Augmented immune response of Atlantic salmon *(Salmo salar)* through the oral delivery of alginate-encapsulated SRS antigens

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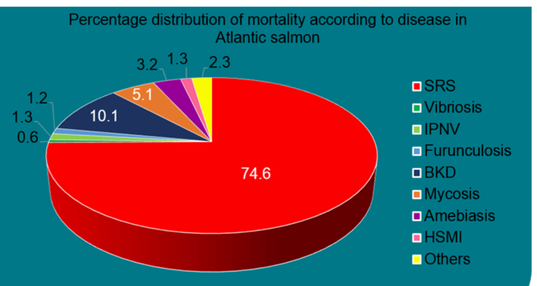
INTRODUCTION

Salmon Rickettsial Septicaemia (SRS) is the infectious disease that produces the highest losses in the Chilean salmon industry (Camussetti, *et al.*, 2015). Disease outbreaks continue to emerge despite the use of vaccines and antibiotics (Mauel & Miller, 2012).



OBJECTIVE

Assess the effect of Alginate-Encapsulated SRS Antigens (AESA) incorporated in the feed as an oral vaccine or booster to improve the adaptive immune response of Atlantic salmon.

MATERIALS & METHODS

1. Preparation of Alginate-Encapsulated SRS Antigens (AESA): Alginate microparticles were produced by ionic gelation using an aerodynamically assisted jetting (AAJ) system (Arumuganathar *et al.*, 2007). Two doses of the oral vaccine were prepared: 1) Low dose: 30% of the recommended injectable dose to each fish for 10 days; 2) High dose: 1 recommended injectable dose to each fish for 10 days, where one dose equals $1x10^7$ *Piscirickettsia salmonis* bacteria inactivated by formaldehyde.

2. Preparation of feed pellets infused with antigen microparticles: The recovered alginate microparticles were suspended into oil mix (800 g). The final feed samples were produced by combining the oil mixture with 4.2 kg base pellet in a vacuum coater.

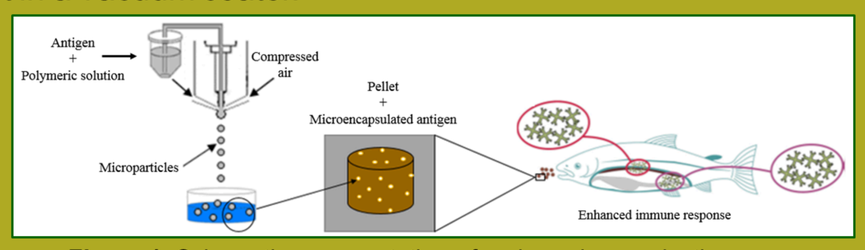
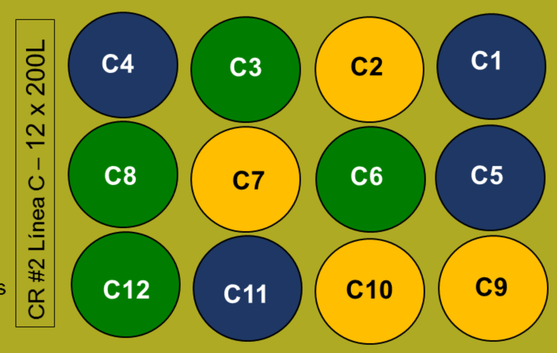


Figure 1. Schematic representation of oral vaccine production.

3. Experimental Design: 960 healthy Atlantic salmon (40 g) were distributed into three groups (Injectable vaccine, oral vaccine high dose, oral vaccine low dose) with four tanks being assigned to each group. The feed intake was assessed during the entire trial. To evaluate the effect of the experimental feed on the fish immune system, blood samples were taken at four sampling points (0 degree days post vaccination (DD), 300DD, 600 DD and 840 DD). The *P. salmonis* specific IgM levels in blood plasma were measured by ELISA.

Total fish: 960
Fish per diet: 320
Fish per tank: 80

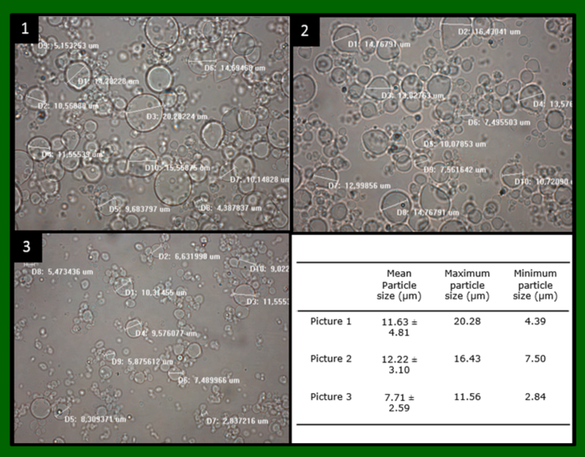
Temperature: 15 °C
Salinity: 5 ppt
Flow: 1,3/h
Fotoperiod: 24 light hours



Diet 1	Low Dose
Diet 2	High Dose
Diet 3	Injectable vaccine

Figure 2. Experimental Design for proof of concept trial.

RESULTS



	Mean Particle size (µm)	Maximum particle size (µm)	Minimum particle size (µm)
Picture 1	11.63 ± 4.81	20.28	4.39
Picture 2	12.22 ± 3.10	16.43	7.50
Picture 3	7.71 ± 2.59	11.56	2.84

Figure 3. Optical microscopy (60X) of alginate encapsulated SRS antigens.

