Influence of 1,3-1,6-β-D-glucan (Leiber® Beta-S) in diets on the effectiveness of anti-Enteric Redmouth Disease (AquaVac ERM) vaccine in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

This study was performed to investigate the influence of 1,3-1,6-β-D-glucan (Leiber® Beta-S), natural product, on the antibody secreting cells (ASC) and specific antibody levels after immunization of rainbow trout (Oncorhynchus mykiss) with anti-enteric redmouth disease vaccine (AquaVac ERM). Fish were fed pellets containing of 1,3-1,6-β-D-glucan (Leiber® Beta-S) at doses 100 mg and 200 mg per kg of pellets per day. After one week of Leiber® Beta-S supplementation, the fish were immunized by immersion of the vaccine. At 7, 14, 21, 28 and 40 days after immunization, blood and pronephros were taken from 20 fish in each group for immunological study. When analyzed by the ELISPOT assay, Leiber® Beta-S at doses 100 mg and 200 mg per kg of pellets increased the number of specific ASC and specific antibody levels in serum analyzed by the ELISA. The highest numbers of ASC and specific antibody levels at a dose 200 mg per kg of pellets were observed. In conclusion, the results of the present study showed that Leiber® Beta-S increased the effectiveness of anti-enteric redmouth disease vaccine in rainbow trout.

Key words: *rainbow trout, 1,3-1,6-β-D-glucan, AquaVac ERM vaccine, ASC, specific antibody level.*

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Introduction

Immunomodulators comprise a group of biological and synthetic compounds that enhance the innate cellular and humoral defence mechanisms in animals. The use of immunomodulators in fish culture for the prevention of diseases is a promising very effective and new development [1-3]. Several types of β-glucans seem especially promising for stimulating the nonspecific immune responses in fish [4, 5]. When a fish initially encounters a pathogenic microorganism, the nonspecific defence mechanisms are more important than the specific immune response, as the latter requires a longer time for antibody build-up and specific cellular activation [1, 3]. In general, fish have short lifespands and most live in cool water environments which slows development of the specific immune response. These factors may not allow fish to develop the complex physiological pathways that are central to development of an adaptive immune response. Immunostimulants may be used in patterns similar to those of chemotherapeutics or chemicals and in combination with vaccine [6, 7]. The fish could be prepared for a predicted event, such as seasonal exposure to pathogens or handling stress, by a treatment prior to the event. Many environmental and physiological variables will influence experiments and protocol for the use of immunostimulators in fish, including timing, dosage requirements, environmental temperature, the characteristics of the vaccine and species of fish [8, 9].

The stimulation of specific protection of fish against infectious diseases by immunization has been developed with limited success, since some immunization techniques

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when actually applied to hatchery conditions are not so highs effective as they should be [1, 10-12]. One of the most frequent uncertainties regarding the use of vaccines is the effective protection over a long time. The immunization techniques by injection or immersion initiate a manipulation stress and consequently negative metabolic effects. In response to stress, the adrenal gland is stimulated to release the hormone cortisol, which has a wide variety of effects, including a decrease in the nonspecific defence mechanisms and suppression of the specific immune response. The application of immunomodulators for the activation of the effectiveness of vaccines is a promising new development in fish culture [3, 6, 7].

In the present study, we determined the influence of 1,3-1,6-β-D-glucan (Leiber® Beta-S) applicated in food (pellets) on the antibody secreting cells (ASC) and specific antibody levels after immunization fingerling of rainbow trout (*Oncorhynchus mykiss*) by immersion with the anti-enteric redmouth disease vaccine (AquaVac ERM).

Material and methods

Animals and immunomodulator

For this experimental study, 300 healthy rainbow trout (*Oncorhynchus mykiss*), weighing 10-15 g were used. They were purchased from Department of Salmonid Culture, Inland Fisheries Institute in Rutki (Poland).

The Leiber® Beta-S preparation (Leiber GmbH, D-49565 Bramsche, Germany) is detached from the cell wall complex of brewers yeast's (*Saccharomyces cerevisiae*) by a patented process that protects the glucan. Highly effective particulate β-glucan structures are not damaged by aggressive treatment using alkalis and acids. 1,3-1,6-β-D-glucan molecules achieve full biological activity in their diverse, native structure, guaranteeing the most effective possible immunomodulating effect during passage through the gut.

Experimental design

The fish were divided into three tanks of 100 fish each (500 l tanks with a recirculation system of water), at a temperature of 10 ±1˚C. Fish were fed daily with pellets (45% protein, 1% body weight) containing 1,3-1,6-β-D-glucan (Leiber® Beta-S) at doses 100 mg and 200 mg per kg of pellets, prepared by protocol used in Inland Fisheries Institute (Poland) for rainbow trout. Control group was fed daily with similar pellets without 1,3-1,6-β-D-glucan. After 4 weeks of fed with 1,3-1,6-β-D-glucan, fish from each group were immunized by immersion of the enteric redmouth disease vaccine (AquaVac ERM Schering-Plough Animal Health, UK), according to the protocol presented by Company. This vaccine is recommended for use in healthy rainbow trout 2 g and larger to reduce mortality due to enteric redmouth disease by the Hagerman type I strain of *Yersinia ruckeri*. In this study we diluted 1 litre of vaccine with 9 litters of clean hatchery water. Drain and weigh a netful of fish and dip fish in the diluted vaccine for 30 seconds ensuring that fish are totally immersed in the vaccine. After 30 seconds exposure lift net, allow draining and returning fish to holding tanks. The blood and pronephros were collected from 10 fish of each group on week 1, 2, 3, 4, 5, 6, 7 and 8 after immunization.

