DIETRY EFFECT OF GINGER *(ZINGIBER OFFICINALE ROSCOE)* ON GROWTH PERFORMANCE, IMMUNE RESPONSE OF NILE TILAPIA *(OREOCHROMIS NILOTICUS)* AND DISEASE RESISTANCE AGAINST *AEROMONAS HYDROPHILA*

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Abstract

The aim of current investigation was to evaluate the effects of graded levels (0, 0.1, 0.2, 0.3, 0.5 and 1 %) of ginger (*Zingiber officinale, Roscoe*) as feed additive in the diets of *Oreochromis niloticus* on growth performance and immunological status. A total of 360 apparently healthy *Oreochromis niloticus* with an average body weight of 30.00 ± 1.00g were used. The fish were randomly divided into 6 equal triplicate groups (each replicate contained 20 fish). The fish were fed isonitrogenous, isocaloric diets 4 times daily at rate of 5% of body weight for 10 weeks.

The results revealed that the fish fed diets contained ginger had a significant ($P < 0.05$) increase in total final body weight, body gain, body gain percent, specific growth rate, and also utilized their feed more efficiently (lower values of FCR) than those fed the control diets. The average daily feed intake wasn't significantly ($P > 0.05$) different with all groups. Lysozyme activity and immunoglobulin M (IgM) were significantly improved due to supplementation of the diets with ginger. Supplemented diets with ginger protected fish against pathogenic strain of *Aeromonas hydrophila*. It could be concluded that supplementation of ginger in fish diets as an alternative to antibiotics and

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

Key words: Ginger, *Oreochromis niloticus*, growth Performance, health status.

INTRODUCTION

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 (Hori *et al.*, 2003); essential oils which has potent anti-inflammatory effects and oleoresin (Zarate and Yeoman, 1996). Ginger is among the spices with reported antiplatelet, antibacterial, antifungal, antiviral, anti-worm, anti-inflammatory, anti-oxidative activity, have effects 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* (Aly *et al*., 2008 and 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 *O.niloticus* fed on ginger supplemented diets.

MATERIALS AND METHODS

1. Experimental fish:

A total number of 360 apparently healthy a live Oreochromis niloticus with an average body weight 30±1.00 g and average body length 11.06 ±0.08 cm obtained from Abassa Fish Hatchery at Sharkia province. Fish were kept in glass aquaria (80 X 60 X 30 cm) filled with 90 L., de-chlorinated 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 20 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. The bulbs were washed, sun dried, cleaned

and milled to powder. It was incorporated in the six diets adopted. The control diet had no ginger additive, while the five supplemented diets contained ginger at 0.1, 0.2, 0.3, 0.5 and 1 %. All fish were fed their respective diets at a level of 5% of body weight four times daily for 10 weeks. Feedstuffs used in diets formulation were analyzed for dry matter, crude protein, ether extract and crude fiber according to the standard procedures of the A.O.A.C (1990) and the data are shown in Table 1.

Isocaloric and isonitrogenous diets were prepared at Fish Research Center, Faculty of Veterinary Medicine, Zagazig University, Egypt. It contained 2940 kcal/kg ME and 30.80% CP in the form of dry pellets and were formulated to meet the nutrient requirements of *Oreochromis niloticus* as set by NRC (1993) and shown in Table 2. The analyzed values were in close agreement with the calculated values.

3. Growth performance parameters:

The fish were weighted at the start and the end of the experiment. Average body weight was calculated by dividing the total weight of fish by the number of fish in each group. Body gain and feed conversion ratio (Siddiqui *et al.*, 1988). Body gain percent (Jauncay and Ross, 1982) and specific growth rate % (Pouomonge and Mbonglang, 1993) were determined.

4. Condition factor:

The condition factor was calculated according to Gjedrem and Gunnes (1978).

5. Health condition:

For evaluation of health condition of the fish during the period of the experiment, escape, defensive, tail and ocular reflexes were regularly observed according to Lucky (1977).

Fish of all groups were regular observed daily, abnormal behaviors and mortality rate were recorded.

6. Blood sample collection:

Blood was obtained from caudal blood vessels into plastic Eppendorf tubes for serum samples preparation. Blood was collected into Eppendorf tubes without anticoagulant in syringe then centrifuged (3,000 r.p.m. for 15 min). The serum samples were collected and stored immediately in deep freezer (-20 ºC) until use (Aly *et al*., 2008).

