Aquaculture 428–429 (2014) 280–283

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

# Efficacy of a pharmaceutical preparation based on glycyrrhizic acid in a challenge study of white spot syndrome in white shrimp (*Litopenaeus vannamei*)

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ARTICLE INFO

*Article history:*
Received 9 January 2014
Received in revised form 5 March 2014
Accepted 7 March 2014
Available online 22 March 2014

*Keywords:*
Shrimp
White spot syndrome
Glycyrrhizic acid
Prevention

ABSTRACT

There is a lack of preventive and therapeutic drug-based treatments for the shrimp viral disease known as white spot syndrome (WSSV). Thus a challenge study inducing WSSV in juvenile white shrimp (*Litopenaeus vannamei*) was established, setting 4 groups: challenged — not treated and unchallenged, untreated control groups and two experimental ones (E1 and E2) both treated with diammonium glycyrrhizic acid, extracted from licorice with added vitamins and oligoelements, and as in-feed medication. Group E1 received diammonium glycyrrhizic acid included in their daily feed, starting 17 days before challenge with WSSV and maintaining the treatment for further 5 days after the end of the trial, which was set on day 18. Group E2 received this medication as group E1 throughout the trial, but starting 1 day before the challenge with WSSV. The group with highest surviving median values was E1, amounting two times the survival median in comparison with the control groups ($P = 0.007$). Also a statistical difference was found in terms of survival means in favor of group E1 as compared to group E2. Macroscopic and histopathological findings revealed lesions compatible with WSSV and similar mortality in the challenged untreated group. These findings were highly reduced or inexistent in mortality analyzed from groups E1 as well as in the unchallenged — untreated control group and greatly reduced in group E2. Considering the apparent high efficacy observed and that glycyrrhizic acid and mineral and vitamin components added as treatment, and taking as an advantage that this preparation has been regarded as nutraceuticals, it is here proposed that large scale trials should be conducted to evaluate the effects here observed in commercial and larger scale shrimp farms.



## 1. Introduction

In spite of difficulties, shrimp production is an important and profitable food producing industry. Even though, in many countries cultured shrimp production has been severely hindered by various viral diseases i.e., white spot syndrome — WSSV (Inouye et al., 1994; Rosenberry, 2001). First signs of this disease, such as sudden reduction in food consumption and red discoloration, are followed by a sharp increase in mortality in shrimp farms over the next 3–10 days, even reaching 100% mortality (Peinado-Guevara and López-Meyer, 2006). First description of WSSV appears to have been from an outbreak in Taiwan in 1992 (Chen, 1995; Chou et al., 1995). This disease seems to have spread world-wide, except perhaps to Australia. It has been suggested that world weather changes have contributed to the dissemination of this disease (Sonnenholzner et al., 2002). As the name of the disease suggests, the main signs of WSSV are 0.5–2.0 mm white spots in the interior part of the shell, appendixes, uropods, telson, pereiopods, pleopods and cuticle of the abdominal segments (Takahashi et al., 1994). The color of shrimps becomes pale red and the lymphoid organ becomes turgid (Takahashi et al., 1994), and it has been described as hypertrophic (Vidal et al., 2001). Diseased shrimps become lethargic, and they show erratic swimming and lack of appetite and die during the next three days.

Viusid® (from Catalysis, S.A. de C.V., Mexico) is the proprietary name preparation based on diammonium glycyrrhizic acid, extracted from licorice with added vitamins and oligoelements. It has been claimed that this drug preparation stimulates production of gamma interferon in human beings (Sugawara, 1986). Glycyrrhizic acid possesses antiviral activity in vitro and in vivo interfering with both DNA and RNA replications, hence interfering with replication of a wide range of viruses, including herpes, influenza A and B, hepatitis B, coronavirus, and SARS (Badam, 1994; Chen, 1995; Durand et al., 1997; Lee et al., 2007; Lin, 2003; Pompei et al., 2009). Glycyrrhizic acid has also demonstrated to be capable of impeding virion eclosion from its capside (Pompei et al., 2009), apparently due to a dose-dependant inhibition

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http://dx.doi.org/10.1016/j.aquaculture.2014.03.005
0044-8486/

**Table 1**
Experimental distribution of control groups and treatments with glycyrrhizic acid.

| Group | Shrimp tank number | Mean shrimp weight (g) | Mean dose of glycyrrhizic acid per shrimp (dose = 20 μL/g) | Inoculum with WSSV |
|---|---|---|---|---|
| CUCH | 1 | 11.8 | 236 | Yes |
| | 2 | 8.6 | 173 | Yes |
| | 3 | 9.6 | 191 | Yes |
| | 4 | 10.2 | 204 | Yes |
| CUUCH | 5 | 9.3 | Saline solution | No |
| | 6 | 8.6 | Saline solution | No |
| | 7 | 9.9 | Saline solution | No |
| | 8 | 9.1 | Saline solution | No |
| E1 | 13 | 10.8 | 216 | Yes |
| | 14 | 10.3 | 187 | Yes |
| | 15 | 10.0 | 199 | Yes |
| | 16 | 10.5 | 209 | Yes |
| E2 | 9 | 9.4 | 188 | Yes |
| | 10 | 11.5 | 230 | Yes |
| | 11 | 9.6 | 192 | Yes |
| | 12 | 9.9 | 197 | Yes |

