg. According to **Citarasu (2010)**, the choice of herbs, their dose and time of application are very important for obtaining higher efficiency.

There was no mortality of the Nile tilapia before infection with *A. hydrophila*. From this result, it can be suggested that ginger caused no harmful effect on fish, at least in our described experimental condition. Hence, it can be considered safe for use in fish feed. However, during 15 days of the bacterial challenge, mortality was observed. Fish mortality was recorded as early as 36hrs of an experiment for control fed. No mortality was recorded until the 3rd day of the experimental period for a fish fed diet containing a different concentration of ginger. In all treatments, mortality began to occur on day three post-challenged and continued until day twelve. The highest mortality rate (70%) was recorded for the control group, followed by those fed 3g/ kg (66.66%) and 12g/ kg (55%) ginger-supplemented diet, respectively. The lowest mortality rate (18.33%) was recorded for 5 g/kg ginger-suplimented diet. In line with this study, **Payung *et al.* (2017)** reported the highest mortality in the control diet, followed by a 3g/ kg ginger-supplemented diet, and the lowest mortality in the 5g/ kg ginger-supplemented diet. The increasing survival rate is related to the increased immune function of the fish. Increased fish immunity will result in increased fish resistance to pathogens. The addition of ginger (*Z. officinale*) extract in fish feed increases the resistance of fish to pathogens because ginger contains

ingredients that can improve the immune system of the fish **(Payung *et al.*, 2017)**. According to **Nugroho *et al.* (2017)**, traditional herbs improve the immune response of fish by increasing granulocytes, macrophages, monocytes and neutrophils. In this respect, **Maqsood *et al*. (2011)** reported that immunostimulants enhanced the general defence system and decreased the mortality against pathogens and increased the viability rate. The results revealed that the Nile tilapia treated with 5, 8 and 12g/ kg of ginger showed a decrease in mortality rates, compared to the control diet after *A. hydrophila* infection. However, as the concentration of *Z. officinale* in fish feed increases, survival appeared to decrease. This indicates that excessive doses will have an immunosuppression effect that suppresses the immune system of the fish (Sakai 1999).

In the present study, before the bacterial challenge, the Nile tilapia presented health characteristics within the normal parameters for the species. However, after two days of infection, the fish showed different clinical symptoms such as erratic swimming behaviour, redness of the skin, darkness on their dorsal body part, fluid accumulation in the scale of the pockets, swelling of tissues, ulcers, haemorrhage, necrosis and exophthalmia. Similar to the present result, **Noor El–Deen *et al*. (2013)** postulated that, bacteria caused acute mortality among infected fishes in which the most visible clinical signs included exophthalmia. Moreover, the present results agree with the findings of **Kaleeswaran *et al*. (2012)**, **Noor El-Deen *et al*. (2014)** and **Workagegn *et al. (*2021)**.

CONCLUSION

The supplementation of ginger (*Z. officiale*) powder in the fish diet showed a significant additive benefit on the immune status of *O. niloticus*, compared to the control diet. Supplementation of ginger with the concentration of 5g/ kg diet provides better protection to *O. niloticus* against *A. hydrophila* infection. Hence, the use of 5g/ kg of dietary ginger powder in juvenile *O. niloticus* diet is recommended.

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Declaration of interest

The authors report no competing interests to declare.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

REFERENCES

Apines-Amar, M.J.S.; Amar, E.C.; Faisan, J.P.Jr.; Pakingking, R.V.Jr., and Satoh S. (2012). Dietary onion and ginger enhance growth, hemato-immunological

responses, and disease resistance in brownmarbled grouper, Epinephelus fuscoguttatus. AACL Bioflux, 5 (4): 231-239.

Awad, E. and Awaad, A. (2017). Role of medicinal plants on growth performance and immune status in fish. *Fish and Shellfish Immunology,* 67:40–54.

Ayotunde, E.O.; Offem, B.O. and Bekeh, A.F. (2011). Toxicity of Carica papaya lim: Haematological and piscicidal effect on Adult catfish (*Clarias gariepinus*). *Journal of Fisheries and Aquatic Science*, 6(3):291-308.

Beveridge, M. C. M.; Thilsted, S. H.; Phillips, M. J.; Metian, M.; Troell, M., and Hall, S. J. (2013). Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture. *Journal of Fish Biology*, *83*, 10671084.

Bichi, M.H,; Agunwamba, J.C.; Muyibi, S.A. and Abdulkarim M. I. (2012). Effect of Extraction Method on the Antimicrobial Activity of Moringa oleifera Seeds Extract. *Journal of American Science*, 8 (9): 450 – 458.