7. Immunological parameters evaluation:

7.1. Assay procedure for of IgM:

Immunoglobulin M (IgM) was determined using ELISA Kit. Catalog No. CSB-E12045Fh (96 test). (CUSABIO BIOTECH CO., Ltd).

7.2. Lysozyme determination:

The lysozyme activity was measured using the turbidity assay. Chicken egg lysozyme (Sigma) was used as a standard and 0.2 mg ml^{-1} lyophilized *Micrococcus lysodeikticus* in 0.04 M sodium phosphate buffer (pH 5.75) was used as substrate. Fifty ml^{-1} of serum was added to 2 ml of the bacterial suspension and the reduction in the absorbance at 540 nm was determined after 0.5 and 4.5 min incubation at 22ºC. One unit of lysozyme activity was defined as a reduction in absorbance of 0.001min^{-1} (Parry *et al.*, 1965).

8. Challenge test:

After 10 weeks of feeding period, 20 fish from each group were challenged by intraperitoneal injection with 0.1 ml of pathogenic *Areomonas hydrophila* (10^8 cfu mL^{-1}) that had previously isolated from moribund fish and confirmed to be pathogenic (Talpur and Ikhwanuddin, 2012). The isolation, culture and maintenance of *Areomonas hydrophila* carried out according to methods described by Collins *et al*. (1991). The

concentration was adjusted to 10^8 cells ml^{-1} by means of optical density (630 nm). Injected fish were observed for mortality over a period of 15 days and mortality was confirmed by re-isolating the microorganism from internal organs of dead fish.

9. 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 AND DISCUSSION

Growth performance:

Growth performance of fish fed the experimental diets is shown in Table 3. The results revealed that the fish fed diets contained ginger had a significant ($P < 0.05$) increase in total final BW, body gain, body gain % and specific growth rate %, while a significant ($P < 0.05$) decrease in the total FCR than those fed the control diets. The average daily feed intake wasn't significantly ($P > 0.05$) different with all groups. Fish fed on diet contained 1% ginger achieved the best significant final average body weight followed by fish groups fed on diet contained 0.5%, 0.3 and 0.2% ginger respectively, while the lowest values were obtained in fish group fed on diet contained 0.1% ginger and control group. Concerning the body gain, body gain % and specific growth rate % followed a similar trend. These results clearly showed that the ginger stimulated fish growth may be respond to ginger supplementation in a dose dependent manner. These results are also in accordance with Talpur *et al.* (2013) who suggested that the growth was dose-dependent; suggesting highest supplementation of ginger at 5 and 10 g/kg feed was most favourable for the growth and survival of Asian sea bass and FCR

was significant which means that the ginger diet acted as an appetizer which led to increase the digestibility and in turn the energetic benefits enhanced the growth rate. Also, Apines-Amar *et al*. (2012) showed that oral administration of ginger in grouper for 12 weeks resulted in either improved growth, or enhanced innate immune defenses or both and improved resistance against *V. harveyi* infection. The positive growth promoting effects of ginger may be due to their chemical and physical properties; their positive immunostimulating effect or stimulates digestion as it influences positively the terminal enzymes of digestive process and improving protein and fat metabolism (Platel and Srinivasan, 2000); bioactive compounds on improving antioxidant status of the fish (Rababah *et al*., 2004), antimicrobial (Mahady *et al*., 2003) and various pharmacological effects (Ali *et al*., 2008). All of these have favorable effects on gut function, which is the primary mode of action for growth promoting feed additives (Windisch *et al*., 2008).

