Influence of dietary ginger (*Zingiber officinale*) on haemato-biochemical parameters, spleen histology and resistance of *Oreochromis niloticus* fingerlings to *Aeromonas hydrophila* infection.

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Key words: Ginger, haemato- biochemical parameters, spleen histology, Nile tilapia, *Aeromonas hydrophila*

ABSTRACT

The effects of dietary ginger on haematological parameters, antioxidant enzymes activites spleen histology and disease resistance of *Oreochromis niloticus* were investigated. Four test diets containing different levels of ginger (0, 1, 2 and 3%) (W/W) were fed for *O. niloticus* fingerlings weighted (7.94± 0.26g) for 4 weeks. A positive correlation was observed between the level of ginger and haemato-biochemical parameters measured. The results revealed that blood parameters [(total leukocytic count (TLC), total erythrocytic counts, packed cell volume (PCV), haemoglobin concentration (HB) and differential leukocytic count (DLC)] and serum (total proteins, albumin and globulin) were significantly higher ($p< 0.05$) in ginger fed groups. Antioxidant enzymes including [Glutathione Peroxidase enzyme (GSH-px), Superoxide Dismutase (SOD)] showed significant increase ($p< 0.05$) in ginger treated groups in relation with control. While, Malondialdehyde (MDA) and liver function enzymes [Glutamic – oxaloacetic transaminase GOT (AST) and Glutamic – pyruvic transaminase GPT (ALT**)**] were significantly lower than the control groups. Spleen histological structure showed time and concentration dependant increase in melanomacrophaphage centers and hemosiderin pigments that reach the highest aggregations at 3rd week post feeding. Challenge infection by *A. hydrophila* recorded highly significant protection reaching (90.2%) in

1% ginger treated groups for 4 weeks. The results suggest that ginger can be recommended as a supplement to *O. niloticus* feed to enhance resistance against *A. hydrophila* pathogen.

Introduction

With the rapid expansion of aquaculture which considered as one of major food production industry and associated fish intensification; as a method for fish rearing which bears stress on fish, rendering it more susceptible to infectious diseases (**Conte, 2004**). Many food supplements were used to counteract the adverse effects associated with culture conditions; probiotics, immunostimulants or plant products (**Newaj-Fyzul** and **Austin, 2015**). Natural plant products have been reported to promote various activities like; anti-stress, growth promoting, appetite stimulation, tonic and immunostimulant and to have aphrodisiac and antimicrobial properties in fin fish (**Immanuel *et al.*, 2009, Apines-Amar et al., 2012, Kanani et al., 2014** and **El-Sayed et al. 2014**) and shellfish culture (**El -Desouky et al., 2012**) due to the active principles such as alkaloids, flavonoids, pigments, phnolics, terpenoids, steroids, and essential oils (**Citarasu et al., 2002** and **Sivaram et al., 2004**). Ginger is considered to be a safe herbal medicine with only a few insignificant side effects. Ginger (*Zingiber officinale*), has been widely used in traditional Chinese, Indian and Japanese medicine for over 25 centuries **(Castleman, 2001**). The main constituents of ginger include zingerone, paradol, and shogaols **(Murray, 1995)** which proved to have antibacterial effect against; *Aeromonas hydrophila* infection **(Nya and Austin 2009; Immanuel et al., 2009**), *Vibrio harveyi* (**Talpur et al., 2013**) and its anti-parasitic effect against *Gyrodactylus turnbulli* in guppy (**Levy et al., 2015**). The purpose of the study was to evaluate the effect of ginger on the haemato-biochemical parameters, spleen histology and resistance of *Oreochromis niloticus* to *Aeromonas hydrophila* infection.

Materials and Methods

Fish:-

Nile tilapia, *Oreochromis niloticus* (*O. niloticus*) fingerlings with an average weight 7.49± 0.26g and average length 7±0.2 cm obtained from a private fish farm (Kafr El Sheikh Governorate, Egypt) were transported to the wetlab at department of Fish diseases and Management, faculty of Veterinary Medicine, Benha University, Egypt. Fish were placed in well

prepared fiberglass (750L) tanks filled with de-chlorinated water. The water temperature was adjusted to 25±2°C and the oxygen level was maintained at optimal level using aerators. Fish were fed twice daily with 5% body weight basal diet (30% protein). Uneaten food and excreta were siphoned and water exchange of about its third volume was done daily. Health conditions of fish were examined for any disease condition (parasitic, bacterial) as described by **Austin and Austin (1989)**.

Preparation of experimental diet:-

Ginger roots were washed, peeled, sliced and shade– dried at room temperature. Then oven–dried at 60°C , powdered by mortar and pestle and sieved using a fine wire mesh house hold sifter following **Nya and Austin (2009)**. The basal diet was divided into four portions; the first three portions were incorporated with ginger at rate of 1, 2 and 3% respectively and the remaining portion used as control (0% ginger). Suitable amount of water was added to form moist dough then pelleted, allowed to dry at room temperature then packed in clean dry tightly closed plastic containers and kept at 4°C till use.

