

Accepted Manuscript

Immune response and protection in gibel carp, *Carassius gibelio*, after vaccination with β -propiolactone inactivated cyprinid herpesvirus 2

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PII: S1050-4648(16)30003-1

DOI: [10.1016/j.fsi.2016.01.003](https://doi.org/10.1016/j.fsi.2016.01.003)

Reference: YFSIM 3772

To appear in: *Fish and Shellfish Immunology*

Received Date: 20 October 2015

Revised Date: 30 December 2015

Accepted Date: 3 January 2016

Please cite this article as: Zhang L, Ma J, Fan Y, Zhou Y, Xu J, Liu W, Gu Z, Zeng L, Immune response and protection in gibel carp, *Carassius gibelio*, after vaccination with β -propiolactone inactivated cyprinid herpesvirus 2, *Fish and Shellfish Immunology* (2016), doi: 10.1016/j.fsi.2016.01.003.

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1 Immune response and protection in gibel carp, *Carassius gibelio*, after 2 vaccination with β -propiolactone inactivated cyprinid herpesvirus 2

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10 **ABSTRACT** Herpesviral haematopoietic necrosis (HVHN) of gibel carp (*Carassius gibelio*) is a
11 newly emerged infectious disease caused by cyprinid herpesvirus 2 (CyHV-2) and has caused
12 huge economic losses in aquaculture operations. Currently, no effective methods are available for
13 the control of the disease. In this study, β -propiolactone inactivated cyprinid herpesvirus 2
14 (CyHV-2) vaccine was prepared, and the immune response and protection in cultured gibel carp
15 after vaccination was thoroughly investigated. This included blood cell counting and classification,
16 phagocytic activity, lysozyme and superoxide dismutase activity, neutralizing antibody titration,
17 immune gene expression analysis, and determination of the relative percent survival in vaccinated
18 gibel carp. The results of blood cell counts indicated that the numbers of the red and white blood
19 cells in the peripheral blood of immunized gibel carp increased significantly at day 4 and day 7
20 after vaccination ($p < 0.01$). The differential leukocyte count of neutrophils and monocytes were
21 significantly different compared to the control group at day 4 and 7 and the percentage of
22 lymphocytes reached a peak at day 21. The phagocytic percentage and phagocytic index peaked at
23 day 4 post-vaccination. The lysozyme activity and superoxide dismutase activity were
24 significantly increased compared to the control group ($p < 0.01$). The serum neutralizing antibody
25 titer peaked (203.03 ± 13.44) at day 21. The qPCR analysis revealed that the expression of the
26 immune genes interleukin 11 and complement component C3 were significantly up-regulated in the
27 immunized group. The challenge test demonstrated that the immunized group had a relative
28 survival rate of 71.4%. These results indicate that the inactivated CyHV-2 vaccine induced both
29 non-specific and specific anti-viral immune responses that resulted in significant protection
30 against HVHN disease and mortality in gibel carp.

31 **Keywords:** Gibel carp (*Carassius gibelio*), Cyprinid herpesvirus 2 (CyHV-2), Inactivated vaccine,
32 Immune responses, Efficacy of protection

33 34 1. Introduction

35 Gibel carp (*Carassius gibelio*) is one of the most popular cultured species in China and is one
36 of the most important fish in freshwater aquaculture [1]. Herpesviral haematopoietic necrosis
37 (HVHN) is a newly emerged infectious disease in gibel carp caused by CyHV-2 and is a
38 significant threat to the development of the aquacultural industry. Cyprinid herpesvirus 2, also
39 known as herpesviral haematopoietic necrosis virus (HVHNV) of goldfish or goldfish
40 haematopoietic necrosis virus (GFHNV), is a member of *Cyprinivirus Alloherpesviridae*, that
41 includes carp pox (CyHV-1) and koi herpesvirus (CyHV-3) [2-5]. CyHV-2 has been reported as

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