Exp Rev Immunol Vaccine Informat
Volume 2 Issue1 March 2015

Original Article

Gelatin Chitin and Carboxy Methyl Cellulose versus Live *Aeromonas hydrophila* Live Bacterin as Immunomodulants in Common Carp *Cyprinus carpio*

Ibrahim Mohamed Seed Shnawa*, Bashar Abdul-Hussain Ellewi Al-Sa'adi**, Khalidah Salim Al-Niaeem**

*College of Biotechnology, University of Kasim, Kasim, Babylon, Iraq

** Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Iraq

***Corresponding author:** Ibrahim Mohamed Seed Shnawa, Professor, College of Biotechnology, University of Kasim, Kasim, Babylon, Iraq, Tel: +9647800188929; E-mail: ishnawa@yahoo.com

Received Date: 27 February 2015; **Accepted Date:** 10 March 2015; **Published Date:** 18 March 2015

Citation: Shnawa MSI, Ellewi AB, Al-Niaeem KS. Gelatin Chitin and Carboxy Methyl Cellulose versus Live Aeromonas hydrophila Live Bacterin as Immunomodulants in Common Carp Cyprinus carpio. Exp Rev Immunol Vaccine Informat. 2015; 2 (1): 62-66.

Abstract

Eleven common carp fish Cyprinus carpio groups each of ten with an average of 100 gm body weight were the study groups. These groups were as, live bacterin, gelatin 1, 2 and 5%, chitin 1,2, and 5%, as well as Carboxy methyl cellulose 1, 2, and 5% and saline control. The test fish groups were constituted within two main categories, the antigen dependent, and combined antigen dependent- independent. The antigen dependent has shown four folds rise in the specific antibody titer than primary response and control. While the combined were showing up to two folds than primary response and control. Thus, *A.hydrophila* bacterin is preferable than chitin, gelatin and carboxy methyl cellulose in their immunomodulatory potentials.

Keywords: Common carp; Bacterin; Antigen; Antibody; Titer; Immunomodulation

Introduction

The immune response in teleost fish as in other mammalian vertebrate can be regulated [1]. The indication for such regulation is either presence of subnormal and abnormal immune functions [2], which, in turn needs augmentation in the first and suppression in the second case [3]. Such regulatory substances are known as immunomodulants and the state as an immunomodulation. The immunomodulants are either potentiators or suppressors [4,5]. The natures of immunomodulants can be chemical, physical, or biological [5]. In the chemical sense, they were; natural, semi-synthetic and synthetic [6]. Chemical immunomodulation is depend on both chemical nature and dose dependent theme. Immunomodulation is either antigen dependent like vaccines, probiotics or antigen independent like levamisole and chitosane [7].

In fish production ponds, bacterial infection in any one of which stands as an ecologic insult in an epiozootic or enzootic pathogenic out-breaks that suggests the use of immunomoduilants in the others [8,9]. The present work aims at the use of two immunomodulating modalities in regulation of fish immune responses.

Materials and Methods

Heat killed *Aeromonas hydrophila* bacterin was prepared and ratified to the density of 1/10 7 in accordance with [10]. The LD50 live challenge infectious dose of *A.hydrophila* was made as in [11]. The applied test immunomodulants were prepared as; *A.hydrophila* live vaccine, gelatin 1, 2 and 5%, chitin 1, 2, and 5% [12] as well as carboxy methyl cellulose 1, 2, and 5%.

Eleven common carp fish *Cyprinus carpio* groups each of ten with an average of 100 grams for each. These fish groups were kept during the experimentation period in an aquaculture systems that were monitored in a week wise manner for PH, salt, weight variations and given minimal feed prepared with continual shift of an O_2 aeration and dynamic continuity as in [13,14]. The fish groups were preconditioned for four weeks through daily minimal feed supplement with gelatin, chitin and carboxy methyl cellulose to the levels of the aforementioned concentrations. The specific immune priming programs were depicted in *Table 1*. Blood samples were collected from the subhead veins by 2.5 ml disposable syringes. Sera were saved and kept at -18°C till use. Qualitative and semi-quantitative slide and standard tube agglutination tests were made to the test and control fish sera with heat killed *A.hydrophila* bacterin [10]. Titers were scored in 2 hr and 24 hr incubation at 37°C.

Goup I : Saline negative control Category One: Antigen dependent immunomodulation	10 Fishes
Goup II : Modulation by bacterin Category Two :Combined Antigen dependent-Independent immunomodulation	10 Fishes
Group III: Chitin	
1%	10 Fishes
2%	10 Fishes
5%	10 Fishes
Group IV: Gelatin	
1%	10 Fishes
2%	10 Fishes
5%	10 Fishes
Group V: Carboxy methyl cellulose	
1%	10 Fishes
2%	10 Fishes
5%	10 Fishes

Table 1: Immunization Protocols of the test carp fish groups.