Bilen, S.; Ünal, S.; Güvensoy, H. (2016). Effects of oyster mushroom (*Pleurotus ostreatus*) and nettle (*Urtica dioica*) methanolic extracts on immune responses and resistance to *Aeromonas hydrophila* in rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 454: 90–94.

Blaxhall, P.C. and Daisley, K. W. (1973). Routine Haernatological Methods for use in Fish blood. *J Fish Biol*., 5:771-781.

Brum, A.; Pereira, S.A.; Owatari, M.S.; Chagas, E.C.; Chaves, F.C.M.; Mouriño, J.L.P. and Martins, M.L. (2017). Effect of dietary essential oils of clove basil and ginger on Nile tilapia (*Oreochromis niloticus*) following challenge with *Streptococcus agalactiae*. *Aquaculture*, 468: 235-243. doi: 10.1016/j.aquaculture.2016.10.020.

Bulfon, C,.; Volpatti, D. and Galeotti, M. (2015). Current research on the use of plant-derived products in farmed fish. *Aquaculture Research,* 46, 513–551.

Campbell, T. W. (2015). Exotic Animal Haematology and Cytology. John Wiley & Sons, Inc. 402pp.

Chelladuria, T.; Mohanraj, J. and Nagrajan, R. (2014). Effect of Herbal extracts supplemented diets on nonspecific immunity and resistance to *Aeromonase hydrophila* Indian catfish (*Mystus Monotanus*). *Journal of Zoological and Bioscience Research*, 1: 10-14.

Chowdhuryl, M.B.R. and Rahman, T. (2008). Efficacy of medicinal plants on microbial fish pathogens. *J. Bangladesh Agril. Univ.*, 6(1): 131-138. ISSN 1810-3030.

Citarasu, T. (2010). Herbal biomedicine: A new opportunity for aquaculture industry. *Aquaculture International*., 18 , 403 - 414.

Devi. K.N.; Dhayanithi, N.B. and Kumar, T.T. (2016). In vitro and in vivo efficacy of partially purified herbal extracts against bacterial fish pathogens. *Aquaculture*, 458: 121–133.

FAO, (2019). The State of Food Security and Nutrition in the World 2019. Safeguarding against economic slowdowns and downturns. Rome, FAO.

FAO, (2015). Tilapia production and the share of the leading producing countries GLOBEFISH- Analysis and information on world fish trade. FAO. [December 2016].

FAO, (2014). The state of world fisheries and aquaculture 2014. Rome: FAO.

Ferri-Lagneau, K.F.; Moshal, K.S.; Grimes, M.; Zahora, B.; Lv, L.; Sang, S. and Leung, T.C. (2012). Ginger stimulates hematopoiesis via bmp pathway in zebrafish. Plos One, 7: e39327. doi: 10.1371/journal. pone.0039327.

Gardner, G.R. and Yevich, P.P. (1970). Histological and haematological response of an estuarine teleost to cadmium. *J of Fisheries Res Board of Canada*, 27:2185-2196.

Goldenfarb, P.B.; Bowyer, F.P.; Hall, E. and Brosious, E. (1971). Reproducibility in the hematology laboratory: the microhematocrit determination. *Am J. clin. Pathol.* 56: 35–39.

Haghighi, M. and Rohani, M. S. (2013). The effects of powdered ginger (*Zingiber officinale*) on the hematological and immunological parameters of raibow trout (*Oncorhychus mykiss*). *Journal of Medicinal Plant and Herbal Theraby Research*, 1 (11), 8-12.

Haniffa, M.A.; Abdul, K. and Mydeen, K.P. (2010). Hematological Changes in *Channa striatus* Experimentally Infected by Aeromonashydrophila. Bioresearch Bulletin 4: 246-253.

Hardi, E.H.; Saptiani, G.; Kusuma, I.W.; Suwinarti, W. and Nugroho, R.A. (2017). Immunomodulatory and antibacterial effects of *Boesenbergia pandurata*, *Solanum ferox*, and *Zingiber zerumbet* on tilapia, *Oreochromis niloticus*. Aquaculture, Aquarium, *Conservation and Legislation-International Journal of the Bioflux Society* (AACL Bioflux), 10(2).

Hardi, E.H.; Kusuma, I.W.; Suwinarti W. (2016). Antibacterial activities of some Borneo plant extracts against pathogenic bacteria of *Aeromonas hydrophila* and *Pseudomonas* sp. Aquaculture, *Aquarium, Conservation and Legislation International Journal of the Bioflux Society* (AACL Bioflux). 9(3): 638–646.

Harikrishnan, R. and Balasundaram, C. (2005). Antimicrobial activity of medicinal herbs in vitro against fish pathogen, *Aeromonas hydrophila*. *Fish Pathol.*, 40(4): 187–189.