Feeding experiment and challenge test:-

O. niloticus fingerlings were acclimated to lab conditions for two weeks. Fish were divided into four groups; one control and three treated groups in three replicates. The control group fed on basal diet and the three treated groups were fed with the diet supplied with ginger at rates of 1, 2 and 3% respectively for a period of four weeks at a rate 5% of body weight.

Determination of the haematological parameters:-

At the end of 1, 2, 3 and 4 weeks from start feeding; blood samples were taken from control and treated groups. Blood was withdrawn from the caudal blood vessels in two portions; one with anticoagulant for measuring blood parameters and the second portion without anticoagulant for separation of serum by allowing the blood to clot at room temperature in a slanting position. The tubes were then centrifuged at 3000×g (–4°C) for 15 min, the serum was collected and stored at (–20°C). Blood elements (RBC_s and WBC_s) were counted according to **Kanaev (1985)** using Neubauer-improved haemocytometer (Neubauer, improved, Germany). Hemoglobin concentration was determined using the cyanomethmoglobin method according to **Stoskopf (1993).** The Packed cell volume (PCV %) was estimated after the method described by **Dacie and Lewis** (**1991).** Differential leukocytic count (DLC) was

carried out according to **Stoskopf** (**1993)** using Giemsa stain (SAS, Mumbai)**.**

Determination of biochemical parameters and antioxidant enzymes activity:-

Serum albumin and total protein were determined according to **Doumas et al., (1981)** using commercial kits (Biodiagnostic Company, Egypt). While serum globulin, was calculated by subtraction of albumin values from total protein. Liver function enzymes; Glutamic – oxaloacetic transaminase GOT (AST) and Glutamic – pyruvic transaminase GPT (ALT) were determined from serum using commercial kits **(**Bio-diagnostic, Egypt).

For measuring Glutathione Peroxidase enzyme (GSH-px), Superoxide Dismutase (SOD) and Malondialdehyde (MDA); weighed liver tissues were homogenized in cool phosphate buffer saline, centrifuged at 4°C at 4000 × g for 15 min and the supernatants were kept at –20°C for determination of (SOD, GSH-px and MDA) using commercial kits **(**Bio-diagnostic, Egypt).

Spleen histological assay:

Spleen specimens were taken from ginger fed fish (1, 2, and 3%) and controls at the end of 1, 2, 3 and 4 week from start feeding, fixed and preserved in formalin buffer saline. The preserved specimens of spleen were subjected to standard dehydration procedure and were embedded in paraffin wax. Histological sections were obtained with the thickness of (5 µm) using a rotatory microtome and stained with haematoxylin and eosin. Tissue structures of the were examined under light microscope **(Banchroft et al., 1996).**

Challenge infection:

Verified *Aermonas hydrophila* (*A. hydrophila*) pathogenic strain (kindly obtained from Central Lab of Aquaculture Research (CLAR), fish disease lab, Abbassa, Egypt) was incubated in Tryptic Soya broth (at 28°C for 24 h) then centrifugation (2500 rpm/ 10 min. at -4°C). The bacterial pellets were re-suspended into sterile slain (0.85% Nacl) with concentration of (1X 10^7 Cells / ml) using hemocytometer slides (Neubar, improved Germany). At the day of challenge (7^{th}, 14^{th} and 28^{th}) no food was supplied to the fish. Ten fish (in three replicates) from each treatment and control were intra-peritoneal (IP) injected with 0.1 ml of bacterial

suspension. Challenged fish were monitored for mortality for 15 subsequent days. All fish of each group were subjected to clinical and post mortem examination. Relative level of Protection (RLP) was calculated according to **Newman and Majinarich (1982**) as the following formula:-

$$RLP = 1- \frac{\text{Percent of Treated Mortality}}{\text{Percent of Control Mortality}} X100$$

Statistical Analysis:-

The data were analyzed by One-way analysis of Variance (ANOVA) and Duncans multiple range tests to determine significant difference between groups using the Statistical package for the Social Sciences (SPSS) software Version 16.00. A value of $p < 0.05$ was considered significant.

Results

The effect of tested ginger incorporated diets on haematological parameters of *O. niloticus*:-

All ginger treated groups with 1%, 2% and 3% showed significant ($p<0.05$) increase in WBCs number (Table, 1). While, RBCs number started to increase significantly from the 2nd week feeding at all treated groups compared with the control (Table, 1). PCV values were significantly increased ($p<0.05$) in all ginger fed groups at the 3rd and 4th week from start feeding (Table, 1). On the same respect, hemoglobin concentrations showed significant increase in its values in all treated groups at the 1st, 2nd , 3rd and 4th week from feeding in relation to the control groups, except 3% fed ginger at the 3rd week (Table, 1). Concerning differential leukocytic count, 1% fed group showed significant increase in lymphocyte number the first week from start feeding followed by those received 3% and 2% ginger compared with control. But at the third week; 3% *Z. officinale* fed group were the highest among all treated groups and control in lymphocyte number (Table, 2). Significant increase in neutrophils was recorded in fish fed 1% ginger