Immunological assays

Pronephros was removed and single cell suspensions were obtained by teasing the tissues in medium through a steel mesh. Cell suspensions were purified on a Gradisol L (density 1.077; Polfa, Poland) gradient. Counts of living cells from pronephros were made with trypan blue using a haemocytometer after three washing in the medium (Leibovitz-15, Sigma, USA). The ELISPOT assays for the quantification of antibody secreting cells (ASC) after immunization were used, according to the protocol presented by Siwicki and Dunier [13].

The anti-*Yersinia ruckeri* (pathogenic bacteria causes of Enteric Redmouth Disease) antibody levels of each fish serum were determined by indirect enzyme-linked immunosorbent assay (ELISA) as described by Vergnet and Dunier [14].

The results were verified statistically by a one-way ANOVA analysis of variance (GraphPadPrism software package) and the significance of differences between the groups was verified with a Bonferroni test.

Results and discussion

The Leiber-Beta S administered before vaccine increased the specific ASC and specific humoral immune response (specific antibody levels), compared to the control group (only vaccined). The influence of 1,3-1,6-β-D-glucan (Leiber® Beta-S) on the kinetics of the specific ASC after immunization are presented in Figure 1, and those on the specific antibody titres in Figure 2. The results showed that Leiber® Beta-S applicated to food 4 weeks before vaccination, at dose 100 mg and 200 mg per kg of pellets, statistically increased ($P < 0.05$) the specific antibody levels and specific antibody secreting cells after immunization by immersion, compared to the Leiber® Beta-S-free group of fish. The levels of specific antibody and specific ASC increased rapidly and the highest levels were observed between 21 and 28 days after vaccination.

In our study, the immunostimulating influence of 1,3-1,6-β-D-glucan (Leiber® Beta-S) on the humoral immune response in rainbow trout was observed. Oral administration of Leiber® Beta-S to fish before immunization enhanced the effectiveness of the vaccine, analyzed by the levels of specific Ig and specific antibody secreting cells. Similarly to the effect of dimerized lysozyme (KLP-602) and HMB applied before the anti-*Yersinia ruckeri* vaccine [3, 7], 1,3-1,6-β-D-glucan increased the levels of the effec-

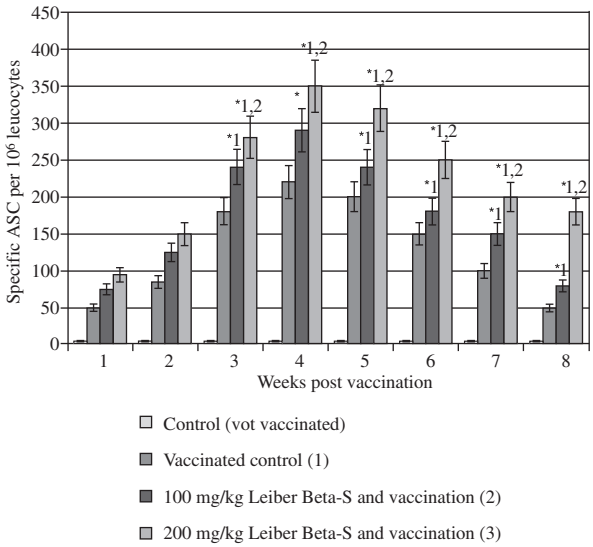

Fig. 1. Influence of Leiber® Beta-S on the specific ASC after vaccination against enteric redmouth disease in fingerling of rainbow trout (*Oncorhynchus mykiss*) (n = 10, mean ±SD; * – statistically significant differences, $P \leq 0.05$)

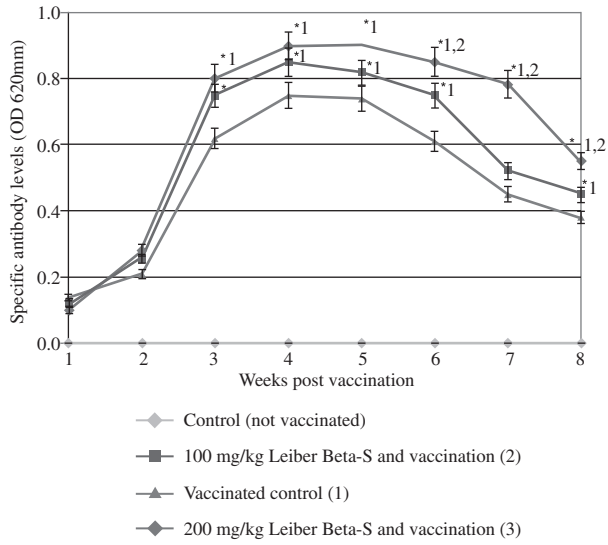

Fig. 2. Influence of Leiber® Beta-S on the specific antibody levels after vaccination against enteric redmouth disease in fingerling of rainbow trout (n = 10, mean ±SD; * – statistically significant differences, $P \leq 0.05$)

tors cells, a very important part of the specific immune response, and had a positive influence on the humoral immune response in fish.

1,3-1,6-β-D-glucan are naturally occurring polysaccharides found in the cell walls of fungi and yeast but alien to the animal kingdom. Throughout evolution, the immune system has learned to recognise its molecular structure as a reliable warning of infection. In purified form, 1,3-1,6-β-D-glucan functions as a signal that alerts the immune system and prepares it to respond quickly and adequately to infections. However, 1,3-1,6-β-D-glucan is more than a potent immune-stimulate that renders animals more resistant to pathogens. Future studies will include determining optimal doses, influence on the cellular and humoral defence mechanisms and protocol for feeding this substance to maximise protection, given the constraints of fish culture and economics.

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