EFFECT OF GINGER ON SOME HEMATOLOGICAL ASPECTS AND IMMUNE SYSTEM IN NILE TILAPIA.

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Abstract

Ginger is the substance which stimulates the specific defense systems of living organism by enhancing the resistance to pathogens during stressful periods. So, this study was carried out to evaluated the immuno-stimulatory effects of dietary powdered ginger rhizome *(Zingiber officinale)*, in Nile tilapia. The fish were hand-fed with a diet containing (3gm/kg, 6gm/kg, 9gm/kg, 12gm/kg and 15gm/kg) on some hematological and immunological status of Nile tilapia. A total of 180 apparently healthy Nile tilapia from Abbasa farm with an average body weight of 100 ± 1gm were used. The fish were randomly divided into 6 equal triplicate groups (control group and 5 treated groups, each replicate contained 10fish). The fish were fed 3 times daily at rate of 3% of body weight for 7 weeks. At the end of the experimental period, various parameters of hematology including haematocrit (Hct), haemoglobin (Hb), red blood cell (RBC) and immunological parameters including white blood cell (WBC leukocytes), lymphocytes, neutrophils, monocytes, basophiles, acidophiles and thrombocytes were determined. The results confirmed that fish fed with powdered ginger rhizome showed significant immune-stimulatory effect, increasing WBC, haematocrit (Hct), RBC values compared with the control group ($p < 0.05$). These results indicate that dietary powdered ginger rhizome stimulates the immune system of the Nile tilapia. The results revealed that the fish fed diets contained ginger had a significant increase in platelets, MCHC but decrease in MCV and MCH. The different doses of ginger causes also increase in the eloctrolytes of blood Nile tilapia. The different doses of ginger causes decreased in the glucose, cholesterol, triglecrides, HDL. CH, LDL CH, creatinine, urea and uric acid in the Nile tilapia. Also, decreased in AST, ALT and ALP in serum of Nile tilapia. Lysozyme activity and immunoglobulin M (IgM) were significantly improved due to supplementation of the diets with ginger. It could be concluded that the ginger effect in fish as antibiotics and

therapeutic agents, had significantly additive benefit in immune status of fish compared with the control.

Key words: Ginger, Nile tilapia, hematology, immunology, electrolytes.

INTRODUCTION

The chemical composition and antioxidant activity (in aqueous and solvent extracts) of Ginger root *(Zingiber officinale)* were determined. The antioxidant components analysed were polyphenols, vitamin C, β carotene, flavonoids and tannins. Antioxidant assays such as free radical scavenging activity, reducing power and total antioxidant activity were carried out for ethanol, methanol, acetone, 80% methanol and 80% ethanolic extracts. Protein and fat of sample were 5.08 and 3.72 g/100 g respectively. Ash, minerals namely iron, calcium, phosphorous, zinc, copper, chromium and manganese) and vitamin C were 3.85 (g), 8.0 (mg), 88.4 (mg), 174 (mg), 0.92 (mg), 0.545 (mg), 70 (µg), 9.13 (mg) and 9.33 (mg) per 100 g of sample, respectively. Antioxidant components (polyphenols, flavonoids and total tannin) were higher in hot water (100°C) extract than other solvent extracts and 30°C water extract. Antioxidant activity by 3 different methods showed higher activity in solvent extract than water extract. Order of antioxidant activity by reducing power and free radical scavenging activity by DPPH was as follows, 80% methanolic > 80%ethanolic > methanolic > ethanolic > 30°C water >100°C water > acetonic extract Shirin and Jamuna (2010). Nutrition plays an important role in intensive fish production depending upon the type of feed availability and its cost. In particular, nutritional status has been increasingly acknowledged as a crucial factor in host defence against pathogens. As such, use of feed supplements aiming to improve not only the growth but also the health of aquaculture species has gained widespread interest and acceptance. Extensive use of antibiotics and biocides in aquaculture leads to the emergence of antibiotic-resistant bacteria and generation of toxicants which may cause risks to the environment (Esiobu *et al.,* 2002). To alleviate these problems, increasing attention is being paid to the use of natural alternative feed additives as ginger for disease-control strategies in aquaculture due to they enhance resistance to

