

New Advances for Warmwater Aquaculture



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AQUAVAC™ ERGOSAN™ in rainbow trout initiates improved vaccine response, stress tolerance and weight

- Key Points
- Increased body weight
- Results after heat shock
- Cytokine levels higher after vaccination

Key Points

- A controlled study on a commercial rainbow trout farm demonstrated that administration of AQUAVAC™ ERGOSAN™, an immunomodulator, initiated a greater immune response to vaccination with AQUAVAC™ ERM, a vaccine for control of Enteric Redmouth Disease (ERM).
- Fish fed AQUAVAC ERGOSAN also demonstrated marked increases in specific stress-control mechanisms such as the heat shock protein response, cortisol response and in the production and modulation of cytokines.
- In addition, fish that received AQUAVAC ERGOSAN demonstrated improved tolerance to various kinds of stress, resulting in improved growth.

The immunomodulator AQUAVAC™ ERGOSAN™ improved tolerance to rearing conditions and heat stress and boosted vaccine response in a study carried out on rainbow trout, as reported by Dr. Oliana Carnevali, of the department of Marine Science at the Università Politecnica delle Marche, Ancona, Italy.

An immunostimulant, she explained, is a naturally occurring compound that modulates the immune system and increases disease resistance. "We already know that oral administration of ERGOSAN in other fish species such as sea bass improves the immune response."¹

Carnevali sought to verify the positive effects of ERGOSAN in rainbow trout (*Oncorhynchus mykiss*) in a study conducted at a commercial farm in Udine, Italy, and supported by Intervet/Schering-Plough Animal Health. Although trout is technically a coldwater species, the study was conducted in a temperate climate.

For the study, 60,000 rainbow trout embryos were divided into six groups. Three of the groups received a commercial diet plus ERGOSAN and the other three served as controls and received the same diet without ERGOSAN, Carnevali said.

In the treated groups, ERGOSAN, which is made from a seaweed-based meal rich in alginates and polysaccharides, was administered to juveniles starting 10 days after the first solid feeding; the supplement was continued for 30 days, stopped for 15 days, fed for 10 days, stopped for 20 days and fed again for another 10 days, she said.

Increased body weight

Compared to controls, fish receiving ERGOSAN had increased body weight during the last sampling, which was conducted at 95 days after the first feeding, Carnevali said (Figure 2).

There were several other parameters examined, all indicating that ERGOSAN improved the immune response. Plasma cortisol levels,

which increase under physiological stress and, ultimately, inhibit infection-fighting antibody production, were analyzed using an enzyme immunoassay (EIA). The levels were significantly reduced in trout that received ERGOSAN when compared to controls, she said.

Stress also causes a cellular response characterized by expression in the liver of Heat Shock Protein 70 (Hsp70). Hsp70 in the rainbow trout was monitored with real-time polymerase chain reaction (PCR) throughout the study, and fish that received ERGOSAN had lower levels compared to controls, she said (Figure 3).

"These results demonstrate that ERGOSAN has a positive impact on the welfare of juvenile trout since fish that received the supplement had lower levels of hepatic Hsp70, lower cortisol levels and higher body weight," Carnevali said.

Results after heat shock

The effects of ERGOSAN were also studied when fish were exposed to heat shock, which consisted of raising the water temperature from 12° C to 18° C (53.6° F to 64.4° F) for 30 minutes, then lowering the temperature back to 12° C (53.6° F). Samples were taken at five different times, ranging from 2 to 72 hours, she said.

Levels of Hsp70 and cortisol were lower in the ERGOSAN-treated fish compared to controls, which demonstrates increased resistance to stressful conditions and quicker recovery from stressful events, Carnevali said.

To analyze the effect of ERGOSAN on innate (natural) immunity — the first line of defense against infection — Carnevali and associates looked at several indicators of an improved immune response, which were interleukin 1, interleukin 8 and tumor necrosis factor. These are cytokines, which are proteins secreted by immune system cells that have an effect on other cells.

All three cytokines were higher in ERGOSAN-treated fish after heat stress, "clearly evidence of the positive role ERGOSAN has on immune-system modulation," she said.

Cytokine levels higher after vaccination

Next the effects of ERGOSAN were evaluated after immersion in AQUAVAC™ ERM, a vaccine for control of Enteric Redmouth Disease (ERM) in rainbow trout. Levels of cortisol and the expression of Hsp70 were significantly lower in fish that received ERGOSAN compared to controls, which were vaccinated under the same conditions but did not receive ERGOSAN, Carnevali said.