CUCH = control untreated challenged.
CUUCH = control untreated unchallenged.
E1 = Experimental 1.
E2 = Experimental 2.
WSSV = white spot syndrome virus.

of kinase-P phosphorylation (Chavali et al., 1987). Additionally, it has been shown to interfere with arylamine N-acetyltransferase bacterial activity, hence exhibiting antibacterial effects at least vs *Streptococcus* spp., *Haemophilus* spp., and *Klebsiella* spp. (Krausse et al., 2004; Lo et al., 1996; Tanaka et al., 2001).

There are no biological or chemical effective treatments to treat WSSV. According to Le Moullac et al. (1998), body defense mechanisms in shrimp are greatly based on the number of circulating hemocytes in their hemolymph, and it has been observed better clinical responses to viral diseases in shrimp with high number of hemocytes. Hence, stimulation of their immune system may be a way to increase shrimp body defense mechanisms, particularly before they face the viral challenge. Thus, considering the apparent immune-modulator and antiviral activities of glycyrrhizic acid, it was set as the aim in this study to assess this preparation for its potential protective effects in a laboratory controlled challenge with WSSV.

## 2. Material and methods

This study was carried out with a total of 960 juvenile white shrimp (*Litopenaeus vannamei*) obtained from a farm free of WSSV as confirmed by PCR analysis 5 days prior to the beginning of the trial. The study lasted for 18 days. Shrimps had a mean weight of 5 g at the beginning of the trial and were randomly distributed in four groups with four replicates each. Thus each replicate was carried out with 60 shrimps and the groups were distributed as follows:

1. Control untreated-challenged group (CUCH), fed with drug-free food throughout the trial and challenged with WSSV on day zero.
2. Control untreated-unchallenged group (CUUCH), fed with drug-free food throughout the trial but not challenged and dosed with saline solution.
3. Experimental 1 (E1), fed as E1, but starting 17 days before challenge as above with WSSV and maintaining the treatment for further 5 days after.
4. Experimental 2 (E2), fed standard diet plus Viusid® throughout the trial and starting 1 day before the challenge with WSSV, as group CUCH.

Once groups were formed five shrimps from each group were randomly selected and their hemolymph obtained and measured to set basal values of total hemocyte counts by direct counting with the Neubauer chamber. Then, additional samples from five shrimps per group were obtained for hemocyte count on days 6, 12 and 18.

Shrimps were maintained in 1000 L tanks with continuous flow of brackish water at an approximate rate of 10 L/h. Temperature was kept at 23–25 °C with a thermostat (LED 200 W Dymax), pH was approximately 7.6–7.8 (Aqualytic, Germany), and continuous aeration was provided at 6.79–6.56 mL/min. Animals were fed twice a day with commercial shrimp drug-free pellets (Camaronina Purina®, Sonora, México), having: 35% protein min, 9% fat min, 3/32 in pellets and considering a 3% feed intake per day with respect to the biomass as established by Alday-Sanz (2010). Lack of ecdysis in shrimp was ensured before initiation of this trial.

Glycyrrhizinic acid was incorporated to pelleted shrimp-feed as liquid Viusid® (Catalysis Spain, distributed by Dermaceutical México, S.A. de C.V. Mexico City). To achieve this, 540 mL of the commercial preparation was diluted in 100 L of demineralized water. Then pellets were dressing-sprayed on big trays. Feed was allowed to dry at room temperature for 8 h, stored in paper bags and fed to shrimps.

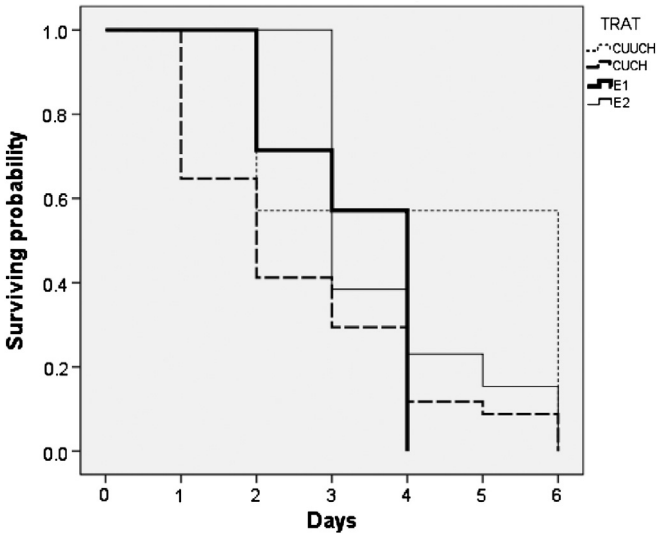


**Fig. 1.** Estimated probability surviving for each group. Log-rank test $\chi^2 = 12.1$; P = 0.007.