infectious disease by increasing the non-specific and specific immune mechanisms (Harikrishnan *et al.,* 2011) contain natural organic materials that any threat to fish health or to the environment or to human health and facilitate growth, anti-stress, environmentally friendly and antimicrobial properties in fish (Maqsood *et al.*, 2011). Ginger (*Zingiber officinalis, Roscoe*), is generally considered as a safe herbal medicine (Weidner and Sigwart, 2000); contains alkaloids, flavonoids, polyphenols, saponin, steroids, tannin, fibre, carbohydrate, vitamins, carotenoids and minerals (Otunola *et al.,* 2010; Shirin and Prakash, 2010) natural antioxidants as gingerols, shogaols and zingerone (Masoud and Mostafa, 2014); essential oils which has potent anti-inflammatory effects and oleoresin (Masoud and Mostafa, 2014; Hassanin, *et al.,* 2014 and Zarate and Yeoman, 1996). Ginger is among the spices with reported antiplatelet, antibacterial, antifungal, antiviral, antiworm, anti-inflammatory, anti-oxidative activity, have effects on Dietry Effect of Ginger *(Zingiber officinale Roscoe)* On gastrointestinal, cardiovascular systems, antilipidemic and antihyperglycemic, anti-tumour properties and are known to be effective as an immuno-modulatory agent in human and animals, including fish (Nya and Austin, 2009; Apines-Amar *et al.,* 2012 and Talpur *et al.,* 2013). Supplementing ginger in fish diets may enhance disease resistance by reinforcing host innate immune functions that are necessary for protection against infectious diseases. Aeromonas hydrophila is known to be one of the most important bacteria associated with diseases in freshwater fishes including *O.niloticus* (Yardimci and Aydin, 2011). Aeromonas hydrophila cause outbreaks in fish farms with high mortality rates and severe economic losses to the aquaculture industry worldwide (Thangaviji *et al.,* 2012). The present study aimed to evaluate the growth performance, immune response and disease resistance of Nile tilapia fed on ginger supplemented diets.

MATERIALS AND METHODS

The study was conducted in Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharkia, Egypy. The fish were transferred to the physiology Laboratory. Fish were kept in glass aquaria (80 X 60 X 30 cm)

filled with 90 L., dechlorinated fresh water and aerator. The water temperature, dissolved oxygen, pH, ammonium (NH4) and nitrite were measured and found to be 27 ± 2° C, 5.4 mg/l, 7.2, 0.20 mg/l and 0.02mg/l respectively. Fish were divided into 6 equal groups; each group was divided into three replicates. Each replicate contain 10 fish. The fish were adapted to the experimental conditions for two weeks before the start of the experiment. 2. Fish diets and feeding: The ginger used for the feeding trial was purchased from market in Sharkia province, Egypt. Fish were exposed to different concentrations of (3, 6, 9, 12 and 15 gm/kg of ginger). Control diet fishmeal was free from ginger. Treated diet were formulated from fishmeal and ginger. Ginger was purchased from the local market, dried and ground to become powder. Ginger and fish meal content transformed into pellet form by Food grinder. After being dried, the pellets were transferred to plastic bags and stored in a freezer at average -3°C until immediately prior to feeding as in table 1. The Nile tilapia (*Oreochromis niloticus*) were divided into 6 experimental groups each containing 10 The Nile tilapia (*Oreochromis niloticus*)with 3 replicate for each as follows Control group and 5 groups of (3, 6, 9, 12 and 15 gm/kg of ginger). The fish treated and fed three times at a level of 3% of body weight daily for 7 weeks.

Table 1. Composition of experimental diets (g/100g dry diet)

	Experimental diets (g/100g dry diet)					
Items	**Control**	**Ginger 3gm/kg**	**Ginger 6gm/kg**	**Ginger 9gm/kg**	**Ginger 12gm/kg**	**Ginger15 mg/kg**
Fish meal	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal	24.00	24.00	24.00	24.00	24.00	24.00
Starch	47.00	46.7	46.4	46.1	45.8	45.5
Corn oil	1.00	1.00	1.00	1.00	1.00	1.00
Ginger	-	0.3	0.6	0.9	1.2	1.5
Chloramphenicol	-	-	-	0.25	0.25	10
Vit mix *	2.00	2.00	2.00	2.00	2.00	2.00
Min mix *	1.00	1.00	1.00	1.00	1.00	1.00
Cellulose	5.00	5.00	5.00	5.00	5.00	5.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

**Vitamin mix:* Each 2.5kg contained vit A 10 mIU, D 3 mIU , E 10gm , B_1 1g , B_2 4g , B_6 1.5g , B_{12} 10mg , Pantothenic 10g , Nicotinic acid 20g , Folic acid 1000 mg , Biotin 50 mg and Choline Chloride 500 mg.

