

## EFFECT OF GINGER DIETARY SUPPLEMENTATION ON GROWTH PERFORMANCE, IMMUNE RESPONSE AND VACCINE EFFICACY IN *Oreochromis niloticus* CHALLENGED WITH *Aeromonas hydrophila*

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**Abstract:** Ginger powder was used in the current study for the improvement of the growth performance and the efficacy of *Aeromonas hydrophila* (*A. hydrophila*) vaccination in *Oreochromis niloticus*. A total of 120 apparently healthy fish were classified into 2 equal groups. The control fish received a basal diet and ginger group received a basal diet enriched with 1% ginger powder. The feeding period lasted for 2 months and the growth performance indicators were measured. Next, each group was allocated into 2 equal groups to be 4 groups. G1: received basal diet, G2: received basal diet then vaccinated with *A. hydrophila* vaccine, G3: received ginger supplemented diet, and G4: received ginger supplemented diet then vaccinated with *A. hydrophila* vaccine. One-week post vaccination, fish were challenged with *A. hydrophila* bacteria. Fish fed 1% ginger supplemented diet had a marked increase in growth performance parameters and utilized their feed more efficiently than those received the basal diet. Nitric oxide, IgM and lysozyme activity were significantly differed among fish groups in order of G4 > G2 > G3 > G1. Ginger and/or *A. hydrophila* vaccine reduced the clinical signs, mortality rate as well as the elevation of alanine aminotransferase and creatinine which induced by *A. hydrophila* infection. It could be inferred that ginger has been suggested as growth promoter and immune-stimulant in *O. niloticus* and increase efficacy of *A. hydrophila* vaccine, which increase protection against *A. hydrophila* infection.

**Key words:** ginger; *O. niloticus*; growth performance; immunity; vaccine

### Introduction

Fish is an important source of protein for human in most countries all over the world and aquaculture represents an important source for fresh water and marine water fish (1). *Oreochromis niloticus* (*O. niloticus*), Nile tilapia, is the most popular cultured species among tilapia in many countries around the world (2). Tilapia are the species of choice in the field of aquaculture due to their high growth rate, high

marketable value, reproduce easily and withstand the environmental conditions and rough handling (3). Motile Aeromonas Septicemia (MAS) is a standout amongst the most well-known bacterial diseases of tilapia which is caused by *Aeromonas hydrophila* (*A. hydrophila*) and causing high mortalities in tilapia (4). Millions of dollars were lost per year due to the diseases caused by this bacterium (5). *A. hydrophila* can affect a variety of fish species including tilapia

(6), common carp (7), eel (8), cat fish (9), and goldfish (10).

Vaccination has a fundamental role in vast scale commercial aquaculture. The use of vaccines in the aquaculture industry has been important in reducing economic losses caused by the disease (11,12) and in the reduction of antibiotic use (13). Excessive use of antibiotics in fish farms results in the emergence of antibiotic-resistant bacteria and creation of toxicants, which may lead to environmental risk (14).

The vaccine is a preparation of killed micro-organisms which are attenuated, fully virulent or non-virulent. Once enhanced by a vaccine, the antibody-producing B-cells still sensitized to the infectious micro-organisms and respond to reinfection by producing more antibodies, so re-stimulating the immune response (15).

Ginger (*Zingiber officinalis*, Roscoe), is as a safe medicinal plant (16); contains flavonoids, alkaloids, polyphenols, steroids, saponin, fibre, tannin, carbohydrate, carotenoids, vitamins, and minerals (17); natural antioxidants as gingerols, shogaols and zingerone and essential oils that has strong anti-inflammatory effect (18). Supplementation of fish diets with ginger may improve the disease resistance by strengthen the host growth and innate immune response that are important for safeguard against infectious diseases (19). Herbs such as ginger have been reported for their biological effects such as growth promotion and immune-stimulation (20). The current experiment delineated to investigate the improvement of the growth performance and the efficacy of *A. hydrophila* vaccination in *O. niloticus* by ginger supplementation.