Harris, J. and Bird, D.J. (2000). Modulation of the fish immune system by hormones. *Vet Immunol. Immunopathol*, 77: 163-176.

Hashimoto, D.; Chow, A.; Noizat, C.; Teo, P.; Beasley, M. B.; Leboeuf, M., D.; Becker, C.; See , P.; Price, J.; Lucas, D.; Greter, M.; Mortha, A.; Boyer, S.; Forsberg, C.; Tanaka M.; Rooijen , N.; García-Sastre, A.; Stanley, E.; Ginhoux, F.; Frenette, P.; and Merad M. (2013). Tissue-Resident Macrophages Self-Maintain Locally throughout Adult Life with Minimal Contribution from Circulating Monocytes. *Immunity*,38(4):792–804.

Heuer, O.E.; Kruse, H.; Grave, K.; Collignon, P.; Karunasagar, I. and Angulo, F.J. (2009). Human health consequences of use of antimicrobial agents in aquaculture. Clin Infect Dis., 49(8):1248-1253. DOI: https://doi.org/10.1086/605667.

Hrubec, T.C. and Smith, S.A. (2010). Hematology of fishes. In: Weiss, D.J. and Wardrop, K.J. (Eds.). Schalm's veterinary hematology. Wiley-Blackwell, Ames, pp. 994-1003.

Kanani, H. G.; Nobahar, Z.; Kakoolaki, S. and Jafarian, H. (2014.) Effect of ginger and garlic–supplement diet on growth performance, some hematological parameters and immune responses in Juvenile *Huso*. *Fish Physiology and Biochemistry*, 40:481-940.

Kaleeswaran, B.; Ilavenil, S. and Ravikumar, S. (2012). Changes in biochemical, histological and specific immune parameters in *Catla* (Ham) by Cynodondactylon (L). *J King Saud Univ Sci.*, 24:139-152.

Laleh, Y.G.,; Mohammad, E.J.Z. and MiLad, A. (2015). The study on effect of temperature stress on occurrence of clinical signs caused by *Aeromonas hydrophila* in *Capoeta damascinain* in vitro condition. *J Adv Anim Vet Sci.*, 3:406-412.

Lee, G.R.; Bithell, T.C.; Foerster, J.; Athens, J.W. and Lukens, J.N. (1993). **Wintrobe's** Clinical Hematology. 9th rominently Edn. Vols 1 and 2. (Pp handbook 2323; £120.). ISBN r Accredit- 0-81211-1885.

Lie, Ø.; Evensen Ø.; SØrensen, A. (1989). Study on lysozyme activity in some fish species. *Dis Aquat Organ*., 6: 1–5.

Maqsood, S.; Singh, P.; Samoon, M.H. and Munir, K. (2011). Emerging role of immunostimulants in combating the disease outbreak in aquaculture. *International Aquatic Research*, 3: 147–163.

Mohammadi, G.; Rashidian, G.; Hoseinifar, S.H.; Naserabad, S.S. and Doan, H.V. (2020). Ginger (*Zingiber officinale*) extract affects growth performance, body composition, haematology, serum, and mucosal immune parameters in common carp (*Cyprinus carpio*). *Fish and Shellfish Immunology*, 99: 267-273. doi: 10.1016/j.fsi.2020.01.032.

Natt, M.P. Herrick, C.A. (1952). A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Poult Sci., 31(4):735–738. doi: 10.3382/ps.0310735.

Nile, S.H. and Park, S.W. (2015). Chromatographic analysis, antioxidant, anti-inflammatory, and xanthine oxidase inhibitory activities of ginger extracts and its reference compounds. *Ind. Crop. Prod*., 70:238–244. doi: 10.1016/j.indcrop.2015.03.033.

Noor El Deen, A.E.; Shalaby, S.I., Zaki, M.S. and Abd Elzaher, M.F. (2013). Some infectious and non infectious eye affection syndrome in fish. *Life Sci J*., 10:1362-1368.

Noor El-Deen, A.E.; Dorgham, S.M.; Hassan, A.H.M. and Hakim, AS. (2014.) Studies on *Aeromonas hydrophila* in cultured *Oreochromis niloticus* at Kafr El Sheikh Governorate, Egypt with reference to histopathological alterations in some vital organs. *World J Fish Mar Sci*,. 6:233- 240.

Nugroho, R.A.; Manurung, H.; Nur, F.M. (2017). *Terminalia catappa* L. extract improves survival, hematological profile and resistance to *Aeromonas hydrophila* in *Betta* sp. *Arch Pol Fisheries*, 25(2): 103–115.

Omoregie, E. (1995). Changes in the haematology of the Nile Tilapia *Orochronis niloticus* under the effect of crude oil. *Hydrobiol*., 40(4):287-292.