In addition, levels of interleukin 1, interleukin 8 and tissue necrosis factor were higher in fish treated with ERGOSAN compared to controls at all 5 times they were evaluated, indicating that ERGOSAN initiated a greater immune response after vaccination, she said.

"All the cytokines we tested were significantly improved after vaccination in animals fed with ERGOSAN," Carnevali said.

"We can conclude that in rainbow trout, ERGOSAN provides a better tolerance to rearing conditions, to heat shock, which can be

figure 1

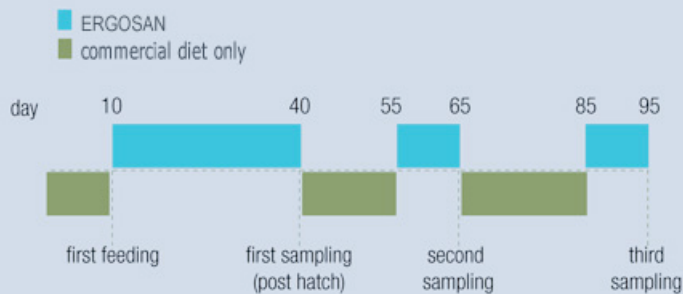


Figure 1: **Feeding schedule**

This shows the feeding schedule in ERGOSAN-treated fish. ERGOSAN administration was initiated in juveniles 10 days after beginning the first solid feeding.

figure 2

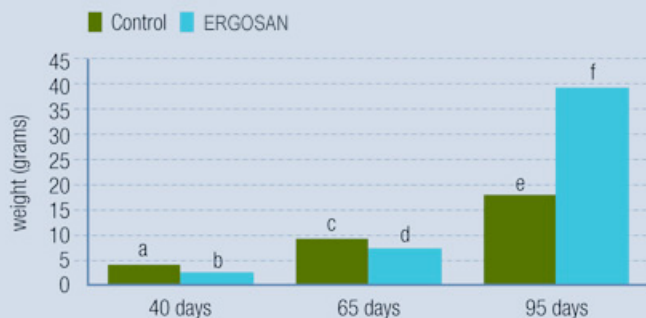


Figure 2: **Body weight**

Body weight increased in the ERGOSAN-treated fish by the 95-day sampling. Values with different letters indicate statistical significance (P < 0.05).

figure 3

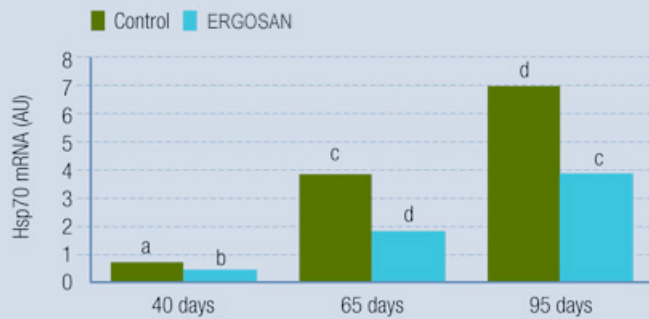


Figure 3: Cellular response

Real-time PCR showed that ERGOSAN-treated fish expressed lower levels of Hsp70, indicating they had less stress than controls. Different letters over the bars mean that the results were statistically significant.

considered an acute stress, and it increases the expression of three cytokines that work as immunomodulators in fish under shock stress," she said.

When asked during a question and answer session why an immunostimulant should be used, Carnevali said the data demonstrate that in trout, increased body weight increases production. "That's the first reason. In the current study, there was no difference in survival between the ERGOSAN-treated fish and controls, but in other species, immunomodulators increase survival, so that's a second reason to use an immunostimulant."

Robin Wardle of Intervet/Schering-Plough Animal Health's Global Aquatic Animal Health Unit added, "Dr. Carnevali's work demonstrates that chronic stress in fish, which results from ordinary farming practices, causes huge losses for producers and is contributing to sub-optimal fish performance. If stress can be modulated, these losses could be minimized. All you need is about a half-percent improvement in performance to pay for the cost of ERGOSAN," he said.

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ⁱ Bagni M, et al, Short- and long-term effects of a dietary yeast beta-glucan (Macrogard) and alginic acid (ERGOSAN) preparation on immune response in sea bass (*Dicentrarchus labrax*). *Fish Shellfish Immunol* (2005) 18: 311-25.