POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2004, Vol. 13/54, SI 2, pp. 59–61

THE EFFECT OF BETA-1.3/1.6 – GLUCAN IN DIETS ON THE EFFECTIVENESS OF ANTI-*YERSINIA RUCKERI* VACCINE – AN EXPERIMENTAL STUDY IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

Andrzej K. Siwicki[1,4], Krzysztof Kazuń[1], Edward Głąbski[1], Elżbieta Terech-Majewska[2], Paweł Baranowski[3], Sylwia Trapkowska[4]

[1]Department of Fish Pathology and Immunology, IFI in Zabieniec near Warsaw, Piaseczno, Poland;
[2]Department of Epizootiology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland;
[3]Sorga LTD, 20-469 Lublin, Poland; [4]Department of Microbiology and Clinical Immunology, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Key words: fish, beta-1.3/1.6 – glucan, anti-*Yersinia ruckeri* vaccine, ASC, Ig level

In the present study, the influence of beta-1.3/1.6 – glucan, a natural product, on the antibody secreting cells (ASC) and specific Ig levels after immunization of rainbow trout (*Oncorhynchus mykiss*) with the anti-*Yersinia ruckeri* vaccine was studied. Fish were fed pellets containing beta--1.3/1.6 – glucan (Macrogard) at a dose of 0.5 g/100 g of pellets (0.5%) per day. After one week of beta-1.3/1.6 – glucan supplementation, the fish were immunized by immersion of the vaccine. At 7, 14, 21, 28 and 40 days after the immunization, blood and pronephros were taken from 20 fish in each group for immunological study. When analyzed by the ELISPOT assay, beta-1.3/1.6 – glucan (Macrogard) was found to increase the number of specific antibody secreting cells (ASC) and specific Ig levels in serum. In conclusion, the results of the present study showed that beta-1.3/1.6 – glucan (Macrogard) increased the effectiveness of anti-*Yersinia ruckeri* vaccine in fish.

INTRODUCTION

The use of immunomodulators in fish culture for the prevention of diseases is a promising new development [Anderson, 1992; Raa, 1996; Siwicki *et al.*, 1998]. Immunomodulators comprise a group of biological and synthetic compounds that enhance the no-nspecific cellular and humoral defence mechanisms in animals and human. Several types of beta-glucans seem especially promising for stimulating the non-specific immune responses in fish [Jørgensen & Robertsen, 1995; Jørgensen *et al.*, 1993; Siwicki *et al.*, 1998]. When a fish initially encounters a pathogenic microorganism, the non-speocific 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 [Anderson, 1992; Siwicki *et al.*, 1998]. In general, fish have short lifespans and mostly live in cool water environments which slow the development of the specific immune response. These factors may not allow a fish to develop the complex physiological pathways that are vital to the development of an adaptive immune response. Immunostimulants may be used in patterns similar to those of chemotherapeutics or chemicals and in combination with vaccines. 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.

The stimulation of specific protection of fish against infectious diseases by immunization has been developed with limited success, since some immunization techniques when actually applied to hatchery conditions are not as effective as they should be [Anderson, 1992; Ellis, 1988]. 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 non-specific 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.

In the present study, we determined the influence of beta-1.3/1.6 – glucan in a diet on the antibody secreting cells (ASC) and specific antibody titres after immunization of rainbow trout (*Oncorhynchus mykiss*) with the anti--Yersiniose vaccine.

MATERIALS AND METHODS

Animals and immunomodulator. For this experimental study, 300 healthy rainbow trouts (*Oncorhynchus mykiss*),

Author's address for correspondence: Andrzej K. Siwicki, Department of Fish Pathology and Immunology IFI, Żabieniec near Warsaw, 05-500 Piaseczno, Poland; tel.: (48 22) 756 24 90; e-mail: aksiw@infish.com.pl

weighing 90–100 g were used. They were purchased from the Inland Fisheries Institute in Olsztyn (Poland).

The Macrogard preparation (KS Biotec-Mackzymal, Tromso, Norway) is described by the manufacturer as a feed-grade beta-1.3/1.6 – glucan derived from the cell walls of *Saccharomyces cerevisiae* yeast.

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 12±1°C. Fish were fed daily with pellets (45% protein, 1% body weight) containing beta-1.3/1.6 – glucan (Macrogard) at a dose of 0.5 g per 100 g pellets (0.5%), prepared following the protocol used at the Inland Fisheries Institute (Poland) for rainbow trout. Control group was fed daily with similar pellets without beta-1.3/1.6 – glucan. After 1 week of beta-1.3/1.6 – glucan application, fish from each group were immunized by immersion of the anti-*Yersinia ruckeri* vaccine (Sanofi, France). The blood and pronephros were colected from 20 fish of each group on day 7, 14, 21, 28 and 40 after immunization.

Immunological assays. Pronephros were 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 threefold 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 [1993].

The anti-*Yersinia ruckeri* antibody levels of each fish sera assayed in ELISPOT were simultaneously determined by micro-agglutination assay as described by Cossarini-Dunier [1985].

Statistical analyses were performed using Student's t-test. Differences between means were considered statistically significant at $p<0.05$. The standard deviation (SD) was always within 10% of the means.