## Material and methods

### *Experimental fish*

A total number of 120 apparently healthy *O. niloticus* with an average body weight  $31 \pm 0.5$  g purchased from Central Laboratory for Aquaculture, Abassa Fish Farm at Sharkia Governorate. Fish kept in glass aquaria filled with 90 L de-chlorinated fresh water. The water temperature, dissolved oxygen, pH, ammonium and nitrite were measured and found to be  $27 \pm 2$

°C, 5.4 mg/l, 7.2, 0.20 mg/l and 0.02 mg/l, respectively. They received normal diet, which was prepared according to NRC (21) to fulfil the nutrient needs of Nile tilapia. Fish supplemented with the basal diet daily at 3% of their body weight throughout the adaptation period (2 weeks).

### *Fish diets and feeding*

The ginger bulbs purchased from local farms in Sharkia Governorate, Egypt. They washed, sun dried, and powdered. The ginger supplemented diet was prepared at Fish Research Center, Faculty of Veterinary medicine, Zagazig University, Egypt. It was prepared by mechanical mixing as 1% ginger powder with the basal diet ingredient, and then finally pelleted. The pellets were dried at room temperature (26°C for 48 h) and stored in a refrigerator at 4°C until use. It contained (2940 kcal/kg ME and 30.80% CP) in the form of dry pellets and prepared to fulfil the nutrient needs of Nile tilapia (21). Feedstuffs used in diets preparation examined according to A.O.A.C. (22). All fish provided with their diets at a level of 3% of body weight three times daily for 60 days.

### *Preparation of vaccine*

Formalin-killed *A. hydrophila* bacterin prepared by the addition of formalin (0.3%) to the bacterial culture, previously incubated at 35°C for 48 hrs according to Baba et al. (23). The formalized bacterial culture kept at room temperature overnight, and then subjected to sterility and safety tests as mentioned by Cardella et al. (24). The sterility test performed by culturing washed bacterin on Tryptic Soy agar and plates incubated at 37°C for 24 hrs and then examined for bacterial growth. The safety test performed by the intraperitoneal injection of the prepared bacterin cells (0.1 ml) to twenty susceptible tilapia fish. The fish kept under observation for 2 weeks post-inoculation and then dead fish subjected to necropsy for re-isolation of *A. hydrophila* using Aeromonas selective base media. Vaccine placed at 4°C. Immediately before use, the prepared vaccine washed twice with sterile saline solution and was prepared at a concentration of 3 mg wet-weight/ml saline.

### *Experimental design*

Fish were classified into two equal groups (each group has three replicates; 20 fish/replicate). The control group received a basal diet and ginger group received a basal diet enriched with 1% ginger powder. The feeding period lasted for 2 months and growth performance indicators measured.

Next, each group was allocated into two equal groups so become four groups each group contain 30 fish. Group 1: supplemented with normal fish diet, group 2: supplemented with normal fish diet then vaccinated with *A. hydrophila* vaccine, group 3: received ginger supplemented diet, and group 4: received ginger supplemented diet then vaccinated with *A. hydrophila* vaccine.

Fish in-group 2 and 4 were vaccinated two times with one week interval. Fish vaccinated with 0.1 ml formalin killed *A. hydrophila* diluted in 0.1 ml sterile saline according to Badran et al., (25).

### *Challenge test*

One week post vaccination, all group were challenged intraperitoneally with 0.1 ml of pathogenic *A. hydrophila* ( $10^8$  CFU mL<sup>-1</sup>) that had previously isolated from moribund fish and confirmed to be pathogenic (26). The groups are, G1 (infected- non-vaccinated group), G2 (infected- vaccinated group), G3 (infected- ginger supplemented group) and G4 (infected- vaccinated- ginger supplemented group). Challenged fish observed for clinical signs and mortality for 14 days. Any dead fish subjected immediately to post-mortem examination and routine bacteriological examination.

### *Blood samples*

Blood samples (n=9/group) were collected 3 days after the 2<sup>nd</sup> vaccination (for evaluation of some immunological parameters) and 3 days post-challenge (for assessment of some biochemical parameters) according to Stoskopf (27). Blood samples were taken without EDTA and were centrifuged at 3000 rpm for 15 minutes for serum separation.

### *Growth performance parameters*

The fish weighed at the beginning and after 2 month of feeding. Average body weight (BW), body gain (g), body gain percent (%), feed conversion ratio (FCR) and specific growth rate (SGR) were determined according to Merrifield et al., (28).