Table (1): RBCs and WBCs counts, PCV % and HB concentrations of O. *niloticus* fed with *ginger (Z. officinale*) at concentration of 0, 1, 2 and 3% for 4 weeks

(Ginger %)	RBC,s ($x10^6$)	WBC,s ($x10^3$)	PCV (%)	HB (g/dl)
		Week1		
0	1.86 ± 0.14^c	73.90 ± 0.10^c	36.00 ± 2.00^c	10.90 ± 0.35^c
1	1.90 ± 0.20^b	$81.60\pm.01^b$	41.33 ± 4.04^a	16.06 ± 0.20^b
2	1.91 ± 0.06^b	81.80 ± 0.10^b	35.66 ± 2.50^c	16.90 ± 1.50^b
3	1.92 ± 0.01^a	82.10 ± 0.12^a	40.83 ± 1.75^b	17.66 ± 0.70^a
		Week2		
0	2.20 ± 0.10^c	74.0 ± 0.10^c	37.33 ± 1.25^c	10.96 ± 1.52^c
1	$2.40\pm.0010^b$	82.4 ± 0.10^b	45.66 ± 4.70^a	14.03 ± 1.40^b
2	2.52 ± 0.02^b	83.6 ± 0.30^b	44.00 ± 2.00^b	15.3 ± 0.60^b
3	2.54 ± 0.01^a	88.1 ± 0.10^a	37.53 ± 1.28^c	15.21 ± 0.72^a
		Week3		
0	2.3 ± 0.1^c	74 ± 0.1^c	38.46 ± 2.50^c	12.19 ± 0.41^c
1	2.43 ± 0.18^b	83.5 ± 0.1^b	45.73 ± 1.77^a	17.5 ± 0.60^a
2	2.53 ± 0.03^b	85.5 ± 0.1^b	44.39 ± 1.52^b	13.95 ± 0.67^b
3	2.56 ± 0.01^a	88.1 ± 0.1^a	43.36 ± 1.87^b	11.86 ± 1.30^c
		Week4		
0	2.4 ± 0.1^c	74.2 ± 0.1^c	40.66 ± 3.05^c	13.18 ± 1.20^c
1	2.55 ± 0.15^b	83.5 ± 0.1^b	46.23 ± 11.10^b	18.13 ± 0.20^b
2	2.57 ± 0.01^b	85.6 ± 6^b	47.00 ± 2.00^b	19.41 ± 0.62^a
3	2.66 ± 0.01^a	88.4 ± 0.1^a	49.43 ± 0.94^a	18.5 ± 1.41^b

containing diet at the first week from start feeding, with no obvious increase at the 2nd, 3rd and 4th weeks (Table, 2). Monocytes were observed in a significant increase with fish fed 1, 2 and 3% ginger at the 1st and 2nd weeks but at the third and fourth week from start feeding there is no significance difference in monocytes number of all *Z. officinale* treated groups (1% , 2% and 3%) and control group (Table, 2).

The effect of tested ginger incorporated diets on biochemical parameters and antioxidant Enzymes activity of *O. niloticus*:-

The serum total protein was most significantly increased in 3% ginger treated groups at 4th week from start feeding (Table, 3). Moreover,

Table (2): The effect of dietary administration of *ginger (Z. officinale)* on differential leukocytic count of *O. niloticus*.

Ginger %	Lymphocyte	Neutrophils	Monocytes	Basophils	Esinophil
Week1					
0	39.30±0.88^{c}	28.00±1.52^{c}	21.00±1.20^{b}	1.00	0
1	46.66±3.71^{a}	33.00±1.15^{a}	23.00±0.81^{b}	1.00	0
2	43.33±0.86^{ab}	31.65±1.45^{ab}	24.00±1.21^{b}	1.00	0
3	45.23±0.85^{ab}	31.00±0.88^{ab}	25.00±0.65^{a}	1.00	0
Week2					
0	40.00±1.76^{b}	30.00±1.15^{a}	21.03±1.20^{c}	1.00	0
1	42.00±1.53^{b}	32.33±0.81^{a}	23.00±1.15^{bc}	1.00	0
2	49.00±2.02a	30.32±1.45^{a}	27.00±1.52^{ab}	2.00	0
3	44.06±1.45a^{b}	31.33±1.86^{a}	30.32±2.02^{a}	1.00	0
Week3					
0	42.00±1.45^{b}	24.30±1.76^{a}	25.32±1.20^{a}	1.00	0
1	41.67±1.45^{b}	26.00±1.70^{a}	23.23±1.24^{a}	1.00	0
2	47.00±1.73^{ab}	26.00±1.45^{a}	27.00±1.55^{a}	2.00	0
3	52.00±2.33^{a}	27.00±1.40^{a}	23.67±1.76^{a}	1.00	0
Week4					
0	45.32±1.76^{a}	23.30±1.42^{a}	26.00±1.23^{a}	1.00	0
1	44..67±1.20^{a}	27.00±1.15^{a}	29.43±2.11^{a}	2.00	0
2	46.00±2.64^{a}	28.00±1.20^{a}	26.12±0.76^{a}	1.00	0
3	44.66±2.02^{a}	25.30±2.33^{a}	26.00±1.02^{a}	1.00	0