***Min mix:* Each kg contains 727.78 g $CaHpo_4$. $2H_2o$, 127.5g $MgSo_4$. $2H_2o$, 60g NaCl, 25g $FeSo_4$. H_2o, 5.5g $ZnSo_4$, 0.48g $CoSo_4$. 7 H_2o, 0.3g Ca $(Io_3)_2$ $6H_2o$, 0.13g $CrCl_3$. 6 H_2o and 50g Kcl

Hematological analysis:

At the end of the experiment, blood samples were collected from the fish caudal vein by a sterile syringe containing heparin as an anticoagulant. Blood samples were placed into microtubes (2.0 mL) containing sodium heparin (50 IU) anticoagulants. All samples were collected in the early morning hours and were processed for hematological analysis. Samples were transported in a refrigerated cooler to the physiology Laboratory. Blood smears were prepared in duplicate and were stained with rapid hematological dye. The total cell count (erythrocytes, leukocytes, and thrombocytes) were performed by the diluent/dye direct method outlined by Natt and Herrick (1952) in a Neubauer chamber at a dilution of 1:100. Following the total cell count of nucleated cells (leukocytes and thrombocytes) in the Neubauer chamber, a differential count of leukocytes and thrombocytes were performed in the stained sample. The packed cell volume was determined by the microhematocrit technique described by Jain (1986). Blood was used for erythrocyte count (DACIE and LEWIS 1984), hemoglobin content (VANKAMPEN, 1961) and hematocrit value (BRITTON, 1963) determination. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formulae mentioned by DACIE and LEWIS (1984). Plasma was obtained by centrifugation at 3000rpm for 15min and the non-hemolyzed plasma was stored in a freezer at -20°C until analysis. Plasma protein content was determined by the Biuret method described by Wootton (1964). Glucose concentration was measured according to Trinder (1969), using Boehring Mannheium kits. Total lipids, cholesterol and triglycerides were determined calorimetrically using a kit supplied by El Nasr Pharmaceutical Chemical Co., according to Knight *et al*. (1972). Electrolytes, Creatinine, uric acid, ALP.Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically using kits supplied by Diamond Diagnostics, according to Reitman and Frankel (1975). Assay procedure for of IgM Immunoglobulin M (IgM) was determined using ELISA Kit. Catalog No. CSB-E12045Fh (96 test). (CUSABIO BIOTECH CO., Ltd).

Statistical analysis:

The obtained data in this study were statistically analyzed for variance ANOVA, LSD (Least significant difference) according to (Snedecor and Cochran, 1982). Differences among treatment means were compared using Duncan's multiple range tests (Duncan, 1995). Data were presented as mean ± SE and significance was declared at ($P < 0.05$).

RESULTS

Table 2 show that the erythrocytes count (RBC), hemoglobin content, and hematocrit percentage were significantly different from those of control. Erythrocyte count and hemoglobin content increased significantly in fish fed on diets containing all doses of ginger.

Table 2. Changes in red blood cells (RBCs), hematocrit HCT and hemoglobin (Hb) of Nile tilapia under the effect of different doses of ginger (3, 6, 9, 12 and 15gm/kg).

Doses / parameters	Control	Ginger 3gm/kg	Ginger 6gm/kg	Ginger 9gm/kg	Ginger 12gm/kg	Ginger 15mg/kg
RBCs ($x10^6/\mu L$)	1.5±0.3	1.7±0.4	1.8±0.4*	2.1±0.3**	2.65±0.2**	2.7±0.2***
HCT (%)	25.2±1.2	28.1±1.5	29.1±1.4 *	30.3±2.3 *	31.5±1.5**	31.1±2.1 **
Hb ($g\ dL^{-1}$)	6.8±1.2	7.5±1.5	7.9±1.5*	8.4±1.5*	8.3±1.1*	8.9±1.6**
Platelets $10^3 mm^{-3}$	289±1.2	290±1.2	294±1.2*	295±1.2**	299±1.2***	300±1.2***

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

The blood indices calculated from the mean values of blood parameters are presented in Table 3. It shows that RBC indices include: Mean cell (or corpuscular) volume (MCV), Mean cell hemoglobin (MCH) were non significant decrease but Mean cell hemoglobin concentration (MCHC) were significantly increased in Nile tilapias fed on diets containing all doses of ginger.

Table 3. Changes in mean corpuscular volume (MCV), Mean cell hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of Nile tilapia under the effect of different doses of ginger (3, 6, 9, 12 and 15gm/kg).