Then at the day 30, 50, 60 fish groups were injected intramuscularly with live *A. hydrophila* challenge doses. Blood sampling were done few days after the 1st, 2nd, and 3rd injections.

Results

The antigen dependent immunomodulation

Saline control sera have shown nil titer with *A. hydrophila* agglutinogens. Primary immune responses were with titers of 320 and secondary immune response was with titer of 5120 in the bacterin group, *Table 2*.

Features	Titers*
Primary immune response	320
Secondary immune response	5120
Control	0

Table 2: The antigen dependent immunomodulation by live *A .Hydrophila* bacterin as indicated by specific antibody titers.
*Mean of two readings.

The combined antigen dependent and independent

Chitin 1, 2, 5% preconditioned fish sera gave anti *A. hydrophila* antibody titers of 10, 80, and 80 respectively in the case of primary immune responses while at the secondary immune responses were 320,640,640 accordingly, *Table 3*.

Features	Titers*
Primary Immune response in carp fish preconditioned with chitin	
1%	80
2%	10
5%	320
Secondary immune response in carp fish preconditioned with	
1%	320
2%	640
5%	0
Control	0

Table 3: Combined chitin-live *A.hydrophila* bacterin modulation as indicated by specific antibody titers.
*Means of two reading

Features	Titers*
Primary immune response of carp fish preconditioned with gelatin	
1%	640
2%	0
5%	0
Secondary immune response of carp fish preconditioned with	
1%	640
2%	0
5%	0
Control	0

Table 4: Combined immunomodulation of gelatin live *A.hydrophila* bacterin as indicated by specific antibody titers.
*Means of two readings.

Features	**Titers***
Primary immune response of carp fish preconditioned with carboxy methyl cellulose	
1%	640
2%	0
5%	0
Secondary immune response of carp fish preconditioned with carboxymethyl cellulose	
1%	640
2%	0
5%	0
Control	0

Table 5: Combined immunomodulation of carboxy methyl cellulose-live *A.hydrophila* bacterin as indicated by specific antibody titers.
*Mean of two readings.

The immunesera of carp fish groups preconditioned with Gelatin 1% and Carboxy methyl cellulose 1% have shown titers of antibodies specific for *A.hydrophila* as 640 for each case. At the days 55, 65 post to secondary immune responses; the fish sera reacted with *A. hygrophila* agglutinogens gave nil antibody titers for 1%, 2%, 5% for gelatin, chitin 1% and carboxy methyl cell 1%.

The post challenge immune protection

Both bacterin and preconditioned challenged groups have shown 100 % immune protection Tables 2-6.

Discussion

The booster doses of bacterin group have led to more than four folds increase than in primary immune response [15], this may be due to the action memory B cells or due to an intrinsic immunologic adjuvanicity of *A .hydrophila* antigens [16].

Features	**Titers***			
	Primary	Secondary	56 day	63 day
Bacterin	320	5120	0	0
chitin				
1%	80	320	0	0
2%	10	640	0	0
5%	320	0	0	0
Gelati 1%	640	640	0	0
Carboxy methyl cellulose 1 %	640	640	0	0
Control	0	0	0	0

Table 6: Carp fish immunomodulation matched in different test fish groups up to 63 days of the initiation of secondary immune response.
*Mean of two readings.

The concentration of 1%, 2% chitin at the primary immune response state may be less than their action threshold as an immunomodulants [17]. While, 5% chitin holds to be the threshold concentration as immunomodulant. At the second injection of the, live *A. hydrophila* bacterin challenge dose, however, chitin 5% concentration may activate T reg cells to inhibit the synthesis and secretion of the *A. hydrophila* specific antibodies from the antigen primed plasma cells [18]. As well as at the days 55 and 65 vanishing the specific antibody titers may be due to a natural decline state of the immune response time curve, or due to *A. hydophila* antigen-anti *A. hydrophila* antibody complexes and/or due to the action of activated macrophage immune clearance happened in vivo in the test carp fish groups [19].

Both preconditioning with gelatin and carboxy methyl cellulose 2%, 5% were found to be inhibitory to *A. hydrophila* specific antibody production. This may be due to a possible immunotoxic action to the antigen primed antibody producing plasma cells [20,21]. The action threshold of these immunomodulants may be between less than or equal to 1%, in the test carp fish groups [17].

The immunostimulating mechanisms can be through antigen targeting, activation of the cytokine network and/ontogenic activation for the developing B lymphocytes [22]. In an analogous situation, the immunosuppressive mechanisms can be; inhibition of cytokine and /or cytokine network, inhibition of B cell affinity maturation, increase of the T reg activity, as well as the inhibition of the ontogenic development of the primed B lymphocyte [23].

The rise of specific antibody titers, absence of pathologic lesions in the both external and internal organs as well as the high survivors of the immunized challenged carp fish are the basic proves for immune protection among the test carp fish groups [24].

Conclusion

The heat killed as well as the live *A. hydrophila* challenge was the better immunomodulating modality among the other test modalities. Post challenge immune protection was evident among the immunized carp groups.