Health status and immunological response:

Condition factor and survival rate is shown in Table 4. It was shown that, both of condition factors and survival rate of the fish were ideal in all groups of the experiment. 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 of *L. calcarifer* after infection with *V. harveyi.* 5. 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 (Talpur *et al*., 2013). 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 presumably be

attributed to a better coordination of their stimulatory and antioxidant scavenging properties (Apines-Amar *et al*., 2012). Dietary supplements had significantly ($P < 0.05$) increased immunity of all groups compared to the control group as shown in Table 5. Lysozymes are catalysing the hydrolysis of peptidoglycans of bacterial cell walls and acts as non-specific innate immunity molecules against the incursion of detrimental bacteria (Saurabh and Sahoo, 2008). The serum lysozyme activity is considered as a defence barrier against bacterial pathogens thus resulting in the reduction of disease (Misra *et al*., 2006). Lysozyme; serum bactericidal activities and enhanced phagocytic activity showed significant increase in all groups given ginger diets which indicates that the immune system was enhanced in the fish (Talpur and Ikhwanuddin, 2012, 2013 and Talpur *et al*., 2013). Moreover, elevated lysozyme activity has been reported in tilapia after supplementing diets with tilapia (GIFT *Oreochromis niloticus*) when fed with *Sophora flavescens* (Wu *et al*., 2013).

Natural Igs 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 LPS, viral and parasitic products. All the supplemented groups especially the ginger-fed fish exhibited significantly increased total Ig levels (Table 3) suggesting better immunocompetence. On the whole, it appeared that the diets supplemented with ginger stimulated lysozyme and total Ig production better than the other groups (Apines-Amar *et al*., 2012).

Infection with *Aeromonas hydrophila*:

The survival and mortality rate of *O.niloticus* infected with pathogenic strain of *A. hydrophila* is shown in Table 6. The results indicated that the highest survival rate was in groups 5 and 6. This may

be due to the excellent effect of ginger in improving the immune response of fish against the infection with *A. hydrophila*. These results are completely agreed with Talpur *et al*. (2013).

CONCLUSION

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 growth performance and immune status of fish compared with the control. High mortalities might be avoided if ginger could be provided to fish before the onset of diseases. Ginger has been suggested as growth promoter and immunostimulatant due to their biological effects. Ginger has been reported that have good effect against the infection with *Aeromonas hydrophila*.

Table 1. Proximate chemical composition of feedstuffs used in formulation of the experimental diets (analyzed).

Ingredient	Nutrient (% as fed basis)					
	DM	CP	EE	CF	Ash	NFE(calculated)
Yellow corn	89.00	8.75	3.70	2.20	1.20	73.15
Ginger	87.40	5.75	1.25	4.53	3.11	72.76
Wheat flour	88.90	12.80	2.50	1.50	1.60	70.50
Soybean meal	90.00	43.70	1.80	6.10	6.50	31.90
Fish meal	94.80	63.40	8.70	0.7	20.70	1.30
Poultry by-product meal	92.60	60.30	12.70	2.10	14.70	2.80

(DM= Dry matter, CP= Crude protein, EE= Ether extract, CF= Crude fiber and NFE= Nitrogen free extract).

Table 2. Chemical composition of the experimental diets.

Ingredient	Experimental diets					
	Control diet	Ginger additive in diets				
		0.1%	0.2%	0.3%	0.5%	1%
Yellow corn	35.00	34.99	34.98	34.97	34.50	34
Ginger	0.00	0.10	0.20	0.30	0.50	1.00
Wheat flour	10.00	10.00	10.00	10.00	10.00	10.00
Soybean meal	10.00	10.00	10.00	10.00	10.00	10.00
Fish meal	18.00	18.00	18.00	18.00	18.00	18.00
Poultry by-product meal	16.00	16.00	16.00	16.00	16.00	16.00
Vegetable oil	14.00	14.00	14.00	14.00	14.00	14.00
Vitamins and Minerals mixture*	5.50	5.50	5.50	5.50	5.50	5.50
Calculated composition	1.50	1.50	1.50	1.50	1.50	1.50
Calculated composition						
DM, %	84.37	84.37	84.37	84.37	84.36	84.36
CP, %	30.79	30.79	30.79	30.79	30.78	30.76
EE, %	10.26	10.26	10.26	10.25	10.25	10.24
CF, %	2.42	2.43	2.43	2.43	2.44	2.45
Ash, %	7.12	7.12	7.12	7.13	7.13	7.14
NFE, %	38.99	38.99	38.99	38.99	38.99	38.99
DE, Kcal/ kg diet**	2944.41	2944.16	2943.90	2943.65	2943.14	2941.86

* Vitamin and Mineral mixture (alfakema):- Each 1 kg contains:-Vit. A 580000 I.U, vit.D3 8600 I.U, vit.E. 720 mg, vit. K3 142 mg, vit C 0.1 mg, vit B1 58 mg, vit B2 34 mg, vit. B6 34 mg , vit.B12 58 mg , Folic acid 86 mg , Pantothenic acid 8 mg , Manganese sulfate 65 mg , Zinc methionine 3000 mg , Iron sulfate 2000 mg , Copper sulfate 3400 mg , Cobalt sulfate 572 mg , Sodium selenite 25 mg, Calcium iodide 25 mg, Calcium carbonate (Carrier substance) till 1000 gm.