a significant increase in serum albumin values were observed in group fed with 3% Z. *officinale* at 1st, 3rd and 4th weeks from start feeding (Table, 3). Also, the results revealed that at 3rd week from start feeding the maximum level of serum globulin found in group fed with 3% *Z. officinale* (Table, 3).Concerning liver function enzymes, group received treatment of 3% Z. *officinale* at all feeding periods showed drastic

significant decrease in AST values compared to control (Table, 4). At the same respect, significant decrease in serum ALT was recorded in the group received 2% *Z. officinale* at 1st, 2nd weeks from starting compared

Table (3): Total protein, albumin and globulin of O. *niloticus* fed with *ginger (Z. officinale*) at concentration of 0, 1, 2 and 3% for 4 weeks.

Ginger (%)	Feeding period (weeks)			
	1	2	3	4
Total protein (g/dl)				
0	3.27±.15.00^{c}	3.51±0.35^{c}	3.66±0.28^{c}	3.82±0.28^{c}
1	3.47±.290^{b}	4.23±0.53^{c}	3.79±0.12^{b}	4.92±0.07^{b}
2	3.84±.17^{b}	3.55±0.29^{b}	4.2±0.60 ^{b}	5.22±0.22^{b}
3	3.96±.06^{a}	3.91±0.07^{a}	4.85±0.05^{a}	5.92±0.03^{a}
Albumin (g/dl)				
0	2.03±0.57^{c}	2.08±0.40^{c}	2.07±0.38^{c}	2.12±0.33^{c}
1	2.13±0.07^{b}	2.9±0.10a	2.23±0.28^{b}	2.46±0.15^{b}
2	2.32±0.28^{b}	2.26±0.32^{b}	2.38±0.14^{b}	2.68±0.32^{b}
3	2.38±0.16^{a}	2.44±0.42^{b}	2.67±0.19^{a}	2.83±0.28^{a}
Globulin (g/dl)				
0	1.23±0.42^{c}	1.42±0.47^{b}	1.59±0.10^{c}	1.95±0.87^{c}
1	1.34±0.26^{b}	2.13±0.61^{a}	1.56±0.35^{c}	2.45±0.08^{b}
2	1.52±0.41^{b}	1.29±0.60^{c}	1.82±0.63^{b}	2.53±0.46^{b}
3	1.57±0.09^{a}	1.47±0.48^{b}	2.17±0.20^{a}	3.09±0.30^{a}

Table (4): Liver and antioxidant enzymes of O. *niloticus* fed with *ginger (Z. officinale*) at concentration of 0, 1, 2 and 3% for 4 weeks.

(Ginger %)	AST (g/dl)	ALT (g/dl)	GSH (mg/tissue)	SOD (mg/tissue)	MDA (mg/tissue)
			Week1		
0	349.26±5.46^{a}	52.96±4.22^{a}	1.88±0.8^{c}	623.33±32.14^{c}	200.00±20.00^{a}
1	241.6±5.04^{b}	42.72±2.94^{b}	16.65±0.65^{b}	2089.32±101.69^{b}	152.67±28.5^{b}
2	238.66±12.66^{b}	37.65±2.64^{c}	19.44±5.4^{b}	2299.45±408.71^{b}	128.00±4.04^{c}
3	167.36±13.37^{c}	48.15±5.36^{b}	37.28±2.24^{a}	2471.03±272.7^{a}	150.00±26.00^{b}
			Week2		
0	360.66±0.57^{a}	48.17±3.62^{a}	1.90±0.1^{c}	630.00±20^{c}	205.00±5.00^{a}
1	300.00±0.57^{b}	46.56±4.69^{b}	21.51±3.99^{b}	3010.76±392.71^{a}	125.00±4.04^{c}
2	250.00±10^{b}	42.89±3.64^{c}	23.36±3.61^{b}	2415.86±319.11^{b}	138.33±4.72^{c}
3	230.00±10^{c}	46.13±2.16^{b}	43.09±2.89^{a}	2656.95±515.8^{b}	136.33±7.09^{c}
			Week3		
0	366.00±11.46^{a}	49.38±3.07^{a}	2.00±0.2^{c}	626.66±25.16^{c}	211.00±11.53^{a}
1	263.66±12.05^{b}	42.69±2.03^{b}	31.67±1.62^{b}	3155.57±435.16^{b}	153.33±26.27^{b}
2	292.66±6.65^{b}	41.33±2.51^{b}	40.67±3.74^{b}	3185.04±325.16^{b}	163.00±6.02^{b}
3	260.66±17.61^{c}	39.40±2.11^{c}	55.20±4.48^{a}	3443.60±302.7^{a}	123.33±2.88^{c}
			Week4		
0	375.00±5.00 ^{a}	53.09±3.56^{a}	2.37±00.4^{c}	637.3±30.29^{c}	205.66±50.13^{a}
1	270.33±15.82^{c}	46.05±0.96^{b}	40.00±50^{b}	3454.44±77.05^{b}	119.00±12.58^{b}
2	202.63±10.27^{c}	46.65±3.73^{b}	52.00±3.60^{b}	3590.37±499.59^{b}	100.67±9.01^{c}
3	294.53±11.49^{b}	42.56±4.06^{c}	64.00±3.60^{a}	3843.46±167.45^{a}	112.00±3.60^{b}