### *Determination of some immunological and biochemical parameters*

Nitric oxide (NO) level was detected as the method described by Rajaraman et al., (29). Lysozyme activity detected according to Parry et al., (30). Immunoglobulin (IgM) measured by an ELISA method according to Fuda et al., (31). Alanine aminotransferase (ALT) assessed as described by Reitman and Frankel (32). Serum creatinine assessed by using the method of Henry (33).

### *Data analysis*

The statistical significance of the immunological and biochemical data was assessed by one way ANOVA using SPSS statistical software package. Data of growth performance parameters were analysed by Student's t test. The level of significance was taken as  $p < 0.05$ .

## **Results**

### *Effect of ginger supplementation on growth performance parameters:*

The results revealed that the fish fed 1% ginger supplemented diet for 60 days had a highly significant ( $p < 0.05$ ) elevation in total final BW (53.45 g), BWG (21.8 g), BG % (68.88%) and SGR (0.87%), and a significant ( $p < 0.05$ ) improvement in the FCR (1.95) than those fed the control diet (48.5 g, 16.88 g, 53.38%, 0.71 and 2.48, respectively). There was non-significant ( $P > 0.05$ ) change in average daily feed intake between control and ginger supplemented group (Table 1).

*Effect of ginger supplementation and/or A. hydrophila vaccine on some immunological parameters:*

Nitric oxide, IgM and lysozyme levels were significantly ( $p<0.05$ ) differ among fish groups.

Regarding to the above-mentioned parameters the positive effect was recorded in group 4 that fed on diet contained 1% ginger and vaccinated with *A. hydrophila* vaccine followed by group 2 and 3 then control group (Table 2).

**Table 1:** Effect of feeding of 1% ginger supplemented diet for 60 days on growth performance parameters of *O. niloticus* (means  $\pm$ SE)

Parameters	Experimental groups		Sig. (2-Tailed)
	Control	Ginger	
Initial BW (g)	31.62 $\pm$ 0.38	31.65 $\pm$ 0.51	0.415
Final BW (g)	48.50 $\pm$ 0.58	53.45 $\pm$ 0.48*	0.000
Body gain (g)	16.88 $\pm$ 0.20	21.80 $\pm$ 0.25*	0.000
Body gain (%)	53.38 $\pm$ 0.17	68.88 $\pm$ 1.45*	0.000
Specific growth rate	0.71 $\pm$ 0.002	0.87 $\pm$ 0.010*	0.001
Feed intake (g)	41.88 $\pm$ 0.68	42.66 $\pm$ 1.11	0.130
Feed conversion ratio	2.48 $\pm$ 0.01	1.95 $\pm$ 0.05*	0.000

Values are represented as the mean $\pm$ SE. \* denote significant difference ( $p<0.05$ ). Control group, fed a basal diet. Ginger group fed a basal diet supplemented with 1% ginger powder.

**Table 2:** Effect of feeding of 1% ginger supplemented diet and/ or *A. hydrophila* vaccine on some immunological parameters of *O. niloticus* (means  $\pm$ SE)

Parameters	Experimental groups				p-value
	G1	G2	G3	G4	
Nitric oxide ( $\mu$ g /ml)	34.35 $\pm$ 1.95 <sup>b</sup>	40.37 $\pm$ 0.90 <sup>a</sup>	36.93 $\pm$ 1.92 <sup>b</sup>	40.25 $\pm$ 0.58 <sup>a</sup>	0.006
IgM ( $\mu$ g /ml)	0.20 $\pm$ 0.003 <sup>b</sup>	0.29 $\pm$ 0.004 <sup>a</sup>	0.28 $\pm$ 0.005 <sup>a</sup>	0.30 $\pm$ 0.0005 <sup>a</sup>	0.000
Lysozyme ( $\mu$ g /ml)	0.22 $\pm$ 0.003 <sup>c</sup>	0.34 $\pm$ 0.003 <sup>ab</sup>	0.31 $\pm$ 0.004 <sup>b</sup>	0.44 $\pm$ 0.039 <sup>a</sup>	0.000

Values are represented as the mean $\pm$ SE. <sup>abc</sup> Within-row different superscript letters denote significant difference ( $P<0.05$ ). G1: fed on basal diet, G 2: fed on basal diet then vaccinated with *A. hydrophila*. G3: fed on ginger supplemented diet. G4: fed on ginger supplemented diet then vaccinated with *A. hydrophila* and infected with *A. hydrophila* bacteria.

