Global Animal Science Journal-GASJ 1(4):94-100, 2014; DOI:10.5281/zenodo.26737
Khafagy et al 2014; Research Article

Effects of some immune-stimulants on Catfish immune response against *Aeromonas hydrophila*

Khafagy A.A.R.; Hamza M.I.; Algammal A.M. and Reham M.T

Department of Bacteriology, Mycology and Immunology .Faculty of veterinary medicine, Suez Canal University, Egypt

Submitted on 2nd January, Accepted on 10th March 2014, published on 1st July 2014

Abstract

In order to determine the effect of levamisole HCL and ginger on the immune response of Catfish against *A.hydrophila* infection, A total number of 80 catfish (*clarias lazera*) of both sexes were obtained alive from el Tamsah lake at Ismailia, Egypt. Fish divided in 8 groups, where group 8 served as control without any additives. groups 1-3 were fed with levamisole HCL ,ginger and mixture of levamisole HCL+ ginger, respectively .Fish of group 4 vaccinated only with *A.hydrophila* formalized-killed vaccine .Fish of groups 5-7 were fed with the same as group 1-3 and vaccinated. Serum samples were collected to determine the level of antibodies by plate agglutination test **,** indirect heamagglutination and estimation of levels of total serum protein fractionation by Polyacrylamide gel electrophoretic analysis of serum proteins **.**Also All fish groups were injected I/P with 0.2 ml of virulent strain of *A. hydrophila* where the relative level of protection among the challenged fish was determined. Briefly, levamisole and ginger help to enhance the immune response of catfish to some vaccines and against infection but levamisole achieve better result than ginger. Ginger and levamisole if added to vaccine achieve best result than levamisole only or ginger only.

Keywords: catfish, levamisole, Aeromonas hydrophila, Tamsah Lake

INTRODUCTION

Aquatic animal diseases control in Egypt includes a limited number of Government approved antibiotics and chemotherapeutics, beside limited vaccines that can be used to assist the environmental management (Aly et al., 2000). Motile Aeromonas Septicemia is one of the most important bacterial diseases affecting fish. the isolation of Aeromonas species from healthy and diseased fish has been reported on world wide basis (Austin and Austin, 1987). Immunotherapy is an approach that has been actively investigated in recent years as a method for disease prevention. It does not involve recognition of a specific antigen or targeting the immune response towards a specific pathogen, but causes an overall immune response that hastens recognition of foreign proteins (Sordello et al., 1997). So the use of immunostimulants for prevention of diseases in fish is considered an alternative and promising area (Sakai, 1999). Levamisole, originally synthesized as an anti-helminthic, has been widely used as an immunomodulator in fish either by injection (Siwicki, 1987), in vitro immunostimulation (Siwicki et al., 1992), or immersion (Siwicki and Korwin 1988), oral administration(Siwicki et al.,, 1989) or in vitro immunostimulation (Siwicki et al., 1992). The use of immunostimulants as an alternative to the drugs, chemicals and antibiotics currently being used to control fish diseases in fish culture is attracting the attention of many researchers. In this context, many have focused on the use of medicinal plant and

Corresponding author: Ahmed Refat Khafagy. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.; e-mail: ahmed_khafagy@vet.suez.edu.eg

animal originated products as potential therapeutic measures for modulating the immune response to prevent and control fish diseases. The possible use of naturally available herbal extract such as Zingiber officinale (Ginger) (Mukesh Kumar Bairwa,2012).

This work was designed to study the effect of levimisole HCL and ginger on the immune response of Catfish with or without vaccination using A. hydrophila vaccine .