Doses / parameters	Control	Ginger 3gm/kg	Ginger 6gm/kg	Ginger 9gm/kg	Ginger12 gm/kg	Ginger15 mg/kg
Mean cell (or corpuscular) volume (MCV)fL10^{-15}	18.3 ±1.1	16.0 ± 2.2**	17.1 ± 1.2*	17.6 ±3.4	15.8 ± 2.1	16.1 ± 1.6**
Mean cell hemoglobin (MCH) pg 10^{-12}	30.3 ±1.5	28.7 ±2.7*	28.5 ±1.5**	29.4 ±2.7*	27.6 ±2.1**	29.2 ±1.5*
Mean cell hemoglobin concentration (MCHC)g/dL	25.2 ±1.1	28.9 ±2.4***	27.5 ±1.5**	26.4 ±2.7*	28.6 ±2.1***	27.2 ±1.5**

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

Numbers of WBCs, neutrophils, lymphocytes, eosinophils, basophils, monocytes and thrombocytes of Nile tilapia are presented in table 4. Among all groups, the total number of WBCs, lymphocytes, eosinophils, basophils, monocytes and thrombocytes of *Oreochromis niloticus* were significantly higher in the all doses of ginger than that control group. WBCs, lymphocytes, eosinophils, basophils, monocytes and thrombocytes of *Oreochromis niloticus* were evaluated between the all doses of ginger were significantly higher than that of the control group as shown in Table 4.

Table 4. Changes in white blood cells (WBCs), neutrophils, lymphocytes, eosinophils, basophils, monocytes and thrombocytes of Nile tilapia under the effect of different doses of ginger (3, 6, 9, 12 and 15gm/kg).

Doses/ parameters	Control	Ginger 3gm/kg	Ginger 6gm/kg	Ginger 9gm/kg	Ginger 12gm/kg	Ginger 15mg/kg
Total Leuk. /µL	5543±133	5566±111*	5576±151*	5575±223*	5578±321*	5587±323*
Seg. Neutro. /µL	1751±145	1768±213*	1876±431**	1887±223**	1999±342**	2121±432***
Lymphocytes/µL	2341±122	2542±213*	2654±223**	2667±244**	2687±212**	2675±133**
Eosinophyls/µL	123 ±11	133±21*	143±14**	148 ±11***	161±12***	169±33***
Basophyils/µL	201±21	211±11*	229±21**	236±21***	238±17***	242±31***
Monocytes/µL	1447±214	1411±111*	1421 ±123*	1422±133*	1355±122**	1441±122*
Thromb/µL	34000±2211	34333±1522*	34456±4311*	34951±4421**	34522±1144**	34883±3411**

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

Total protein content in fish body was significantly higher in the group fed on diet containing (3, 6, 9, 12 and 15gm/kg) of ginger than that control group (Table 5). Total plasma protein increased significantly in all groups when compared to control group. The mean value of total plasma lipid level decreased significantly in fish fed on diets containing all doses of ginger than that control group. Cholesterol, high density lipoprotein(HDL), low density lipoprotein (LDL) and triglycerides levels in fish body decreased significantly in fish fed on diet containing all doses of ginger than that control group (Table 5). Also, there are significant decreases of plasma glucose in fish fed on diets containing all doses of ginger than that control group.

Table 5. Changes in serum total protein, lipids profile, glucose of Nile tilapia under the effect of different doses of ginger (3, 6, 9, 12 and 15gm/kg).

Doses / parameters	Control	Ginger 3gm/ kg	Ginger 6gm/ kg	Ginger 9gm/ kg	Ginger 12gm/ kg	Ginger 15mg/ kg
Glucose (mg/dl)	101 ±1.3	101 ±1.7	100 ±1.3*	99±1.5*	97 ±1.4**	97±1.2**
Total protein (g/dl)	6.0±0.2	6. 3 ± 0.1	6.8 ±0.5*	6.9±0.6*	6.9 ±0.7**	7.1 ±0.8**
Total lipid	4.8±0.2	4.5±0.3	4.4±0.5*	4.3±0.1*	4±0.2**	4±0.2**
Cholesterol (mg/dl)	191 ±12	190±14	186±12**	173±16***	172±23***	172 ±22***
Triglycerides (mg/dl)	3.9 ±0.3	3.8 ±0.3	3.6±0.3*	3.6±0.1*	3.2 ±0.2**	3.1 ±0.5**
HDL CH mg/dl	3.5±0.2	2. 7 ± 0.3*	2.9 ±0.4*	2.7 ±0.5*	2.8 ±0.5*	2.7 ±0.2*
LDL CH mg/dl	1.6±0.1	1.5±0.1*	1.41±0.2*	1.4±0.1**	1.3±0.2**	1.3±0.2**

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

Table 6 show that the level of aspartate aminotransferase (AST) activity in plasma was decreased significantly with increasing levels of ginger. Plasma ALT activity after treatment with ginger varied significantly between groups, whereas a significant reduction and was recorded in fish fed on diets containing all doses of ginger, respectively. The levels of ALP, uric acid urea and creatinine in plasma were decreased significantly with increasing levels of ginger. The highest values were obtained in control group.

Table 6. Changes in serum liver functions and kidney functions of Nile tilapia under the effect of different doses of ginger (3, 6, 9, 12 and 15gm/kg).