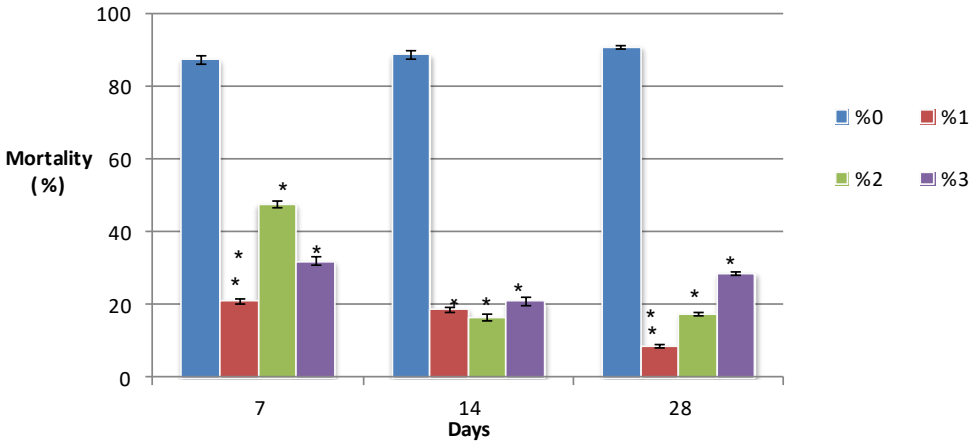

Figure 1: Mortality (%) of *O. niloticus* fed ginger incorporated diet at concentration of (0, 1, 2 and 3%) for 7, 14 and 28 days and challenged with *A. hydrophila.* Data are average of three replicate (10 fish each) ±SD. Columns with stars means significantly different ($p<0.05$) from the control group.

