Aquaculture 454 (2016) 35–43

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Dietary effects on immunity, stress, and efficacy of two live attenuated *Flavobacterium psychrophilum* vaccine formulations

P.S. Sudheesh [a], J.K. Zimmerman [b], K.D. Cain [a,*]

[a] Department of Fish and Wildlife Sciences and the Aquaculture Research Institute, University of Idaho, 875 Perimeter Drive MS1136, Moscow, ID 83535, USA
[b] Skretting, 712 East 2400 North, Tooele, UT 84074, USA

ARTICLE INFO

Article history:
Received 25 August 2015
Received in revised form 7 December 2015
Accepted 9 December 2015
Available online 10 December 2015

Keywords:
Flavobacterium psychrophilum
Coldwater disease
Attenuated vaccine
Iron limited
Immunity
Functional feed
Immunostimulant
Stress

ABSTRACT

The effects of feeding a commercially available health enhancing aquaculture feed (Bio-Oregon's BioPro/Protec® diet) on the efficacy of two coldwater disease (CWD) vaccine formulations consisting of *Flavobacterium psychrophilum* 259-93B.17 (B.17) and B.17 grown in iron limited medium (ILM) were carried out in two separate trials. The primary aim of this study was to compare efficacy of the B.17 and ILM formulations in rainbow trout (*Oncorhynchus mykiss*). A secondary aim was to determine if dietary alterations could enhance the immune response and/or elicit greater levels of protection. Therefore, a basal diet (BioOlympic fry diet; control feed) and an immunostimulatory diet (BioPro; IS feed) were fed to rainbow trout at 2% body weight (bwt) per day for 2 weeks prior to immersion immunization with the B.17 vaccine, ILM vaccine, or a TYES media control. A booster immunization was administered two weeks post initial vaccination, and feeding of the respective feeds continued for one additional week at which time all groups received the control diet until the end of the experiment. In trial 1, an acute chlorine spike occurred just prior to pathogen challenge (week 7) resulting in substantial mortality among treatment groups. Interestingly, fish fed the IS feed had significantly lower mortality (36.7%) than fish fed the control feed (67%). Vaccinated fish had significantly higher antibody titers than the control fish at week 2 through 7 in trial 1. In trial 2, antibody titers for all vaccinated fish were significantly greater than controls at week 4, but at weeks 6 and 8 the titers of B.17 vaccinated fish were not significantly different from controls. However, average titers for ILM vaccinated were significantly greater than control fish at weeks 6 and 8. Cumulative percent mortality (CPM) in B.17 and ILM vaccinated groups was significantly lower than control groups at the point where CPM reached 60% (mid-way through outbreak), but by the end of the challenge (28 days) ILM vaccinated fish had significantly lower CPM than either control or B.17 vaccinated fish. When comparing dietary effects, CPM was not significantly different but RPS values for fish fed the BioPro IS feed were consistently elevated over groups fed the control diet regardless of the vaccine formulation administered. This study demonstrates increased efficacy of the ILM vaccine in rainbow trout, and provides evidence that a commercial "health enhancing" diet can provide benefits in response to an acute stress event and may contribute to increased vaccine performance.

Statement of relevance: This manuscript addresses an important and very practical problem in aquaculture. Coldwater disease is a major disease affecting salmonid aquaculture around the world. There is no approved vaccine available in the US. This manuscript reports the beneficial effects of feeding commercial immunostimulatory functional feeds on the efficacy of two formulations of a coldwater disease vaccine. The results presented here are highly relevant to the development of aquaculture and finding sustainable and non-antibiotic based aquatic animal health management strategies. This study will contribute towards coldwater disease management and identifies potential benefits of commercial functional feeds commonly used in the aquaculture industry.