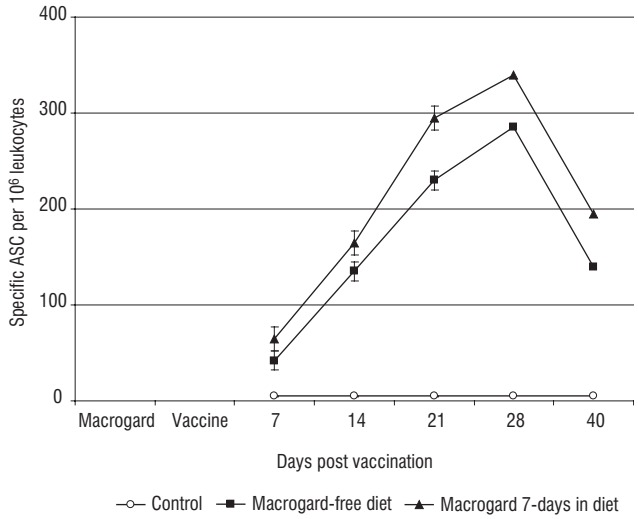

FIGURE 1. The influence of beta-1.3/1.6-glucan (Macrogard) on the kinetics of the numbers of specific antibody secreting cells (ASC) per 10^6 pronephros leucocytes from rainbow trout immunized with anti-*Yersinia ruckeri* vaccine and in non-vaccinated fish (Control); (mean±SD; n=20).

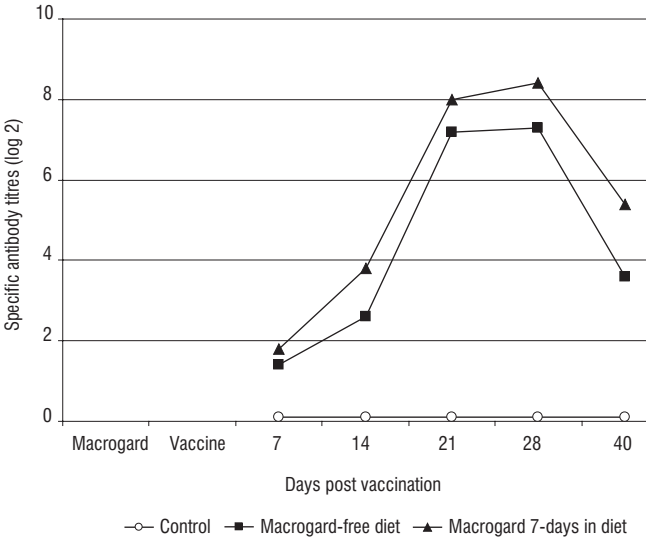

FIGURE 2. The influence of beta-1.3/1.6-glucan (Macrogard) on the kinetics of the specific antibody titres in serum from rainbow trout immunized with anti-*Yersinia ruckeri* vaccine and in non-vaccinated fish (Control); (mean; n=20).

RESULTS AND DISCUSSION

Beta-1.3/1.6 – glucan (Macrogard) administered before vaccine increased the specific antibody secreting cells (ASC) and specific humoral immune response (specific antibody levels), compared to the control group (only vaccinated). The influence of beta-1.3/1.6 – glucan on the kinetics of the specific ASC after immunization is presented in Figure 1, and that on the specific antibody titres – in Figure 2. The results showed that beta-1.3/1.6 – glucan applicated to food one week before vaccination, at a dose of 0.5% of feed, increased statisically significantly ($p<0.05$) the specific antibody levels and specific antibody secreting cells after immunization by immersion, compared to the Macrogard-free group of fish. The titres 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 beta-1.3/1.6 – glucan (Macrogard) on the humoral immune response in rainbow trout was observed. Oral administration of beta-1.3/1.6 – glucan 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 [Siwicki *et al.*, 1998, 2001], beta-1.3/1.6 – glucan increased the levels of the effector cells, a very important part of the specific immune response, and had a positive influence on the humoral immune response in fish.

Beta-1.3/1.6 – glucans 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 recognize its molecular structure as a reliable warning of an infection. In a purified form, beta-1.3/1.6 – glucan functions as a signal that alerts the immune system and prepares it to respond quickly and adequately to infections. However, beta-1.3/1.6 – glucan is more than a potent immune-stimulant 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 maximize protection, given the constraints of fish culture and economics.

REFERENCES

1. Anderson D.P., Immunostimulants, adjuvants, and vaccine carriers in fish: application in aquaculture. Annu. Rev. Fish Dis., 1992, 2, 281–307.
2. Cossarini-Dunier M., Indirect enzyme-linked immunosorbent assay (ELISA) to titrate rainbow trout serum antibodies against two papthogens: *Yersinia ruckeri* and Egtved virus. Aquaculture, 1985, 49, 197–208.
3. Jørgensen J.B., Robertsen B., Yest α-glucan stimulates respiratory burst activity of Atlantic salmon (*Salmo salar*) macrophages. Dev. Comp. Immunol., 1995, 19, 43–57.
4. Jørgensen J.B., Sharp G.J.E., Secombes C.J., Robertsen B., Effect of yeast-cell-wall glucan on the bactericidal activity of rainbow trout macrophages. Fish Shellfish Immunol., 1993, 3, 267–277.
5. Raa J., The use of immunostymulatory substances in fish and shellfish farming. Rev. Fish. Scien., 1996, 4, 229–288.
6. Siwicki A.K., Dunier M., Quantification of antibody secreting cells to *Yersinia ruckeri* by ELISPOT assay after *in vitro* and *in vivo* immunization of rainbow trout (*Oncorhynchus mykiss*). Vet. Immunol. Immunopathol., 1993, 37, 73–80.
7. Siwicki A.K., Morand M., Terech-Majewska E., Niemczuk W., Kazun K., Glombski E., Influence of immunostimulants on the effectiveness of vaccine in fish: *in vitro* and *in vivo* study. J. Appl. Ichthyol., 1998, 14, 225–227.
8. Siwicki A.K., Morand M., Fuller J.C., Nissen S., Kazun K., Glombski E., Influence of HMB on antibody secreting cells (ASC) after *in vitro* and *in vivo* immunization with the anti-*Yersinia ruckeri* vaccine of rainbow trout (*Oncorhynchus mykiss*). Vet. Res., 2001, 32, 491–498.