Payung, C. N.; Tumbol, R. A. and Manoppo, H. (2017). Dietary ginger (*Zingiber officinale*) enhance resistance of Nile tilapia (*Oreochromis niloticus*) against *Aeromonas hydrophila*. *AACL Bioflux,* 10(4):962-968.

Plumb, J.A. and Bowser, P.R. (1983). Microbial fish disease laboratory manual, Alabama: Auburn University, Alabama Agriculture Experiment Station, p. 95.

Puisford, A.L.; Lemaire-gong, S.; Tomlinson, M.; wood, N. and Glynn, P.J. (1994.) Effects of acute stress on the immune system of the Dab, Limanda. *Comp. Biochem Physi*, 109:129-139.

Ravikumar, S.; Raja , M. and Gnanadesigan , M. (2012.) Antibacterial potential of benzoate and phenylethanoid derivatives isolated from *Acanthus ilicifolius* L. leaf extrac. *Natural Product Research*, 26(23):2270-3. DOI:10.1080/14786419.2011.652962

Reverter, M.; Tapissier-Bontemps, N.; Sasal, P. and Saulnier, D. (2017). Use of medicinal plants in aquaculture. pp. 223–261 in Austin, B and Newaj-Fyzul, A (Eds) Diagnosis and control of diseases of fish and shellfish.

Reverter, M.; Bontemps, N.; Lecchini, D.; Banaigs, B. and Sasal. P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current statue and future perspectives. *Aquaculture,* 433:50-61.

Sakai, M. (1999). Current research status of fish immunostimulants. *Aquaculture*, 172(1–2): 63–92.

Schaperclaus, W.; Kulow, H. and Schreckenbach, K. (1992): Fish Diseases, Vol. I. A.A.Balkema / Rotterdam.

Skariyachan, S.; Prasanna, A.; Sirisha, P.; Karanth, S.S. and Nazre, A. (201). Exploring the Medicinal Potential of the Fruit Bodies of Oyster Mushroom, Pleurotus ostreatus (Agaricomycetes), against Multidrug Resistant Bacterial Isolates, *Int J MedMushrooms*,18(3):245-52. doi: 10.1615/IntJMedMushrooms.v18.i3.70.

Stanley, C.; Iheanacho, Johnny, O.; Emmanuel, O.; Lucy, A.; Ifebundu, O.; Stephen, N.; Christian, E.; Ibrahim, B. and Musa, H. (2017). Comparative assessment of ampicillin antibiotic and ginger (*Zingiber officinale*) effects on growth, haematology and biochemical enzymes of *Clarias gariepinus* juvenile. *Journal of Pharmacognosy and Phytochemistry*, 6(3): 761-767.

Stoner, G.D. (2013). Ginger. Is it ready for prime time? *Cancer Prev. Res*. 6:257–262. doi: 10.1158/1940-6207.CAPR-13-0055.

Sutili, F.J.; Gatlin, D.M.; Heinzmann, B.M. and Baldisserotto, B. (2018). Plant essential oils as fish diet additives: benefits on fish health and stability in feed. *Reviews in Aquaculture*, 10: 716-726. doi: 10.1111/ raq.12197.

Talpur, A. D.; Ikahwanuddin, M. H. D. and Bolong, A. M. A. (2013). Nutritional effect of ginger (*Zingiber officinale*) on immune response of Asian sea bass, *Lates calcarifer* (Bloch) and disease resistance against *Vibrio harveyi*. *Aquaculture,* 400: 46-52.

Tavares-Dias, M. and Faustino, C.D. (1998). Parâmetros hematológicos da tilápia-do-Nilo *Oreochromis niloticus* (Cichlidae) em cultivo extensivo. *Ars. Vet.*, 14 (3), 254-263.

Van Hai, N. (2015). The use of medicinal plants as immunostimulants in aquaculture: A review. *Aquaculture,* 446, 88–96.

Waite, R.; Beveridge, M.; Brummett, R.; Castine, S.; Chaiyawannakarn, N.; Kaushik, S.; Mungkung, R.; Nawapakpilai, S. and Phillips M. (2014). "Improving Productivity and Environmental Performance of Aquaculture." Working Paper, Installment 5 of Creating a Sustainable Food Future. Washington, DC: World Resources Institute.

Workagegn, K.B.; Lema, B.; Natarajan, P. and Prabadevil, L. (2021). *Aeromonas* Spp. Infection in Farmed Nile Tilapia, *Oreochromis Niloticus*. *J Aqua Res Dev.*, 10:465.

Yardimci, B. and Aydin, Y. (2011). Pathological findings of experimental *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*). *Ankara Üniv Vet Fak Derg*., 58:47-54.