*Effect of ginger supplementation and/or A. hydrophila vaccine on clinical signs post-mortem lesions, mortality rate and some biochemical parameters of A. hydrophila infected fish:*

Fish in control infected group showed loss of equilibrium, ascites, skin darkness, exophthalmia and ulcers. Enlargement and congestion of internal organs were appeared in post-mortem examination. The severity of clinical signs and post-mortem lesions differed following the order of G1 (infected- non-

vaccinated group) > G2 (infected- vaccinated group) and G3 (infected-ginger supplemented group)>G4 (infected-vaccinated- ginger supplemented group). The survival rate was 76.7% in G1, 90% in G2, 83.4 in G3 while 96.7 % in G4 (Table 3).

The vaccinated fish group (2<sup>nd</sup>) or groups fed on 1% ginger with or without vaccination revealed a marked ( $p<0.05$ ) lowering of renal injury marker (creatinine) and hepatic injury biomarker (ALT) concentrations compared to infected non vaccinated group (Table 3).

**Table 3:** Effect of feeding of 1% ginger supplemented diet and/ or *A. hydrophila* vaccine on mortality rate and serum levels of ALT and creatinine of *A. hydrophila* infected *O. niloticus* (means  $\pm$ SE)

Parameters	Experimental groups				p-value
	G1	G2	G3	G4	
ALT (IU/L)	40.67 $\pm$ 2.02 <sup>a</sup>	34.33 $\pm$ 1.20 <sup>b</sup>	33.67 $\pm$ 1.20 <sup>b</sup>	28.67 $\pm$ 0.88 <sup>c</sup>	0.000
Creatinine (mg/ dl)	0.48 $\pm$ 0.02 <sup>a</sup>	0.47 $\pm$ 0.02 <sup>a</sup>	0.41 $\pm$ 0.01 <sup>b</sup>	0.38 $\pm$ 0.01 <sup>b</sup>	0.014

Values are represented as the mean $\pm$ SE. <sup>abc</sup> Within-row different superscript letters denote significant difference ( $P < 0.05$ ). G1: fed on basal diet and infected with *A. hydrophila* bacteria, G 2: fed on basal diet then vaccinated with *A. hydrophila* and infected with *A. hydrophila* bacteria. G3: fed on ginger supplemented diet and infected with *A. hydrophila* bacteria. G4: fed on ginger supplemented diet then vaccinated with *A. hydrophila* and infected with *A. hydrophila* bacteria.

## Discussion

Our results revealed that the fish fed 1% ginger supplemented diet for 60 days had a significant ( $p < 0.05$ ) elevation in total final BW, BWG, BG % and SGR %, as well as a significant ( $p < 0.05$ ) improvement in the total FCR than those fed the control diet. These results clearly showed that the ginger stimulated fish growth. Our data are supported with those of Talpur et al., (34) who suggested that supplementation of Asian sea bass diet with 5 and 10 g ginger /kg feed was most effective for the growth and FCR. In addition, Sukumaran et al., (19) showed that dietary enrichment with 0.8% ginger enhanced the growth performance of *L. rohita*. The positive growth promoting effect of ginger might be due to their chemical and physical properties; their positive immune-stimulating effect or acted as an appetizer which led to stimulates digestion and improving protein and fat metabolism (34) or contains bioactive compounds which improving anti-oxidant status of the fish (35), anti-microbial (36), various pharmacological effects (37) and favourable effects on gut function, which is the primary mode of action for growth promoting feed additives (38). Additionally, growth performance was affected by supplementation of *Onchorhynchus mykiss* (39) or *Lates calcarifer* (34) diets with the ginger.

Fish vaccination is one of the strategies that can be applied to prevent the infectious diseases by means of inducing the immune response (40). *A. hydrophila* Vaccine significantly

increased the immunity of vaccinated groups compared to the parallel control groups as shown in our results. These results supported by those of Sumiati et al., (41) who detected improvement of the specific immune response in *O. niloticus* vaccinated against *A. hydrophila* and the increase of antibody in fish involved in the elevation of the survival rate fry fish. Moreover, Sukenda et al., (42) stated that vaccination in tilapia brood stock succeeded in improving the specific and non-specific immune responses, and protecting fry tilapia from *A. hydrophila* infection. The mechanism of its action, the antigen slowly releases into the blood or tissue, stimulating and prolonging the humeral response (42). The success of vaccine is often regulated by the individual antigens concentration, cross reactivity and competition among different antigens (43).