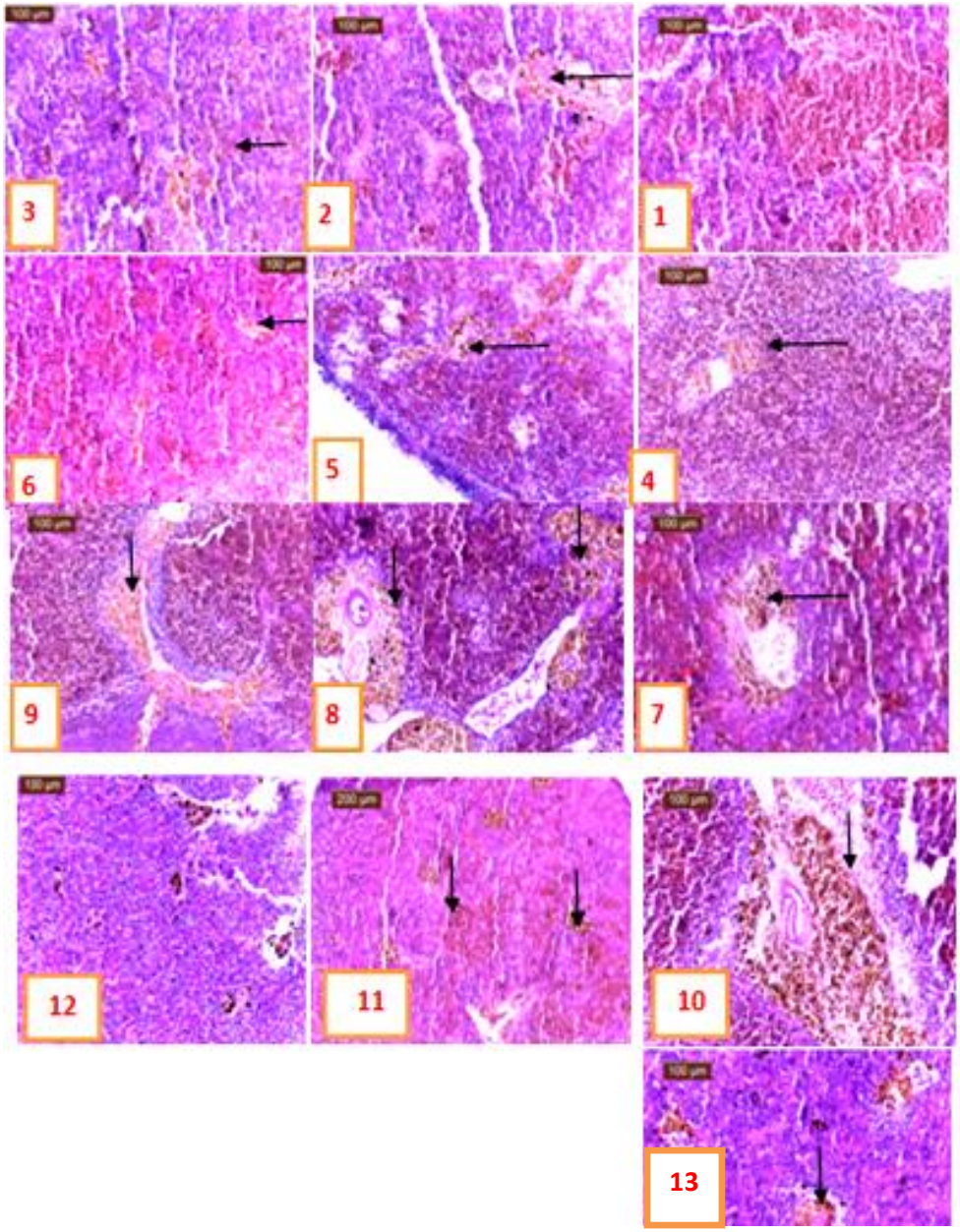

Plate 1. Spleen histology of *O. niloticus* fingerlings fed ginger. At 1st week concentration 0 (1) showing normal spleen histological structure, 1% (2), 2% (3) and 3% (4) showed increase MMCs and hemosiderin pigment began to appear (arrow). But, at 2nd week, 1% (5), 2% (6) and 3% (7) showing ascending increase in MMCs and hemosiderin pigments (arrow). While, at 3rd week, 1% (8), 2% (9), 3% (10) huge amount of MMCs are collected mainly around blood vessels (arrow). And at 4th week, 1% (11), 2% (12), 3% (13) showing reduction in the amounts of MMCs and hemosiderin pigments around blood vessels (arrow).

to control, while 3% ginger incorporation significantly reduced ALT at the 3rd and 4th weeks (Table, 4).

GSH-px enzyme level of *O. niloticus* fed ginger were significantly increased at all groups, with the most significant increase ($P<0.05$) was observed in 3% *Z. officinale* at 4th week from start feeding (Table, 4). Moreover, the level of SOD enzyme of *O. niloticus* fed with 1%, 2% and 3% *Z. officinale* showed significant increase at 1st, 2nd, 3rd and 4th weeks from start feeding than control groups (Table, 4).With respect to concentration, the most significant lower results of MDA enzyme were observed in fish fed 2% *Z. officinale* at 4th week from start feeding followed by 3% at the same feeding period (Table, 4).

Effect of ginger on spleen histological structure

Spleens of control tilapia showed normal histological features as they were covered with connective tissue capsule and their parenchyma were consisted of red and white pulps with presences of melanomacrophage centers (MMCs) (plate, 1). Spleens of ginger fed groups showed ascending weekly increase in amount of MMCs and hemosiderin pigments begun to appear (plate, 1), with huge amount of MMCs and hemosiderin pigments were collected mainly around blood vessels at 3rd week (plate, 1). The fourth week showed slight reduction in the amounts of hemosiderin pigments and MMCs (plate, 1)

Effect of ginger on resistance of *O. niloticus* to artificial infection by *Aeromonas hydrophila*:-

Results revealed that addition of ginger to *O. niloticus* diet enhance the body health and their resistance to infection with *A. hydrophila*. Regarding concentration, the mortality rates of 1% ginger-fed fish was reduced to (20-10%) with relative level of protection (RLP) (75-92.2%) compared to 90% mortalities in the controls at 7, 14 and 28 days feeding times. Also, 2% ginger was effective in reducing the mortality rates of fish to (47-17%) with RLP (45-80.46%). In addition the present work showed that ginger incorporation at a rate of 3% in tilapia diet and fed for 7, 14 and 28 days reduced the mortalities of *A. hydrophila* infected fish to (32-21%) with RLP reaching (63-76.26%) compared with 90% mortalities in the control group (Fig, 1).