MATERIALS AND METHODS

Fish:

A total numbers of 80 catfish (*clarias lazera*) of both sexes with 120+ - 10 g body weight were obtained alive from el Tamsah lake during winter season (2013) transported to eight glass aquaria each , 50 liter capacity were used for performing the expiremental infection. Aquaria were supplied with continous aeration using air pumps and chlorine free water used according to (**Innes ,1966*).***

Immunostimulants:

1-LEVAMISOLE® HCL (Memphis)

It's a commercial product available in the pharmacy manufactured by Memphis Pharmaceutical, Cairo, Egypt. Each one ml contains 0.1g levamisole HCL in form of tablet. The dose was calculated to be 150mg /kg diet then mixed with the basal diet and kept at room temperature and given to fish daily. **(Nevien K. M. Abdelkhalek2008)**

2- Ginger:(Market)

It's a commercial powdered product available in markets .It's obtained from market in Portsaid , Egypt. The dose was calculated to be 10g /kg diet then mixed with the basal diet and kept at room temperature and given to fish daily.

Experimental design (Table 1):

Fish divided in 8 groups, group 8 served as control without any additives. groups 1-3 were fed with levamisole HCL (150mg /kg diet),ginger (10 g/ kg diet) and mixture of levamisole HCL (150mg /kg diet) and ginger(10 g/ kg diet) , respectively .Fish of group 4 vaccinated only with formalized-killed vaccine.Fish of group 5-7 were fed with the same as group 1-3 and vaccinated with formalized-killed vaccine (levamisole HCL (150mg /kg diet) +vacc-ginger (10 g/ kg diet) +vacc.and mixture of levamisole HCL (150mg /kg diet) and ginger(10 g/ kg diet)+vacc.) , respectively. The experiment extended for 4 weeks after acclimatization. Serum samples were taken at 0, 7,14,21,28 day of experiment

Table (1): Showing groups and their treatment.

Groups	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
levamisole+ginger+vaccine							+	
Levamisole	+							
Ginger		+						
Levamisole+ginger			+					
Vaccine				+				
Levamisole+vaccine					+			
Ginger+vaccine						+		
Control								+

Determination of non-specific immune response:

Serum samples were collected in Eppendorf tubes and kept in refrigerator to determine level of antibody by plate agglutination test **(Franc and Westwood 2002)** and indirect heamagglutination **(carter, 1955)** and estimation of levels of total serum protein fractionation by **Polyacrylamide** gel electrophoretic analysis of serum proteins **(Jovin et al., 1971).**

Corresponding author: Ahmed Refat Khafagy. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.; e-mail: ahmed_khafagy@vet.suez.edu.eg

Bioassay against *A. hydrophila*

All fish groups were injected with 0.2 ml of virulent strain of ***A. hydrophila*** (containing 10^8 bacteria ml^{-1}) via intraperitoneal route. The challenged fish were kept under observation for 1 week and the dead fish were counted and subjected for bacterial reisolation. The relative level of protection (RLP) among the challenged fish was determined **(Newman and Majinarich 1982)** using the following equation.

RLP = 1- percent of immunized mortality – percent of control mortality X 100.

Statistical analysis:

Statistical analysis was performed using the one-way analysis of variance (ANOVA)**.** Multiple Range Test was done to determine differences between treatments (mean at significance level of $P< 0.004$). Standard errors were also estimated. All analysis was run on the computer using the SAS program **(SAS, 2000)**.

RESULTS

1- Results of plate agglutination test in examined fish:

Table (2): Collective Table of plate agglutination test at 0th, 7th, 14th, 21th and 28th day:

Group	Treatment	0th day	7th day	14th day	21th day	28th day
1	Levamisole HCL	0.5	20	40	40	160
2	Ginger	1	10	20	40	40
3	Mixture of Levamisole HCL and Ginger	1	20	40	160	160
4	Vaccine	0.5	40	80	160	320
5	Levamisole HCL + vaccine	1	40	160	320	640
6	Ginger + vaccine	0.5	40	80	160	320
7	Mixture of Levamisole HCL and Ginger + vaccine	1	80	320	640	1280
8	Control	1	1	1	1	1

2- Results of indirect Hemaglutination test in examined fish:

Table (3) Collective Table of indirect Hemaglutination test at 0th, 7th, 14th, 21th and 28th day:

Group	Treatment	0th day	7th day	14th day	21th day	28th day
1	Levamisole HCL	0.5	20	40	80	160
2	Ginger	0.5	10	20	40	80
3	Mixture of Levamisole HCL and Ginger	1	40	80	160	320
4	Vaccine	1	80	80	160	320
5	Levamisole HCL + vaccine	1	80	320	320	640
6	Ginger + vaccine	0.5	80	160	320	320
7	Mixture of Levamisole HCL and Ginger + vaccine	0.5	160	640	640	1280
8	Control	1	1	1	1	1

3-Effects of levamisole and ginger on the level of serum proteins in examined fish:

A-serum pre albumin and albumin:

Table (4) Effects of levamisole and ginger on the level of-serum pre albumin and albumin:

Group	Treatment	No. of fish tested	Pre-albumin	Albumin
1	Levamisole HCL	6	1.745±0.333	24.500±0.122
2	Ginger	8	1.368±0.564	23.132±0.476
3	Mixture of Levamisole HCL and Ginger	8	1.93±0.562	24.890±0.134
4	Vaccine	8	2.490±0.0567	26.789±0.0314
5	Levamisole HCL + vaccine	8	2.87±0.134**	27.823±0.045**
6	Ginger + vaccine	8	2.654±0.365*	27.543±0.673*
7	Mixture of Levamisole HCL and Ginger + vaccine	8	3.310±0.146***	28.782±0.560***
8	Control	8	0.822±0.105	21.120±0.142

The result were statistically interpretated as : * highly significant(P<0.001); ** moderate significant(P<0.01); * slightly significant(P<0.05); non-significant(P<0. 5)**

Corresponding author: Ahmed Refat Khafagy. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.; e-mail: ahmed_khafagy@vet.suez.edu.eg

B-Alpha globulins:

Table (5) Effects of levamisole and ginger on the level of Alpha globulin

Group	Treatment	No. of fish tested	1 globulin	2 globulin
1	Levamisole HCL	6	2.147±0.243	5.756±0.122
2	Ginger	8	1.836±0.652	4.653±0.673
3	Mixture of Levamisole HCL and Ginger	8	2.96±0.562	5.990±0.356
4	Vaccine	8	2.89±0.765	6.323±0.135
5	Levamisole HCL +vaccine	8	3.654±0.429**	7.563±0.743**
6	Ginger + vaccine	8	3.290±0.0553*	6.799±0.234*
7	Mixture of Levamisole HCL and Ginger +vaccine	8	4.210±0.166***	8.710±0.560***
8	Control	8	1.576±0.135	3.120±0.232

The result were statistically interpreted as : * highly significant(P<0.001); ** moderate significant(P<0.01); * slightly significant(P<0.05); non-significant(P<0. 5)**

C-Beta globulins:

Table (6) Effects of levamisole and ginger on the level of Beta globulins

Group	Treatment	No. of fish tested	B1 globulin	B2 globulin	B3 globulin
1	Levamisole HCL	6	14.789±0.0354	12.256±0.542	1.674±0.897
2	Ginger	8	14.136±0.532	11.780±0.123	1.365±234
3	Mixture of Levamisole HCL and Ginger	8	15.243±0.565	13.043±0.346	1.934±0.356
4	Vaccine	8	16.290±0. 556	13.299±0.123	2.145±0. 967
5	Levamisole HCL +vaccine	8	17.454±0.429**	13.823±0.165**	2.900±0.567**
6	Ginger + vaccine	8	16.890±0.765*	13.563±0.834*	2.563±0.678*
7	Mixture of Levamisole HCL and Ginger +vaccine	8	18.543±0.166***	14.720±0.560 ***	3.564±0.234** *
8	Control	8	13.584±0.339	10.150±0.324	1.178±0.235

The result were statistically interpretated as :* highly significant(P<0.001); ** moderate significant(P<0.01); * slightly significant(P<0.05); non-significant(P<0. 5)**

D-Gamma globulins:

Table (7) Effects of levamisole and ginger on the level of Gamma globulins

Group	Treatment	No. of fish tested	gamma1 globulin	gamma2 globulin	gamma3 globulin	Gamma 4 Globulin
1	Levamisole HCL	6	0.785±0.0534	3.596±0.897	17.674±0.886	2.435±0.564
2	Ginger	8	0.536±0.832	3.480±0.763	17.165±984	2.245±0.675
3	Mixture of Levamisole HCL and Ginger	8	1.043±0.056	3.298±0.533	18.145±0. 853	2.578±0. 546
4	Vaccine	8	1.110±0. 896	3.643±0.336	18.834±0.457	2.690±0.754
5	Levamisole HCL +vaccine	8	1.652±0.529**	4.463±0.054**	19.563±0.978**	3.406±0.684**
6	Ginger + vaccine	8	1.290±0.895*	3.823±0.845*	18.900±0.567*	2.789±0.564*
7	Mixture of Levamisole HCL and Ginger +vaccine	8	1.943±0.166***	4.720±0.893 ***	25.786±0.234***	3.976±0.034***
8	Control	8	0.234±0.323	3.150±0.345	14.034±0.135	1.805±0.675

Corresponding author: Ahmed Refat Khafagy. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.; e-mail: ahmed_khafagy@vet.suez.edu.eg

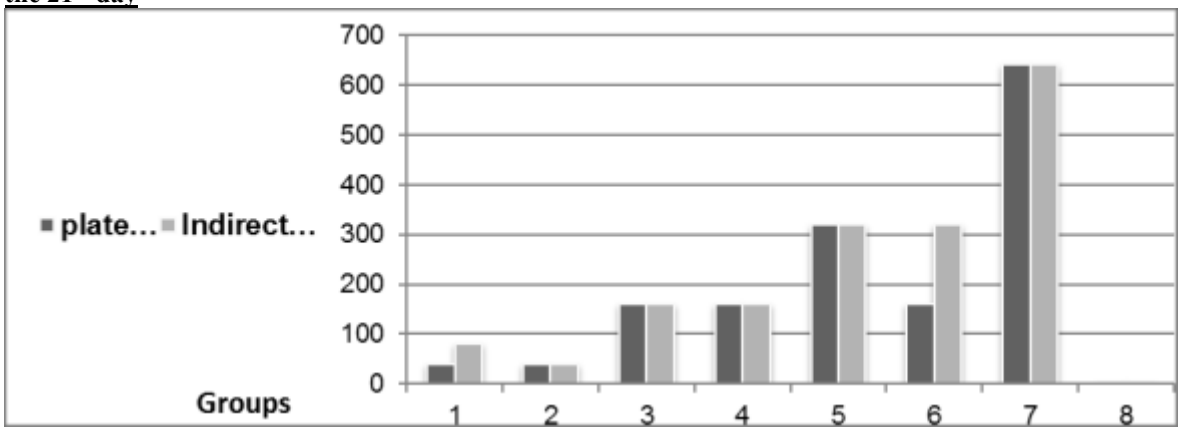
The result were statistically interpretated as : * highly significant(P<0.001); ** moderate significant(P<0.01); * slightly significant(P<0.05); non-significant(P<0. 5)**

4-Results of protection against virulant *A.hydrophila*:

Table (8) Relative level of protection after challenge among all examined groups:

Group	Treatment	No. of challenged fish	challenged dose	living	Dead	RLP(%)
1	Levamisole HCL	6	0.2 ml	3	3	50.2
2	Ginger	6	0.2 ml	3	3	50.2
3	Mixture of Levamisole HCL and Ginger	6	0.2 ml	3	3	50.2
4	Vaccine	6	0.2 ml	5	1	89.3
5	Levamisole HCL + vaccine	6	0.2 ml	4	2	66.6
6	Ginger + vaccine	6	0.2 ml	4	2	66.6
7	Mixture of Levamisole HCL and Ginger + vaccine	6	0.2 ml	6	0	100
8	control	6	0.2ml	1	5	16

Figure (1): Comparative studies between plate agglutination test and indirect Hemaglutination test at the 21th day