Acknowledgements

The authors wish to thank the department heads of biology, College of Science, University of Babylon, Fish pathology and sea resources, College of Agriculture, University of Basrah and Fish resources department, College of Agricultural Technology, Board of Technology/Babylon, for their help extended during conduction of this work.

References

1. Uribe C, Floch H, Enriquez R, Moran M. Innate and adaptive immunity in teleost fish: A review. Veterina Med. 2011; 36 (10): 486-503.
2. Selvaraj V, Sampath K, Sekar V. Use of glycan from Sacchramyces cerevisiae as an immunostimulants in carp. J Aquacul. 2005; 57(1): 39-48.
3. Maqsood S, Samoon MH, Singh P. Immunomodulating and growth promoting effects of dietary levamisole in Cyprinus carpio fingerlings against the challenge of Aeromonas hydrophila. 2009; Turk J Fish Aquacul 9: 111-120.
4. Marzouk MS, Moustafa MM, Mohamed MM. Evaluation of immunomodulatory effects of some probiotics on cultured Orechromis niloticus. 8th international symposium on Tilapia in aquaculture. 2008; 1043–1058.
5. Jones SRM, Woo PTK, Stevenson RMW. Immunostimulation in rainbow trout Salmo gairderi R, caused by C. salmonica. J Fish Disease. 1986; 9(5): 431-438.
6. Pell JM, Ashton R. Principles of immunomodulation. Liv Prod Sci 1995; 42: 123-133.
7. Stites DP, Stobo JD, Wells JV. Basic and Clinical Immunology, 6th ed. Appleton and Lange. 1987; Middle East Edition, California.
8. Holvald LB. Immunostimulants connects innate and adaptive immunity in atlantic salmon, salmo solar. College Fishery Science, University of Tormso, 2007.
9. Kiron J. Fish immune system and its nutritional modulation for preventive health care. Anim feed Sci Tech. 2012; 173(1-2): 111-133.
10. Stevensons R. Clinical Immunology and Serology: A Practical Perspective. 2010; Davis Co. Philadelphia.
11. Reed LJ, Muench H. A Simple method for estimating fifty percent end point. Am J Hyg. 1938; 27(3): 493-497.
12. Islama Md AI, Shaikhe AA, Roy SK. Preparation of chitosane from shrimp shell and investigation of its properties. Int J Appl Sci 11(1).
13. Jagadeesh JD, Ahmed RM, Chethan N, Kamani HVD, Adija NS. Effect of dietary supplement of manan oligosaccharide on water quality parameters recalculating aquaculture system. Eu J Exp Biol. 2013; 3(3): 48-55.
14. Olsen R, Ringo E, Svihus B. Criteria for use of plant ingredients in diet of aquiculture of fish: Opinion of the panel of the feed of the Norwegian Scientific committee for feed safety. 2009.
15. Fletcher TC. Fish immunology, Citable Reviews in life Science. Encyclopedia of Life Science. 2010.
16. Desser DW, Philips JW. The cellular targets for action of adjuvants: T cell adjuvants and B cell adjuvants, immunopotentiation, Ciba Foundation Symposium 18 (New Series). 1973; Elsever, Amstrdam.
17. Duffy JE, Zelikoff JT. Approaches and Models for assessment of chemical induced immunomodulation in fish, in Tryhonas H, Founier M ,Balkley B R ,Smits J E G,Brousseau P ed.,Investigative immunotoxicology, CRC Press, 2005; 49-61.
18. Zuo W. Characterization of fish CD4 molecules and further identification of regulatory T cells in lower vertebrate. 2009; Ph.D. Thesis, Net document/1505077.
19. Jimeno CD. A transcriptomic approach towards understanding PAMP-driven macrophage activation in dietary immunostimulation in fish. Dissert from universed Autonomia de Barcelona department cellular, physiology biology and immunology, faculty of science. 2008.
20. Wester PW, Vethaak AD, van Muiswinkel WB. Fish as biomarkers in immunotoxicology. Toxicol. 1994; 86(3): 213- 232.
21. Zelikoff JT, Rymond A, Carison E, Beaman JR, Anderson M. Biomarkers of immnotoxicity in fish from laboratory to ocean. Toxicol Lett. 2000; 112-113:325-331.
22. Bomford R. Adjuvants for anti-parasite vaccines. Parasitol Tod 1989; 5: 41-46.

23. Rombout JH, Huttenchus HB. Picchietti S, Scapigilati G. Phylogeny and Ontogeny of fish leukocyte. Fish and Shellfish immunol. 2005; 19 (5): 441-455.
24. Bai SD, Reddy DC, Kalarani V. Influence of Spirulina on growth and immunity of the fingerlings of common carp Cyprinus carpio 1.1758, challenged with pathogenic Aeromonas hydrophila. Int J Pharm Lif Sci. 2014; 5(6): 3590-3602.