Discussion

Ginger bioactive molecules are; gingerol, flavonoids, zingerone, shogols and phenolic acids (**Ademola et al., 2004 and Ghasemzadeh et al., 2010**) which were evaluated for its immune modulatory , anti – inflammatory , anti- apoptotic, antimicrobial, anti – ulcer and anti-oxidant activities (**Ali et al., 2008**). The total and differential leukocytic counts are important indices of non-specific defense activities in fish **(Pedro *et al.*, 2005, Fazlolahzadeh et al., 2011)** as leukocytes responses to parasitic, bacterial, viral and similar challenges **(Houston et al., 1990)**. In the present study, there are significant increases in leukocytic counts, in all ginger treated groups (1, 2 and 3%) along the experimental periods. These findings were supported with the results of several investigators (**Sivagurunathan et al., 2011, Haghighi and Rohani, 2013**, **Chelladuria et al**., **2014**) **Talpur et al**., **2013** and **Kanani et al., 2014**). Total red blood cells of *O. niloticus* fed ginger, revealed significant increase that was directly proportional with increasing ginger dosage (1, 2 and 3%) in all periods of experiment. These results are supported by the findings of **Talpur et al., (2013)** who found significant increase in the erythrocyte number in Asian sea bass ginger fed groups than control. Moreover, **Kanani et al., (2014)** reported an elevation of total erythrocyte count when added ginger at dose of 1g/kg to *Huso huso* feed for period of 60 days. Similar observations were recorded in other studies results (**Immanuel et al., 2009; Sivagurunathan et al., 2011; Haghighi and Rohani, 2013 and Chelladuria et al., 2014).** The obtained findings may be attributed to polyphenols and flavonoids in ginger affect erythrocyte membrane fragility by protecting cells from possible damage against oxidative radicals (**Sivonova et al., 2004)**. Hemoglobin (Hb) concentration was significantly increased at all experimental periods, these findings came in accordance with that result of **(Sivagurunathan *et al.*, 2011; Haghighi and Rohani, 2013; Kanani et al., 2014; Chelladuria et al., 2014)**

PCV values also, revealed significant increase at all treatments. Similar observations were recorded by (**Apines- Amar et al., 2012 and Kanani et al., 2014).** For the differential leucocytic count; lymphocyte and nuetrophil counts revealed an increase from the first week of ginger feeding. These results come in accordance with **(EL Asely et al**., **2014)** who recorded an increase in phagocytes ten days post bee pollen feeding to tilapia.

Commonly increase in the level of serum total protein; albumin and globulin in fish are thought to be associated with a stronger innate immune response (**Wiegerijes et el., 1996**). In the present study feeding of *O. niloticus* with *Z. officinale* at 1, 2 and 3% significantly increased total protein values in all treated group along the whole experimental period. These results supported by the results of (**Immanuel et al., 2009, Talpur et al., 2013 and Kanani et al., 2014**). Regarding to the albumin concentration in this study; feeding of *O. niloticus* with *Z. officianle* incorporated diet at (1, 2 and 3%) resulted in significant increase the albumin level than control group especially with 1% *Z. officinale* at second week of experimental period. Nearly similar observation was detected in *Asian sea bass* fed ginger for 15 day (**Talpur et al., 2013**). The same, ginger fed fish showed significant increase in globulin levels especially at 3% at 3 and 4 weeks from start feeding. In the same respect (**Kanani et al., 2014**) revealed that feeding *Huso huso* fish with *Z. officinale* at dose of (1g/kg diet) for 60 day significantly increased globulin concentration in treated fish.

Liver enzymes, are included in trans-amination represents one of the main pathways for synthesis and de-amination of amino acid, thereby allowing interplay between carbohydrate and protein metabolism during the fluctuating energy demands of the organism in various adaptive situations. It is also considered to be important in assessing the state of the liver and some other organs **(Verma and Delela., 1981)**. In this study there was a marked decrease in level of AST and ALT of all ginger treated groups along the whole of experiment period than control, these results indicate that the fish treated with ginger have healthy liver tissue than control at the same condition. Similarly feeding of Indian catfish with *Z. officinale* incorporated diet at dose of 0.5g with other herpes resulted in lower level of AST and ALT in ginger treated group than control infected groups (**Kumar et al., 2014**). These results are supported with **Kanani et al., (2014**) in addition to the findings of (**Abbass et al., 2012**) that found significant decrease in ALT activity in tilapia spawners fed proplis or honey bee pollen at 2.5% for 3 weeks.

In this study all groups of *O. niloticus* fed with ginger showed significant increase in GSH-P**x** level than control groups. Similar results observed when feeding rainbow trout two phytogenic feed additives, one rich in carvacrol (CARV containing 12g/ kg carvacol) and the other rich in thymol THYM containing 6g/kg thymol) for 8 weeks (**Giannenas et al., 2012**). Malondialdehyde (MDA) showing a significant decrease than control for all ginger treated groups (1, 2 and 3%) along the whole

experiment period. Malondialdehyde is the major and the most studied toxic byproduct of polyunsaturated fatty acid peroxidation. Exposure to MDA induces intracellular oxidative stress leading to membrane lesions in erythrocyte. So its decrease than normal level indicate good health condition. A significant reduction in MDA levels were noticed in ginger fed groups, and these results were in accordance with **Giannenas et al.,** (**2012**). Superoxide dismutase (SOD) of all ginger treated groups showed significant increase than control groups along the whole experimental period. These results supported with the finding of **Kumari and Sahoo,** (**2006**) when fed pacific red snapper with β1, 3/1, 6- glucan at concentration of 0.1 and 0.2% for 6 weeks showing significant increase. Increased SOD level could be explained by the role of ginger to increase the number of circulating neutrophils and activate phagocytic cells associated with response to reactive oxygen species.