Nitric oxide has serum potent bactericidal activities and showed a variety of biological functions as microbicidal and tumoricidal activity, and a range of immunopathologies (44). In our results, a significant increase NO level was recorded group 2 and 4, which indicate the stimulation of the fish immune system. This may be due to ginger has a special phytochemical properties which increase efficacy of vaccine to activate fish macrophage-inducible NO synthase and stimulate NO generation in response to infection. NO can inactivate several respiratory terminal oxidases and aconitase or react with reactive oxygen intermediates (such as superoxide) to form several powerful oxidizing reactive nitrogen intermediates (such as peroxynitrite) that can effectively kill

microorganisms (45). Further, the serum NO is elevated in the *Renibacterium salmoninarum*-infected rainbow trout with (46) or in the *Edwardsiella ictaluri* infected channel catfish (45).

The soluble IgM is present in the blood and other fluids and act as an immune stimulator molecule (47). Current work clarified an elevation of serum IgM level in ginger supplemented groups (3<sup>rd</sup> and 4<sup>th</sup>). These results coincide with those of Hassanin et al., (48) who demonstrated that IgM were significantly improved in Nile tilapia due to supplementation of the diets with ginger.

Lysozyme is considered a serum bactericidal activity thus resulting in the reduction of disease (49). Our findings revealed a significant increase of lysozyme activity in 3<sup>rd</sup> and 4<sup>th</sup> groups which indicates that the immune system was enhanced in the fish (34). This may be due to lysozymes are catalysing the peptidoglycans hydrolysis of bacterial cell walls and acts as non-specific innate immunity molecules against the incursion of detrimental bacteria (50). Also, Hassanin et al., (48) demonstrated that lysozyme activity in Nile tilapia was significantly improved due to supplementation of the diets with ginger. Moreover, Sukumaran et al., (19) showed that 0.8% ginger dietary supplementation could strengthen immunity of *L. rohita*. Similarly, Clara et al., (20) recorded a significant advancement of fish resistance against *A. hydrophila* infection by dietary *Zingiber officinale* supplementation. Positive immunological results of ginger could be due to the bioactive compounds polyphenols, flavonoids, tannins and saponins that present in ginger may protect fish from bacterial infection by activating immune system (34) or have a better coordination of their stimulatory and anti-oxidant scavenging properties (51). However, unlike this study, lysozyme activity wasn't influenced in juvenile beluga fed with ginger (52).

Increased serum ALT level may indicate hepatic cellular damage (53) and elevated creatinine level signifies impaired kidney function or kidney disease. The vaccinated fish group (2<sup>nd</sup>) or groups fed on 1% ginger with or without vaccination revealed a marked

reduction of ALT and creatinine concentrations compared to control infected group. The active ingredients such as flavonoids, polyphenols, saponins and tannins that present in ginger may safeguard fish from bacterial infection by stimulating immune system and its supplementation might prevent lipid peroxidation of cell membranes and inhibit the hepatic and renal damage (34). Similarly, feeding of Indian catfish with *Zingiber officinale* incorporated diet at dose of 0.5g resulted in lowering the level ALT in ginger treated group than control-infected groups (54). Furthermore, the immune-stimulating effect of 1% ginger and /or *A. hydrophila* vaccine could increase the resistance of *O. niloticus* to *A. hydrophila* infection which manifested by reduction of the mortality rate and the severity of the clinical symptoms and post-mortem lesions in G2, G3 and G4 when compared to infected–non vaccinated group.

## Conclusion

The findings of the current study revealed that addition of 1% ginger to diet improved growth performance and immune status in *O. niloticus*. Ginger increases the efficacy of *A. hydrophila* vaccine, which increase protection against *A. hydrophila* infection and could represent a suitable way against this infection in *O. niloticus* aquaculture.

## Conflict of interest

The authors declare that they have no conflict of interest.

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