** digestible energy calculation based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm, NFE 2.5 kcal/gm (Santiago *et al.*, 1982).

(DM= Dry matter, CP= Crude protein, EE= Ether extract, CF= Crude fiber and NFE= Nitrogen free extract).

Table 3. Effect of dietary supplementation with different levels of ginger on growth performance of *O. niloticus*.

Ingredient	Experimental diets					
	Control diet	Ginger additive in diets				
		0.1%	0.2%	0.3%	0.5%	1%
Initial body weight (g)	30.66 ±0.37[a]	30.62 ±0.48[a]	30.61 ±0.39[a]	30.62 ±0.54[a]	30.69 ±0.45[a]	30.62 ±0.53[a]
Final body weight (g)	43.57 ±0.59[e]	44.32 ±0.86[e]	46.58 ±0.56[d]	50.44 ±0.46[c]	53.47 ±0.64[b]	56.80 ±0.64[a]
Body weight gain (g)	12.90 ±0.21[f]	13.70 ±0.39[e]	15.97 ±0.25[d]	19.81 ±0.15[c]	22.78 ±0.19[b]	26.18 ±0.22[a]
Body weight gain (%)	42.08 ±0.18[e]	44.73 ±0.60[e]	52.17 ±0.76[d]	64.76 ±1.55[c]	74.22 ±0.49[b]	85.54 ±1.41[a]
Specific growth rate %	0.39 ±0.001[f]	0.41 ±0.004[e]	0.47 ±0.005[d]	0.56 ±0.01[c]	0.62 ±0.003[b]	0.69 ±0.008[a]
Feed consumption (g)	41.88 ±0.78[a]	43.66 ±0.25[ab]	43.32 ±0.77[a]	42.66 ±1.11[ab]	43.18 ±0.79[ab]	44.33 ±0.48[ab]
Feed conversion ratio	3.24 ±0.01[a]	3.19 ±0.08[a]	2.71 ±0.08[b]	2.15 ±0.05[c]	1.89 ±0.01[d]	1.69 ±0.01[e]

[a-f] Mean in the same row with different superscripts are significantly different at ($P < 0.05$).

Table 4. Effect of dietary supplementation with different levels of ginger on condition factor and survival rate of *O. nilioticus*.

Ingredient	Experimental diets					
	Control diet	Ginger additive in diets				
		0.1%	0.2%	0.3%	0.5%	1%
Initial body length (cm)	11.06 ±0.08	11.06 ±0.14	11.00 ±0.15	11.06 ±0.06	11.10 ±0.11	11.06 ±0.03
Final body length (cm)	13.50 ±0.86	13.60 ±0.01	13.8 ±0.05	14.2 ±0.08	14.4 ±0.16	14.7 ±0.50
Condtion factor (K)	1.77 ±0.12	1.76 ±0.1	1.77 ±0.02	1.76 ±0.03	1.79 ±0.1	1.79 ±.01
Survival rate %	97	98	97	98	98	97

Table 5. Effect of dietary supplementation with different levels of ginger on immune status of *O. nilioticus*.

Ingredient	Experimental diets					
	Control diet	Ginger additive in diets				
		0.1%	0.2%	0.3%	0.5%	1%
Initial IgM value (µg /ml)	21.53 ±0.28[a]	21.16 ±0.33[a]	20.75 ±0.37[a]	21.85 ±0.42[a]	21.00 ±0.76[a]	21.32 ±0.44[a]
Final IgM value (µg /ml)	27.50 ±0.57[f]	30.20 ±0.15[e]	32.05 ±0.24[d]	33.83 ±0.33[c]	38.41 ±0.01[b]	41.40 ±0.66[a]
Initial lyzozyme value (µg /ml)	14.00 ±0.25[a]	13.50 ±0.57[ab]	12.55 ±0.51[ab]	12.83 ±0.33[ab]	12.26 ±0.69[b]	12.88 ±0.38[ab]
Final lyzozyme value(µg /ml)	17.83 ±0.33[f]	20.00 ±0.26[e]	22.11 ±0.33[d]	24.57 ±0.04[c]	27.50 ±0.22[b]	30.33 ±0.44[a]

[a-f] Mean in the same row with different superscripts are significantly different at ($P < 0.05$).