Doses / parameters	Control	Ginger 3gm/ kg	Ginger 6gm/ kg	Ginger 9gm/ kg	Ginger 12gm/ kg	Ginger 15mg/ kg
AST (u/l)	38 ± 0.21	37 ± 0.43	36 ± 0.27*	36 ± 0.56*	34 ± 0.55**	31 ± 0.65**
ALT(u/l)	21± 0.3	20± 0.5	19 ± 0.11*	18 ± 0.22**	17.3± 0.33**	17± 0.4**
ALP (u/l)	26± 0.6	26± 0.5	23 ± 0.22**	24 ± 0.3**	22± 0.6***	21± 0.4***
Uric acid (mg/dl)	15 ± 0.2	14 ± 0.4*	13± 0.1*	11± 0. 3**	12± 0.5**	11± 0.4**
Creatinine (mg/dl)	0.37± 0.2	0.35± 0.01*	0.32± 0.02**	0.3± 0.02**	0.24± 0.03***	0.23± 0.02***
Urea (mg/L)	29.8± 4.7	26.5± 3**	26.6± 5**	23.6± 2***	19.6± 4***	19.6± 3***

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

The levels of Ca, Na and K and immunoglubin (**IgM value**) in plasma were increased significantly with increasing levels of ginger. The lowest values were obtained in control group as shown in Table 7.

Table 7. Changes in serum electrolytes and immunoglobin gM of Nile tilapia under the effect of different doses of ginger (3, 6, 9, 12 and 15gm/kg).

Doses/ parameters	Control	Ginger 3gm/kg	Ginger 6gm/kg	Ginger 9gm/kg	Ginger 12gm/kg	Ginger 15mg/kg
Ca mmol/ L	12±1	12.3±2*	12.7±1*	12.9±2**	12.6±1*	12.7±2*
Na mmol/ L	151±3	156±4*	158±2**	159±4**	156±3*	157±5**
K mmol/ L	5±0.2	5.6±0.3*	5.7±0.3*	5.9±0.4**	5.5±0.2*	5.6±0.4**
IgM value (µg /ml)	27±0.5	30±0.1*	32±0.2**	33±0.3***	38±0.7***	41±0.6***

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

Tables 8 & 9 indicate that ginger administration led to severe hypoglycaemia after all periods of the experiment accompanied by liver glycogen depletion and decreased of white muscle glycogen at the end of the experiment. The tables also show that ginger administration led to significant hypolipidaemia, accompanied by decreases in liver total lipids and a

significant decreased in the white muscle total lipids. Ginger administration led to the liver and muscle TFAA in response. This result was accompanied with a transitory decreased in the liver cholesterol and significant changes in its levels in white muscle. There were significant decreases in both serum triglyceride and cholesterol levels in response to ginger administration.

Table 8. Changes in some liver contents of Nile tilapia under the effect of different doses of ginger (3, 6, 9, 12 and 15gm/kg).

Doses/ parameters	Control	Ginger 3gm/ kg	Ginger 6gm/ kg	Ginger 9gm/ kg	Ginger 12gm/ kg	Ginger 15mg/ kg
Glycogen (mg/g dry wt)	114±8.2	120±8.2*	119±6.4*	125±11**	122±6.7**	124±12**
Total lipids (mg/g dry wt)	70±6.2	70±2	69±2	68.6±4*	66±6**	62±4***
Triglycerides	25±2.4	24.6±2	22±1*	21.2±1**	19±1.9***	19.3±2***
Cholesterol (mg/g dry wt)	4±0.2	3±0.3*	3.2±0.2*	3.1±0.1*	3.3±0.3*	3±0.3*
TFAA (µg/g dry wt)	488±41	489±50	490±52*	493±41*	499±51**	499±53**

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

Table 9. Changes in some muscle contents of Nile tilapia under the effect of different doses of ginger (3, 6, 9, 12 and 15gm/kg).

Doses parameters	Control	Ginger 3gm/ kg	Ginger 6gm/ kg	Ginger 9gm/ kg	Ginger 12gm/ kg	Ginger 15mg/ kg
Glycogen (mg/g dry wt)	13±1.3	14±1*	14±0.7*	15.0*±1**	15.7±1.1**	15.2±0.8**
Total lipids (mg/g dry wt)	9.7±0.5	9.3±0.4	9±0.7*	8.7±0.3*	8±0.51**	8.1±0.52**
Triglyceride (mg/g dry wt)	35±2	34±3	31±3.1*	30±3.5**	29±2.6**	28.2±4.5**
Cholesterol (mg/g dry wt)	4.1±0.2	3.5±0.3*	3.3±0.2*	3.1±0.26*	3±0.3**	3±0.1**
TFAA µg /g dry wt	442±21	440±20	432±41*	386±48**	382±32**	373±22***