1. Introduction

Infectious diseases are recognized as one of the most serious challenges facing salmonid aquaculture worldwide (Pridgeon and Klesius, 2012; Zagmutt et al., 2013). *Flavobacterium psychrophilum* is an important bacterial pathogen responsible for bacterial coldwater disease (CWD) and rainbow trout fry syndrome (RTFS) (Nematollahi et al., 2003; Cipriano and Holt, 2005; Barnes and Brown, 2011). This pathogen is a serious cause of mortality and economic loss especially in species such as rainbow trout (*Oncorhynchus mykiss*) and Coho salmon (*O. kisutch*) as well as several other fish species (Rucker et al., 1953; Bernardet and Kerouault, 1989; Wakabayashi et al., 1991; Ekman et al., 1999; Dalsgaard and Madsen, 2000; Madetoja et al., 2001; Nematollahi et al., 2003; Chen et al., 2008; Nilsen et al., 2011; Austin and Austin, 2013).

* Corresponding author.
E-mail address: kcain@uidaho.edu (K.D. Cain).

http://dx.doi.org/10.1016/j.aquaculture.2015.12.004
0044-8486/

Currently practiced methods to control CWD include appropriate management practices along with chemical and antibacterial treatment, which are often ineffective and costly (Branson, 1998; Cipriano and Holt, 2005). Two approved antibiotics (Aquaflor® and Terramycin®) are available in the United States to treat *F. psychrophilum* infections in freshwater salmonids (www.fda.gov/cvm). However, there continues to be concern over use of antibiotics and potential development of resistant bacterial strains (Fryer and Bartholomew, 1996; Bruun et al., 2000, 2003; Schmidt et al., 2000; Álvarez et al., 2004; Kum et al., 2008; Sundell and Wiklund, 2011). Development of efficient, non-antibiotic based prevention and control methods for CWD are required. Although vaccination is a promising and sustainable alternative to the use of antibiotics, approved commercial vaccines are not presently available for CWD. An attenuated strain (259-93B.17) of *F. psychrophilum* (referred to here as B.17) has been developed using a rifampicin selection approach and is a promising candidate vaccine for use against CWD (LaFrentz et al., 2008). Initial work showed that immersion vaccination of rainbow trout with the live attenuated vaccine and further challenge with the virulent parent strain (CSF-259-93) provided significant protection in vaccinated fish following adipose fin clipping (LaFrentz et al., 2008). Further work, demonstrated that expression of several immunogenic genes were upregulated in the B.17 strain when grown in iron limited medium (ILM), and protein profiles were similar to *F. psychrophilum* grown in vivo (LaFrentz et al., 2009). This led to recent work that demonstrated enhanced efficacy of the ILM vaccine where RPS value reached 73% in Coho salmon (Long et al., 2013). The efficacy of this improved ILM formulation over the original vaccine remains to be evaluated in other susceptible species such as rainbow trout. Furthermore, there is potential to affect immune response and vaccine efficacy by altering the diet prior to vaccination, and studies have shown that the quality and composition of diets can have a profound influence on the incidence of CWD (Post, 1987; Daskalov et al., 2000). Feed is one of the major inputs in aquaculture production and its composition, quality and quantity can profoundly influence fish health, especially under stressful conditions (Kiron, 2012). Current industry and research trends are focusing on enhancement of disease resistance and improving overall immunity of host fish through novel immunomodulatory feed ingredients, probiotics and vaccines (Sealey and Gatlin, 2001). The use of commercial functional aquaculture feeds containing enhanced nutritional and immunostimulatory properties are increasing and may provide an alternative to antibiotic use in some cases (Tacchi et al., 2011). Several aquaculture feeds are marketed as functional feeds with added immunostimulants/immunomodulators and micro-nutrients that are claimed to provide improvements in overall fish health and performance. Beneficial effects of immunostimulants and feed additives such as β-glucans, nucleotides, and probiotics have been well documented for aquaculture (Irianto and Austin, 2002a; Li and Gatlin, 2006; De et al., 2014). It is well recognized that nutrient composition of diets can significantly influence different components of the fish immune response (Blazer and Wolke, 1984; Landolt, 1989; Kiron et al., 1995a, 1995b) and adequate and well balanced nutrition is essential to ensure the health and disease resistance of fish reared in intensive aquaculture.