Table 6. Results of experimental infection of *O. niloticus* with *Aeromonas hydrophila*.

Ingredient	Experimental diets					
	Control diet	Ginger additive in diets				
		0.1%	0.2%	0.3%	0.5%	1%
Survival percentage (%)	35	40	60	75	80	90
Mortality rate percentage (%)	65	60	40	25	20	10

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التأثير الغذائي للجنزبيل على كفاءة النمو، الاستجابة المناعية لأسماك البلطي النيلي ومقاومة الاصابة بالايروموناس هيدروفيلا

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الملخص العربى

اجريت هذه الدراسه لتوضيح تاثير الجنزبيل علي معدلات النمو والحاله المناعيه لاسماك البلطي وتوضيح مدي قدرته علي زياده قدره الاسماك البلطي المناعيه علي مواجهه العدوي بميكروب الايروموناس هيدروفيلا. اجريت هذه الدراسة على عدد ٣٦٠ سمكه من اسمكا البلطي النيلي بمتوسط وزن ٣٠ ± ١ جم من مفرخ العباسه بمحافظه الشرقيه. تم تقسيم الاسماك الي ٦ مجموعات متساوية وكل مجموعه تحتوي علي ٣ مكررارات . غذيت الأسماك علي سته علائق متساوية في نسبة البروتين (٣٠ %) والطاقة المهضومة (٢٩٤٠ كيلو كالوري/كجم) بنسبة ٥% من متوسط الوزن الكلي للمجموعات قسمت على اربع مرات يوميا لمدة ١٠ اسابيع . كانت المعاملات كالتالي: ١- عليقة ضابطة، ٢- عليقة ضابطة أضيف إليها الجنزبيل (١ جرام / كيلوجرام عليقة)، ٣- عليقة ضابطة أضيف إليها الجنزبيل (٢ جرام / كيلوجرام عليقة) و ٤- عليقة ضابطة أضيف إليها الجنزبيل (٣جرام / كيلوجرام عليقة). ٥- عليقة ضابطة أضيف إليها الجنزبيل (٥جرام / كيلوجرام عليقة) . ٦- عليقة ضابطة أضيف إليها الجنزبيل (١٠جرام / كيلوجرام عليقة). تم وزن الاسماك فى بداية ونهاية التجربة وحساب متوسط وزن الجسم ، معدل ونسبة الزيادة اليومية في وزن الجسم ، معدل استهلاك العلف ومعامل التحويل الغذائي.

في نهاية التجربة تم اختيار ٥ اسماك عشوائيا من كل مجموعة للحصول على الدم لقياس نسبه الليزوزيم وايضا الاجسام المناعيه. ثم عدوي الاسماك بميكروب ضروي للايروموناس هيدوفيلا السابق عزلها وتنصنيفها ومعرفه تاثيره علي الاسماك السابق ذكرها و قد بينت النتائج ان اضافة الجنزبيل خاصه بنسب ٥ و ١٠% ادت إلى ظهور تأثير معنوي على وزن الاسماك، معدل ونسبة الزيادة اليومية ومعامل التحويل الغذائي مقارنة بالعليقة الضابطة. حاله الاسماك الصحية كانت جيده ومعدلات الاعاشه مرتفعه وايضا عدم وجود اي اعراض مرضيه علي الاسماك كما انها كانت تستجيب لكافه المؤثرات الخارجية وخاصه الهروب. ادي إلي تحسين معنوي في مستوي الليزوزيم وايضا الاجسام المناعية مقارنة بالعليقة الضابطة. زياده مقاومه الاسماك لميكروب الايروموناس هيدروفيلا مقارنه بالاسماك التي تمت تغذيتها علي العليقه الضابطه.