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

DISCUSSION

The present study revealed that the erythrocytes count (RBC), hemoglobin content, and hematocrit percentage were significantly different from those of control. Erythrocyte count and hemoglobin content increased significantly in fish fed on diets containing all doses (3, 6, 9, 12 and 15gm/kg) of ginger as agreement with (Talpur *et al.,* 2013; Hassanin, *et al.,* 2014 and Masoud, *et al.,* 2014). Mean cell (or corpuscular) volume (MCV), Mean cell hemoglobin (MCH) were non significant decrease but Mean cell hemoglobin concentration (MCHC) were significantly increased in Nile tilapias fed on diets containing all doses (3, 6, 9, 12 and 15gm/kg) of ginger.these result agreement with Aysel *et al.*, 2016 reported that 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 Nile tilapia. Numbers of WBCs, neutrophils, lymphocytes, eosinophils, basophils, monocytes and thrombocytes of Nile tilapia. Among all groups, the total number of WBCs, lymphocytes, eosinophils, basophils, monocytes and thrombocytes of *Oreochromis niloticus* were significantly higher in the all doses (3, 6, 9, 12 and 15gm/kg) of ginger than that control group as reported by Aysel *et al.*, 2016. WBCs, lymphocytes, eosinophils, basophils, monocytes and thrombocytes of *Oreochromis niloticus* were evaluated between the all doses (3, 6, 9, 12 and 15gm/kg) of ginger were significantly higher than that of the control. These results were supported by Talpur *et al.* (2013) who reported that ginger induced beneficial effects such as disease protection due to improved immune response which was supported by the higher survival of the treated groups. Ginger at certain levels in the diet of fish could improve the non-specific immunity of fish and displayed encouraging health benefits in terms of a reduction in mortalities after challenge (Hassanin, *et al.,* 2014). Better survival rate could be explained that the bioactive compounds polyphenols, flavonoids, tannins and saponins found in ginger prevented fish from infection by triggering immune system (Shirin and

Prakash, 2010 and Talpur *et al.,* 2013) or the immunomodulatory effects of ginger in grouper can attributed to a better coordination of their stimulatory and antioxidant scavenging properties (Apines-Amar *et al.,* 2012). WBCs, lymphocytes, eosinophils, basophils, monocytes and thrombocytes of *Oreochromis niloticus* were evaluated between the all doses (3, 6, 9, 12 and 15gm/kg) of ginger were significantly higher than that of the control group. Dietary supplements had significantly increased immunity of all groups compared to the control group. Ginger diets which indicates that the immune system was enhanced in the fish Total protein content in fish body was significantly higher in the group fed on diet containing all doses (3, 6, 9, 12 and 15gm/kg) of ginger than that control group as showed by Aysel, *et al.*, 2016. Total plasma protein increased significantly in all groups when compared to control group. The mean value of total plasma lipid level decreased significantly in fish fed on diets containing all doses (3, 6, 9, 12 and 15gm/kg) of ginger than that control group as reported by Aysel, *et al.*, 2016. Cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides levels in fish body decreased significantly in fish fed on diet containing all doses (3, 6, 9, 12 and 15gm/kg) of ginger than that control group. Also, there are significant decreases of plasma glucose in fish fed on diets containing all doses (3, 6, 9, 12 and 15gm/kg) of ginger than that control group are agreement with (Talpur *et al.,* 2013 and Aysel, *et al.*, 2016). The level of aspartate aminotransferase (AST) activity in plasma was decreased significantly with increasing levels of ginger. Plasma ALT activity after treatment with ginger varied significantly between groups, whereas a significant reduction and was recorded in fish fed on diets containing all doses (3, 6, 9, 12 and 15gm/kg) of ginger, respectively. The levels of ALP, uric acid urea and creatinine in plasma were decreased significantly with increasing levels of ginger. The highest values were obtained in control group. The levels of Ca, Na and K and immunoglubin (IgM value) in plasma were increased significantly with increasing levels of ginger. The lowest values were obtained

in control group. These results are agreement with (Talpur and Ikhwanuddin, 2012, 2013 and Talpur *et al.*, 2013) also, reported that natural IgM are considered as components of the innate immune system since they are produced without any apparent antigenic stimulation, are found in the serum of healthy vertebrates and are polyreactive showing reactivity for non-self associated molecular patterns like viral and parasitic products. And Talpur *et al.,* 2013 Hassanin, *et al.,* 2014; Masoud, *et al.,* 2014 reported that all the supplemented groups especially the ginger-fed fish exhibited significantly increased total Ig levels that led to better immunocompetence. On the whole, it appeared that the diets supplemented with ginger stimulated total Ig production better than the other groups these result agreements with (Apines-Amar *et al.,* 2012 and Aysel, *et al.,* 2016). It could be concluded that the supplementation of ginger in fish diets as an alternative to antibiotics and therapeutic agents, had significantly additive benefit in immune status of fish compared with the control.