DISCUSSION

In our results, the addition of levamisole to the diet of catfish at small doses 150mg/kg of diet stimulated the humoral immune response against ***A. hydrophila*** bacterin, the increase was significant at the first week and highly significant at the fourth week of experiment as shown in (Tables 6, 7). These results are parallel to those reported by **(Siwicki et al., 1990)**. The immune stimulation of levamisole at small doses may be attributed to the activation of the non-specific immune response particularly macrophages, this activation could enhance the antigen trapping and processing **(Fischer, et al. 1975)**. The results of the present study revealed that the total serum protein content in the control (infected) group increased significantly (P<0.001) when fish were intra-peritoneally challenged with ***A. hydrophila***. Total serum protein content at the end of experimental trial was lowest in control (infected) group and highest in group7 which vaccinated and feeded with levamisole and ginger. **Dina *et al.* (2007)** also found that total serum protein content was significantly enhanced in levan fed common carp fingerlings against the infection of ***A. hydrophila*** while the lowest values were found in control (infected) group. (Bruno and Munro, 1986) obtained similar

Corresponding author: Ahmed Refat Khafagy. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.; e-mail: ahmed_khafagy@vet.suez.edu.eg

results in rainbow trout and Atlantic salmon experimentally infected with ***Renibacterium salmonarium***.

In the present study, the serum biochemical parameters like total serum protein, albumin and globulin were significantly ($P<0.05$) enhanced in the levamisole supplemented groups 1,5, 7 particularly in 150 mg levamisole /kg of diet. The highest values were recorded in levamisole supplemented groups with chitosan. and this result agreed with **(Sajid , 2009)** who used different doses of levamisole (125 mg,250 mg,500 mg) and(**Mesalhy , 2010**) who used the same dose of this study and found group of vaccine and levamisole has the best result than group of levamisole or vaccine only. The increase in serum total protein, albumin and globulin is similarly in line with previous work involving immunostimulants, namely chitosan and β-glucan (**Siwicki 1989)**. Ginger conferred health benefits in terms of a reduction in mortalities after challenge and a heightened effect on non-specific immune mechanisms. Ginger is recognized to have broad-spectrum activity including activation of phagocytic cells, which is an important component of the non-specific immune system of fish (**Ahmed, Seth, Pasha & Banerjee 2000)**. A possible mode of action of ginger is in immunostimulation as a result of its bioactive constituent, gingerol, which has been reported to induce the activity of interleukin-6 (IL-6) (**Benny *et al.* 2004)**.

In the current study, the groups challenged with ***A. hydrophila*** after vaccination and giving immunostimulants showed better results (lower mortalities), in the groups received vaccine or mixture of levamisole and ginger than other levamisole or ginger group only, these results agree with that obtained by (**Erdal and Reitan , 1992**) .

In conclusion, levamisole and ginger help to enhance the immune response of catfish to some vaccines and against infection but levamisole achieve better result than ginger. Ginger and levamisole if added to vaccine achieve best result than levamisole only or ginger only.

References

Austin B., and Austin D.A.(1987): Bacterial fish pathogens .Diseases of farmed and wild fish. 2nd ed. Chichester: Simon and Schuster .pp .111-117.

Aly S., Hekmat M. Tantawy, Badran A. and Magda A. EL-Baz(2000a): Histopathologic and immunologic response of *Clarias Lazera* to the injection of ***Aeromonas hydrophila*** vaccine . Suez Canal Vet.Med J., III (1), 133-144.

Ahmed R.S., Seth V., Pasha S.T. & Banerjee B.D. (2000) Influence of dietary ginger Zingiberofficinale Roscoe, on oxidative stress induced by malathion in rats. Food and Chemical Toxicology 38, 443–450.

Bruno, D.W. and Munro, A.L.S. (1986): Haematological assessment of rainbow trout, *Salmo gairdneri* Richardson, and Atlantic salmon, *Salmo salar* L., infected with *Renibacterium salmoninarum*. Journal of Fish Disease, 9: 195-204.

Carter, G.R. (1955) Studies on *Pasturella multocida* .I.A haemagglutination test for the identification of seriological types .Am. J. Vet .Res .16,481-484.