Many variables can affect vaccination success and include such things as vaccine production conditions, delivery, host species, pathogen virulence, environment, and general management practices related to aquaculture production conditions (Bly et al., 1997; Magnadottir, 2010). Very often, the method of vaccination is the most limiting factor with injection vaccination being the most reliable and widely practiced delivery method. The efficacy of such vaccines can be substantially improved by including adjuvants or other immunostimulants in the injected vaccine (Nikl et al., 1991; Chen and Ainsworth, 1992; Anderson, 1992; Aakre et al., 1994; Nakanishi and Ototake, 1997; Figueras et al., 1998). Immersion vaccination is a more feasible mass vaccination strategy for diseases such as CWD due to the small size of affected fish, which precludes injection as an effective delivery method (Decostere et al., 2001; Hastein et al., 2005). It has been speculated that adipose fin clipping in previous trials provided a portal of entry for the attenuated *F. psychrophilum* vaccine and that efficacy of the B.17 or ILM formulation would be reduced if fish were not fin clipped. Such a strategy also has issues due to the practicality of incorporating during most production situations. Since immersion vaccines can pose different challenges and demand alternate strategies to improve potency and efficacy, this study attempted to address delivery and efficacy by determining if an adjuvant effect could be induced by feeding a diet containing immunostimulants and other functional ingredients prior to and just after vaccination. Such dietary alterations should contribute to heightened non-specific responses that in turn would enhance antigen presentation and long-term adaptive components of the immune system (Gómez et al., 2014).

One of the most common ingredients used as an immunostimulant in aquaculture feeds is β-glucan. These have been studied extensively and it is well documented that β-glucans are able to enhance innate immune responses in fish (Meena et al., 2013). This is important for combatting infections since many innate responses provide an immediate response to an invading micro-organism and the body is better able to respond and eliminate the threat. However, it is clear now that the innate and adaptive arms of the immune response function together and that enhanced innate responses, such as increased macrophage activity, can lead to long-term adaptive immunity (Tafalla et al., 2013). To determine the extent of adaptive immunity it is common to characterize specific antibody responses following antigen exposure or vaccination. The potential for immunostimulatory feeds to improve long-term adaptive immunity has not been well characterized, but their use has potential to act as an adjuvant and improve vaccine performance. It is therefore hypothesized that feeding a more nutritionally complete diet containing immunostimulants such as β-glucans and nucleotides along with elevated vitamins & minerals, and antioxidants just prior to or during vaccination will result in an enhanced immune response. Therefore, this study was designed to compare the efficacy of two different live attenuated CWD vaccine formulations, and characterize growth, antibody response, and mortality following pathogen challenge of rainbow trout fed a basal (BioOlymic fry) and immunostimulatory (BioPro/Protec) commercial trout diet.

2. Materials and methods

2.1. Bacteria and culture conditions

The virulent strain, *F. psychrophilum* CSF-259-93 strain was originally isolated from moribund rainbow trout during an outbreak of CWD in Southern Idaho, and earlier works have demonstrated it to be highly virulent in rainbow trout (LaFrentz et al., 2002, 2003, 2008). The virulent strain was grown for 72 h at 15 °C in tryptone yeast extract salts broth (TYES; 0.4% tryptone, 0.04% yeast extract, 0.05% calcium chloride, 0.05% magnesium sulfate, pH 7.2) following published protocols (LaFrentz et al., 2003). To harvest bacteria for challenge experiments, 72 h grown broth culture was centrifuged (4300 $\times g$) for 15 min, the supernatant was poured off, and the pellet resuspended in sterile phosphate buffered saline (PBS, pH 7.0). To estimate colony forming units (CFU mL^{-1}), a 6 × 6 drop plate method was used (Chen et al., 2003) using TYES agar plates (TYES broth containing 1.5% agar, pH 7.2). Plates were incubated at 15 °C for 96 h and colonies were counted. Typical yellow convex colonies with smooth or thin spreading margins were presumptively identified as *F. psychrophilum.* The species identity was confirmed either by a fluorescent antibody technique (FAT) (Lindstrom et al., 2009), using a FITC labeled monoclonal antibody (MAb FL43, Immunoprecise Antibodies Ltd., Victoria, BC, Canada) or by a nested PCR (Taylor and Winton, 2002).