CONCLUSION

The results of this study indicate that powdered ginger rhizome is able to enhance the non-specific immune response in Nile tilapia. In general, this study suggests that ginger can be applied as an alternative and a supplement in diet to boost immune system for Nile tilapia. Ginger has been suggested as immunostimulatant due to their biological effects. Ginger has been reported that have good effect against the infection with diseases. It could be concluded that the supplementation of ginger in fish diets as an alternative to antibiotics and therapeutic agents, had significantly additive benefit in immune status of Nile tilapia compared with the control.

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REFERENCES

Apines-Amar, M.J.S.; E.C Amar; J.P.Jr.Faisan; R.V.Jr. Pakingking and S. Satoh, 2012. Dietary onion and ginger enhance growth, hemato-immunological responses, and disease resistance in brown- marbled grouper, *Epinephelus fuscoguttatus*. AACL Bioflux, 5 (4): 231-239.

Aysel Ş.; S. Özütok and E.B. Kurutaş, 2016. Determination of Some Hematological Parameters and Antioxidant Capacity in Nile Tilapia (Oreochromis Niloticus Linnaeus, 1758) Fed Ginger (Zingiber Officinale Roscoe) to Aeromonas hydrophila Turkish Journal of Fisheries and Aquatic Sciences, 16: 197-204.

Britton, C.J., 1963. Disorders Of The Blood. 9.Ed. London: J&A Churchill. 332p.

Dacie, J.V. and S.M. Lewis, 1984. Practical Hematology. 6.Ed. New York: Churchill. 22p.

Duncan, D.B., 1995. Multiple range and multiple F-tests. Biometrics, 11: 1-42.

Esiobu, N.; L. Armenta and J. Ike, 2002. Antibiotic resistance in soil and water environments. International Journal of Environmental Health Research, 12: 133–144.

Harikrishnan, R.; C. Balasundaram and M.S. Heo, 2011. Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. Aquaculture, 317: 1–15.

Hassanin, M. El-Sayed; Y. Hakim and M. E. Badawi, 2014. Dietry Effect of Ginger *(Zingiber Officinale Roscoe)* on Growth Performance, Immune Response of Nile tilapia *(Oreochromis niloticus)* and Disease Resistance Against Aeromonas Hydrophila. Abbassa Int. J. Aqua., 7 (1): 35- 52.

Jain, N.C., 1986. Schalm's Veterinary Hematology. 4th Ed. Lea And Febiger, 600. Washington Square, Philadelphia, Usa.

Knight, J.A.; S. Anderson and J.M. Rawle, 1972. Chemical Basis Of The Sulfo-Phospho-Vanillin Reaction For Estimating Total Serum Lipids. Clin. Chem., 18: 199-202.

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

Masoud, H. and M.S. Rohani, 2014. The effects of powdered ginger rhizome (Zingiber officinale) on haematological and immunological parameters of rainbow trout *Oncorhynchusmykiss.* Asian Journal of Marine Sciences, P. 7-11

Natt, M.P. and C.A. Herrick, 1952. A New Blood Diluent For Counting The Erythrocytes And Leucocytes Of The Chicken. Poultry Science, 31: 735-738.

Nya, E.J. and B. Austin, 2009. Use of dietary ginger, Zingiber officinale Roscoe, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases, 32: 971–977.

Otunola, G.A.; O.B. Oloyede; A.T. Oladiji and A.J. Afolayan, 2010. Comparative analysis of the chemical composition of three spices part 1: five sulfonated compounds from Zingiberis rhizome (Shokyo). Phytochemical, 62: 613-617.

Reitman S. and S. Frankel, 1975. Colorimetric Determination of Glutamic Oxaloacetic and Glutamic Pyruvic Transaminase. J. Clin. Pathol., 28-56.

Shirin. A.P.R. and J. Prakash, 2010. Chemical composition and antioxidant properties of ginger root (Zingiber officinale). Journal of Medicinal Plants Research, 4 (24): 2674-2679, 18.

Snedecor, G.W. and W.G. Cochran, 1982. Statistical methods. 8th Ed., Ames.

Lowa state university.

Talpur, A.D. and M. Ikhwanuddin, 2012. Dietary Effects Of Garlic (Allium Sativum) On Haematoimmunological Parameters, Survival, Growth, And Disease Resistance Against Vibrio Harveyi Infection In Asian Sea Bass, Lates Calcarifer (Bloch). Aquaculture, 365: 6-12.

