EFFECTS OF ADJUVANTED *AEROMONAS SALMONICIDA* VACCINES ON SELECTED PHYSIOLOGICAL AND IMMUNOLOGICAL VARIABLES IN JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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The effects of seven injectible vaccines against *Aeromonas salmonicida* on oxygen consumption, growth, lysozyme activity and antibody titres of juvenile rainbow trout (*Oncorhynchus mykiss*) were investigated. The vaccines were: *A. salmonicida* bacterin only, bacterin adjuvanted with levamisole, bacterin in oil emulsion, microencapsulated bacterin, microencapsulated bacterin with the addition of muramyl dipeptide, bacterin microencapsulated with β-1,3 glucan, and bacterin microencapsulated with *Vibrio anguillarum* lipopolysaccharide (LPS). The highest level and broadest range of responses were affected by the bacterin which was microencapsulated with LPS.

Table 1. Effect of vaccination on specific growth rate (SGR), oxygen consumption ($\dot{M}O_2$) and haematocrit (Hct) at 5 weeks post vaccination. Data are presented as means (± 1 SE). Different subscripts denote significant differences ($P<0.05$).

Treatment group	SGR (% wt per day)	$\dot{M}O_2$ (mg/kg/h)	Hct
Saline	1.06 ± 0.01 a	318.8 ± 22.4 a	39.0 ± 1.11 a
Bacterin	1.09 ± 0.05 a	311.6 ± 24.9 a	37.1 ± 1.20 a
Bacterin with levamisole	1.28 ± 0.06 ab	401.9 ± 24.9 ab	37.3 ± 1.19 a
Oil adjuvanted bacterin	1.36 ± 0.03 ab	400.1 ± 26.6 ab	36.9 ± 1.41 a
Microencapsulated bacterin	1.37 ± 0.03 ab	365.1 ± 49.7 a	40.1 ± 1.33 a
Microencapsulated bacterin with muramyl dipeptide	1.49 ± 0.14 b	329.8 ± 39.9 a	40.4 ± 1.31 a
Microencapsulated bacterin with β-1,3 glucan	1.31 ± 0.09 ab	336.0 ± 35.2 a	37.5 ± 1.19 a
Microencapsulated bacterin with *V. anguillarum* LPS	1.76 ± 0.09 c	512.5 ± 41.7 b	45.3 ± 1.42 b

Fish immunized with the LPS adjuvanted vaccine displayed significantly higher growth rates and Hct values than any other treatment group at 5 weeks (Table 1). MDP injection also resulted in growth rates which were significantly greater than those fish injected with saline or the plain bacterin. No differences were seen among treatments at 12 or 24 weeks.

Metabolic rates for the LPS treated group at 3 weeks were significantly greater than all other treatment groups with the exception of the levamisole and the bacterin in oil emulsion (Table 1). No differences were seen among treatment groups at 6 or 12 weeks.