Duncan D (1955): Multiple range and multiple F test Biometrics 11:1-2.

Dina Rairakhwada, A.K. Pal, Z.P., Bhathena, N.P., Sahu, A. Jha and Mukherjee, S.C. (2007). Dietary microbial levan enhances cellular non-specific immunity and survival of common carp (*Cyprinus carpio*) juveniles. Fish and Shellfish Immunology, 22(5): 477-486. (1)68-75.

Corresponding author: Ahmed Refat Khafagy. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.; e-mail: ahmed_khafagy@vet.suez.edu.eg

Fischer, G.W., Podgore, J.K. & Bass, J.W. (1975): Enhanced host defense mechanisms with levamisole in suckling rats. Journal of Infectious Diseases. 132 (5), pp. 578-581.

Frank C.Hay and Olwya M.R. Westwood (2002):: Library of congress cataloging-in-Publication Data. Practical Immunology 4th ed. 103 page.

Jovin, T.N.; Dante, M.L.and Chrumback, A. (1971) some use-ful polyacrylamide gel electrophoresis system U.S. Gov. Res. And Dev. Reports PB – 196085 through PB 196092.

Mukesh Kumar Bairwa Jitender Kumar Jakhar, Satyanarayana Y and A. Devivaraprasad Reddy (2012) Animal and plant originated immunostimulants used in aquaculture. *J. Nat. Prod. Plant Resour*., 2012, 2 (3): 397-400 .

Newman S.A. and Majinarich J.J. (1982): Direct immersion vaccination of Juvenile rainbow trout, Slam gairidi and juvenile Coho salmon (Oncorhynchu kisutch) with Yersinia ruchari bacterin J. of Diseases, 5:338-341.

Nevien K. M. AbdELkhalek, Viola H. Zaki and M. A. A.(2008) Effect of some innunostimulants on health status and disease resistance of Nile Tilapia (*OREOCHROMIS NILOTICUS*) *8th International Symposium on Tilapia in Aquaculture 2008*.

Sakai,M.(1999): Current research status of fish Immunostimulants. Aquaculture 172: 63-92.

Siwicki, A. (1987):Immunomodulatory activity of levamisole in carp spawners, *Cyprinus cariop L.* Journal of Fish Biology (Supp. A) 31:242-246.

Siwicki, A.K. and M. Korwin-Kossakowski. (1988): The influence of levamisole on the growth of carp *(Cyprinus Carpio)* larvae. Journal of Applied Ichthyology 4: 178-181.

Siwicki, A. K. (1989) Immunostimulating influence of levamisole on non- specific immunity in carp *(Cyprinas carpio)*. Dev. Comp. Immunol. 13, 87-91.

Siwicki, A., D. P. Anderson and O. W. Dixon. (1992) In vitro effect of Levamisole on the neutrophil activity in rainbow trout *(Oncorhynchus mykiss)*. Arch. Immunol. Ther. Exp. 40(5-6). 253-256.

SAS (2000); Statistical analysis system North caroline 37513, USA.

sordillo. and Campos, M., D. Godson, H. Hughes, L. Babiuk (1997). The role of biological response modifiers in disease control. Journal of Dairy science, 76: 2407-2417.

Sajid Maqsood, M.H. Samoon, Prabjeet Singh(2009) Immunomodulatory and Growth Promoting Effect of Dietary Levamisole in *Cyprinus carpio* Fingerlings Against the Challenge of *Aeromonas hydrophila* Turkish Journal of Fisheries and Aquatic Sciences 9: 111-120

Salah Mesalhy Aly; Osama Abd-Allah; Amina Mahmoud and Hala Gafer(2010) Efficiency of Levamisole in Improving the Immune Response of Catfish (*Clarias gariepenus*) to *Aeromonas hydrophila* Vaccine: Clinico- Pathological Studies *Mediterranean Aquaculture Journal* 2010 1(1); 8-17

Corresponding author: Ahmed Refat Khafagy. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.; e-mail: ahmed_khafagy@vet.suez.edu.eg