Talpur, A.D. and M. Ikhwanuddin, 2013. *Azadirachta indica* (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *Lates calcarifer* challenged with *Vibrio harveyi*. Fish & Shellfish Immunology, 34: 254–264.

Talpur, A.D; M. Ikhwanuddin and A.B. Abol-Munafi, 2013. Nutritional effects of ginger (Zingiber officinale Roscoe) on immune response of Asian sea bass, *Lates calcarifer* (Bloch) and disease resistance against *Vibrio harveyi*. Aquaculture, 401: 46–52.

Thangaviji, V; M. Michaelbabu; S.B.; P. Gunasekaran and T. Citarasu, 2012. Immunization with the *Aeromonas* OMP provides protection against *Aeromonas hydrophila* in Goldfish (*Carassius auratu*) .J. Microbial Biochem. Technol., 4: 219-233.

Trinder P., 1969. Determination of Glucose in Blood Using Glucose Oxidase With An Alternative Oxygen Acceptor. Ann. Clin. Biochem., 6: 24-7.

Vankampen, E.J., 1961. Determination of Haemoglobin. Clin. Chem. Acta., 6: 538-44.

Weidner, M.S. and K. Sigwart, 2000. The Safety of a Ginger Extract in the Rat. J. Ethnopharmacol, 73 (3): 513–520.

Wootton, L.I., 1964. Micro-Analysis In Medical Biochemistry In Micrometer. 4.Ed. London: J&A Churchill, 264p.

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

Zarate, R. and M.M. Yeoman, 1996. Change in the amounts of gingerol and derivatives during a culture cycle of ginger, *Zingiber officinale*. Plant Sci., 121 (1): 115-12.

تأثير الزنجبيل على بعض مكونات الدم والجهاز المناعي في أسماك البلطي النيلي

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الملخـص العربـي

الزنجبيل هو نباتيحتوي علي مادة تحفز أنظمة الدفاع عن الكائنات الحية من خلال تعزيز مقاومة العوامل الممرضة خلال فترات الدراسة. لذلك، أجريت هذه الدراسة لتقييم التأثيرات التحفيزية المناعية لطحين الزنجبيل المجفف (نبات الزنجبيل علي البلطي النيلي. تم تغذية الأسماك بنظام غذائي يحتوي على (٣ جم / كجم ، ٦ جم / كجم ، ٩ جم / كجم ، ١٢ جم / كجم و ١٥ جم / كجم) على بعض مكونات الدم والمناعة للبلطي النيلي. وقد استخدم ١٨٠ من البلطي النيلي من مزرعة العباسة بمتوسط وزن يبلغ ١٠٠ ± ١ جم. تم تقسيم الأسماك إلى 6 مجموعات متساوية مكررة من ثلاث مجموعات (مجموعة ضابطة و ٥ مجموعات معالجة، كل تكرار يحتوي على 10 اسماك). تم تغذية الأسماك ٣ مرات يوميًا بمعدل ٣٪ من وزن الجسم لمدة ٧ أسابيع. في نهاية الفترة وجد ان الهيماتوكريت والهيموجلوبين وخلايا الدم الحمراء والمعلمات المناعية بما في ذلك خلايا الدم البيضاء (كريات الدم البيضاء والخلايا الليمفاوية). أكدت النتائج أن الأسماك المغذية بالزنجبيل أظهرت تأثير محفز للمناعة، وزيادة في WBC وقيم الهيماتوكريت و RBC مقارنة مع المجموعة الضابطة. هذه النتائج تشير إلى أن الزنجبيل يحفز الجهاز المناعي للبلطي النيلي. كشفت النتائج أن الأسماك التي غذيت على الزنجبيل زادت فيها عدد الصفائح الدموية، MCHC ولكن انخفاض في MCV وMCH. اكدت الدراسة ان الزنجبيل ادي الي زيادة في eloctrolytes في سيرم البلطي النيلي. كما أوضحت النتائج تحسن في وظائف الكبد و الكلي وذلك بانخفاض مكونات السيرم مثل الجلوكوز، والكوليسترول، triglecrides، HDL. CH، LDL CH، الكرياتنين في دم البلطي النيلي أيضا ، انخفض AST، ALT و ALP في مصل البلطي النيلي. تم تحسين نشاط والجلوبيولين المناعي M (IgM)بشكل ملحوظ بسبب إضافة الحمية بالزنجبيل. يمكن الاستنتاج أن تأثير الزنجبيل في الأسماك كمضادات حيوية وعوامل علاجية، كان له الفائدة الكبيرة في الحالة المناعية للأسماك مقارنة بالأسماك التي لم تعالج.