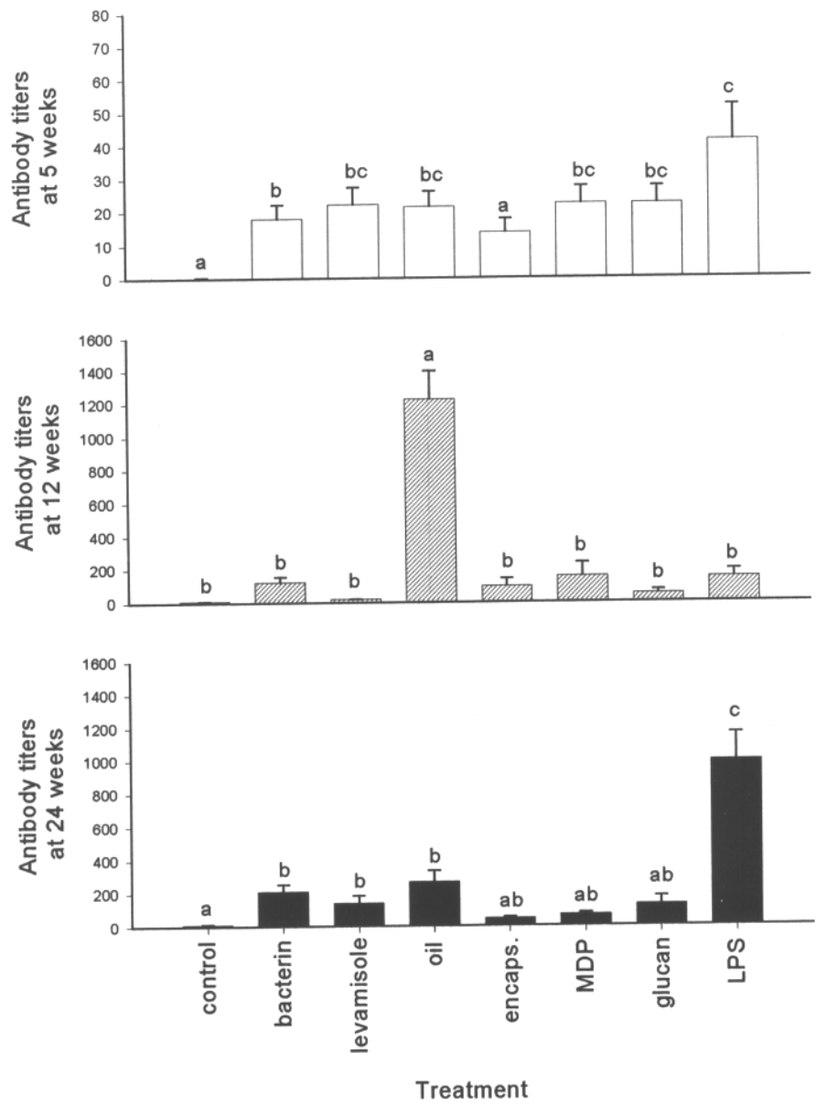

Figure 1. Mean agglutinating antibody titres in immunized juvenile rainbow trout over three time trials. Means (± 1 SE) with different superscripts differ significantly ($P < 0.05$) by all pairwise multiple comparison.

A lack of differences in the final weights of fish in this study seems to indicate that the differences in specific growth rates were not enough to result in a sizable difference in final body weight among treatment groups. While fish were not maintained to harvest size, a computer simulation demonstrated the possibility that those fish which received the LPS vaccine could

exceed the weight of both the bacterin immunized and the control fish by as much as 60% at harvest size.

There were significant differences in antibody titres among treatment groups in all three trials (Figure 1.). At 5 weeks, fish immunized with the LPS vaccine exhibited the highest level of circulating antibodies, a level significantly greater than those seen in the control fish, the microencapsulated bacterin and the simple bacterin. However, all vaccines except the microencapsulated bacterin resulted in levels significantly greater than controls. Fish immunized with the bacterin in oil emulsion vaccine had significantly greater antibody titres at 12 weeks than all other treatment groups while at 24 weeks, the LPS treated fish had the highest levels of circulating antibodies.

Antibody titres are not always indicative of the protection of a vaccine or the survival of a fish. It has been demonstrated that the outcome of a challenge may be independent of the agglutinating antibody titer present in the serum of fish (Hastings and Ellis 1990; Thuvander et al. 1993) and that it is the specificity rather than the quantity of antibodies which are produced that is important (Olivier et al. 1985). Antibodies must act in conjunction with other factors in the fish's immune system in order to combat a pathogen. Only a successful furunculosis challenge could have provided a clearer picture of the protection of the vaccines in this study.

Table 2. Effect of vaccination on kidney lysozyme activity at 5, 12 and 24 weeks post vaccination. Data are presented as means (± 1 SE). Different subscripts denote significant differences within columns ($P<0.05$).

Treatment Group	Lysozyme Activity (U/g) 5 weeks	12 weeks	24 weeks
Saline	1792.7 ± 155.5 a	644.4 ± 35.7 a	1344.4 ± 102.6 b
Bacterin	2381 ± 199.0 ab	685.4 ± 46.3 ab	902.5 ± 93.7 a
Bacterin with levamisole	2743.4 ± 285.3 ab	720.5 ± 59.1 ab	1309.8 ± 180.1 ab
Oil adjuvanted bacterin	1740.4 ± 129.6 a	988.5 ± 105.9 b	847.6 ± 67.8 a
Microencapsulated bacterin	4389.1 ± 1205.0 b	768.4 ± 46.9 ab	1263.8 ± 128.0 ab
Microencapsulated bacterin with muramyl dipeptide	3119.1 ± 509.4 ab	965.0 ± 101.8 b	1061.4 ± 65.4 ab
Microencapsulated bacterin with β-1,3 glucan	2406.9 ± 218.5 ab	724.9 ± 55.2 ab	1441.3 ± 292.0 ab
Microencapsulated bacterin with *V. anguillarum* LPS	1883.5 ± 168.3 a	897.3 ± 88.3 ab	2154.0 ± 532.6 b

Significant differences in lysozyme activity were observed at all three time periods (Table 2). At 5 weeks, fish immunized with the microencapsulated bacterin had significantly higher lysozyme activity than did fish injected with saline, oil emulsion, and LPS. At 12 weeks, immunization with the bacterin in oil and the MDP vaccine resulted in significantly higher lysozyme activity than was present in control fish. LPS treated fish exhibited the highest levels of lysozyme

activity at 24 weeks, but the levels were only significantly different from the plain bacterin and the bacterin in oil which were both significantly lower than control values.

The results of these trials demonstrated that vaccines which were encapsulated and adjuvanted with LPS and MDP had a positive effect on specific growth rates. Fish immunized with the LPS treatment displayed a higher initial growth rate than all other groups and the MDP treated fish exhibited the same response to a lesser degree. LPS treated fish also demonstrated an increased metabolic rate over the course of a month while maintaining increased plasma antibody titre and kidney lysozyme activity for a substantially longer period than other immunizations.

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