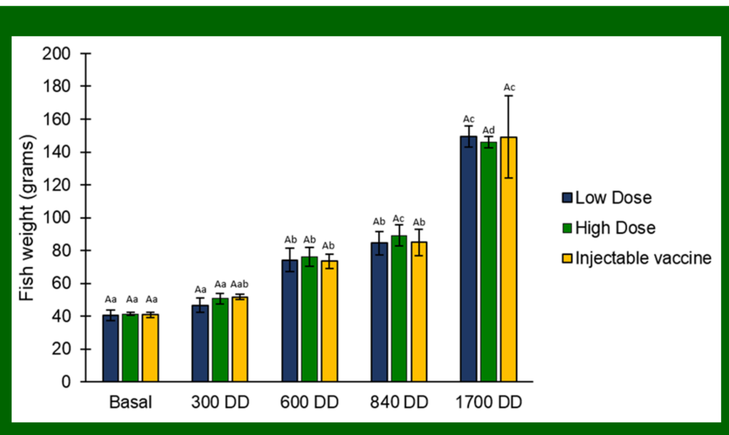


Figure 4. Fish weight at different sampling points during the trial.

Different capital letters indicate significant differences (P ≤ 0.05) of the weight between the different experimental groups (Low Dose, High dose, Injectable vaccine) within the same sampling point. Different lowercase letters indicate significant differences (P ≤ 0.05) of the weight between the different sampling points (baseline, 300 DD, 600 DD, 840 DD and 1700 DD) within the same experimental group.

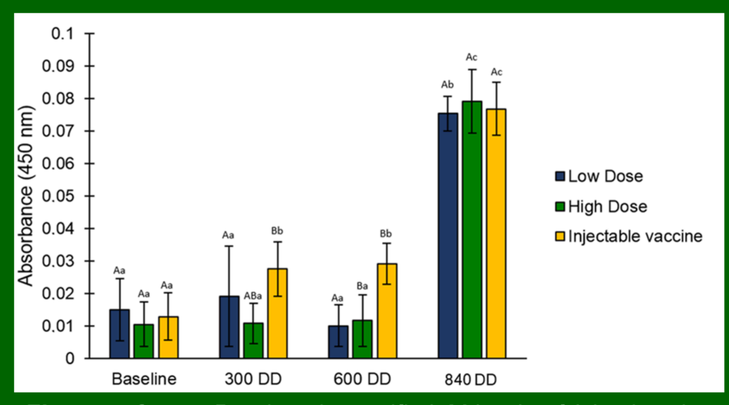


Figure 5. Serum *P. salmonis* specific IgM levels of Atlantic salmon vaccinated with: oral vaccine low dose, oral vaccine high dose and injectable vaccine.

Different capital letters indicate significant differences (P ≤ 0.05) of the absorbance between the different experimental groups (Low Dose, High dose, Injectable vaccine) within the same sampling point. Different lowercase letters indicate significant differences (P ≤ 0.05) of the absorbance between the different sampling points (baseline, 300 DD, 600 DD and 840 DD) within the same experimental group.

DISCUSSION & CONCLUSIONS

- Microparticles containing the SRS antigens were effectively produced using the AAJ system (Figure 3). Microparticles had a round smooth morphology and a mean particle size of 10.52 µm, diameters between 2.84 µm and 20.28 µm were observed by optical microscopy.
- During the vaccination period, the feed intake rates were 100% for all groups indicating that the addition of AESA did not affect the palatability of the fish feed.
- Figure 4 shows that orally vaccinated fish had no significant differences in terms of weight with the ones injected with the vaccine at all stages of growth. This result suggests that administration of the oral vaccine induces no effect in nutrient assimilation in orally vaccinated fish (Tobar, *et al.*, 2011).
- There was a significant increase in the IgM levels at 840 DD for both experimental groups. IgM is the major systemic antibody in teleost fish (Hordvik, 2015), therefore, the oral vaccine effectively enhanced the immune response of fish. Furthermore, there were no significant differences when comparing the IgM levels of the experimental groups with those of the injectable vaccine (Figure 5).
- Alginate encapsulated SRS antigens given orally can be an effective alternative to enhance the immune response in Atlantic salmon as a secondary vaccination or booster .

REFERENCES

Arumuganathar, S., Jayasinghe, S.N., Suter, N. (2007). Aerodynamically assisted jet processing of viscous single- and multi-phase media. Soft Matter. 3: 605–612; Camussetti, M., Gallardo, A., Aguilar, D. Larenas, J. (2015). Análisis de los costos por la utilización de quimioterápicos y vacunas en la salmonicultura. Salmonexpert. 4: 46-49; Hordvick, I. (2015). Review. Immunoglobulin Isotypes in Atlantic Salmon, Salmo Salar. Biomolecules. 5: 166-177; Mauel, M.J., Miller, D.L. (2002). Piscirickettsiosis and piscirickettsiosis-like infections in fish: a review. Veterinary Microbiology. 87: 279–289; Tobar, J.A., Jerez, S., Caruffo, M., Bravo, C., Contreras, F., Bucarey, S.A., Harel, M. (2011). Oral vaccination of Atlantic salmon (*Salmo salar*) against salmonid rickettsial septicaemia. Vaccine. 29: 2336–2340.

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