In the present work; spleen of all ginger treated groups 1,2 and 3% showing ascending weekly increase in amount of melanomacrophage (MMCs) and hemosidrin pigments and overall third week in all treated groups showed the highest aggregations of MMCs and hemosidrin pigment. This may be due to increase number and activity of immune cells (Macrophages) due to action of ginger to stimulate immune system (non-specific) to activate immune cells (macrophages). Similarly; feeding Indian major carp *Catla catla* with ethanolic extract of *Cynodan dactylon* mixed diet with 0.05, 0.5 and 5% extract for 60 day showed aggregation of MMCs and considerable modification were observed in the histological analysis of the spleen of *A. hydrophila* infected fish (**Kaleeswaran et al., 2012**). Also feeding of Nile tilapia diet containing two types of probiotics one is called Diamond–V yeast which is composed of *Saccharomyces cerevisae* at dose of 10g/kg feed and the other called megalo composed of *S. crevasse and Bacillus subtilis* at dose of 1.5 g/kg fed for a period of 6 weeks showed great activation of MMCs and kuffer cells in spleenic tissue of probiotic treated fish (**Marzouk et al., 2008**).

Concerning the effect of ginger in protection against artificial infection by *Aeromonas hydrophila*, the results revealed that the groups of *O. niloticus* treated with 1, 2 and 3% ginger showed a decrease in mortality rates compared to controls. With the highest record in protection 4 weeks post feeding at 1% *Z. officinale* fed groups (90.2%) followed by 2% (81.21) then 3% (76.26%) . These results supported by the results of (**Talpur et al., 2013)** who observed reduction in the mortality rate of Asian sea bass fed ginger containing and the results of

(**Immanuel et al., 2009** and **Nya and Austin, 2009).** The rhizome of ginger (*Zingiber officinale***)** has been reported to possess abroad – spectrum of prophylactic and therapeutic activities **(Ernst and Pittler, 2000).** And it is effective in the control of arrange of bacterial, viral, fungal and parasitic diseases **(Endo et al., 1990 and Martins et al., 2001).** This may be also attributed to that *Z. officinale* contains gingerols and shogaols and over 50 components of the oils have been characterized these are mainly monoterpenoids, sesquiphellandrene (15-20%), B-biasbolene (10-15%) and the main pharmacological actions of ginger and compounds isolated from it are immune–modulatory, anti-hyperglycemic, anti–inflammatory, anti- apoptotic, antitumourgenic, antimicrobial anti- platelet, anti–ulcer and anti-oxidant **(Ali et al., 2008)**.

It could be concluded that; inclusion of ginger in fingerlings diet is effective in enhancing the fish health status, rendering it more resistant to infectious diseases.

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تأثير تغذية إصبعيات البلطى النيلى بالزنجبيل على خصائص وكيمياء الدم وانسجة الطحال ومقاومتها للعدوى ببكتريا الايروموناس هيدروفيلا

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الملخص العربى

تهدف هذه الدراسة الى تقييم استخدام الزنجبيل كاضافات غذائية لاصبعيات البلطى النيلى ومدى تاثيره على خصائص وكيمياء الدم وكذلك التغيرات الهستولوجية فى انسجة الطحال ومدى مقاومتها للامراض. وذلك من خلال استخدام ثلاث جرعات (١و٢و٣%) من وزن العليقة بالاضافة الى الجرعة الضابطة (٠%). حيث تم تغذية اصبعيات البلطى (٠,٢٦±٧,٩٤) جرام لمدة اربعة اسابيع.. وقد اوضحت النتائج وجود زيادة معنوية فى قياسات خصائص الدم وكيمياء الدم فى معظم المعاملات مقارنة بالمجموعة الضابطة وانخفاض معنوى فى انزيمات الكبد وانزيم MDA مقارنة بالمجموعة الضابطة. اما بالنسبة لانسجة الطحال فقد وجد زيادة مرتبطة بمدة التغذية والجرعات فى عدد مراكز الميلانين وكمية صبغة الهيموسيدرين بانسجة الطحال واعلى زيادة سجلت بعد ٣ اسابيع من التغذية. وقد اوضحت نتيجة العدوى الصناعية ان تركيز ١% كان قادرا على حماية الاسماك بنسبة اعاشة ٩٠,٢% بعد ٤ اسابيع من التغذية. وبناءا عليه فانه يوصى باستخدام الزنجبيل كاضافات للعلف لرفع حالة الاسماك للحد من العدوى بميكروب الايروموناس الهيدروفيلا.