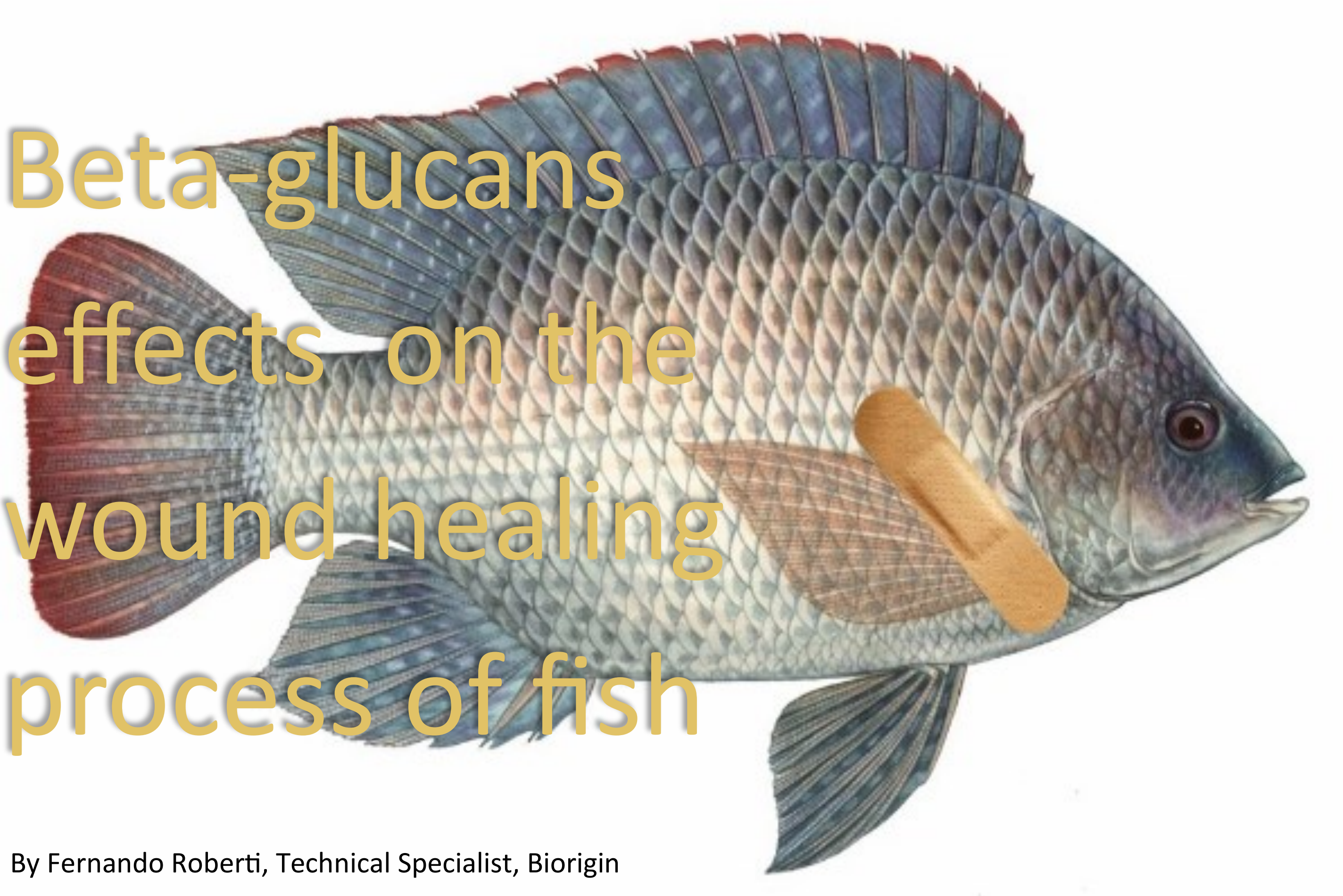

Beta-glucans effects on the wound healing process of fish

By Fernando Roberti, Technical Specialist, Biorigin

Studies confirm the beneficial effects of beta-glucans on wound healing in fish and suggest that beta-glucans are capable of accelerating the beginning of this process.

Skin lesion in fish have several causes: inadequate transport, fights due to high rearing density, skin parasite infestation, presence of sharp structures in tanks and cages, and predator attack[1].

These lesions affect fish health and development, as they may suffer locomotion problems and feeding difficulties, in addition of being a site for the entrance of pathogenic microorganisms in the body.

Beta-glucans are polysaccharides present in the cell wall of yeasts, such as *Saccharomyces cerevisiae*, which were proven to enhance the resistance against several diseases[2,3,4.] These compounds have direct action on macrophages, which are immune cells responsible for removing dead tissue and secrete growth factors that promote wound healing. Therefore, a beneficial effect of beta-glucans on wound healing may be expected.

Aiming at evaluating the potential effects of beta-glucans on the wound healing of fish, two distinct protocols were designed.

Experimental set up:

The studies were conducted at the Institute of Veterinary Medicine Research of Chungnan National University, South Korea, in partnership with Pisa University, Italy.

Adult Zebrafish, free from ectoparasites, were adapted to lab conditions (10 fish/tank, fed 4 times daily, lighting cycle of 12 h light/12 h of dark) for one month. During this period, fish received a commercial feed.

- **Protocol 1 – fin amputation:**

Fish were anesthetized with a 0.2% tricaine solution and photographed to determine total fin size before amputation. The fin was amputated 1 mm below the fin bifurcation, and fish were again photographed to determine the total amputated area. Fish were then transferred to recovery tanks until they recovered from anesthesia, after which they were placed in the original tanks. Regenerated area (relative to the amputated area) was evaluated 1, 4, 5, 6, 7, 12, and

14 days post amputation (DPA) by the use of the software Image J®.

Three treatments were applied: control and two beta-glucan sources: MacroGard® (Biorigin, Brazil) and a “R&D beta-glucan”. Beta-glucan feed inclusion dose was calculated based on daily feed intake in order to ensure an intake of 12.5 mg beta-glucans/kg live weight. Each treatment included 3 replicates (tanks). Fish started to be fed the experimental diets immediately after fin amputation.

Results of fin regeneration are shown in Figure 1. On 6 DPA, the regenerated area (RA) in the MI group was significantly larger ($P < 0.05$) than that of the control group. No differences were observed between MI and MII or between MII and C groups. This was also observed on 7 DPA.

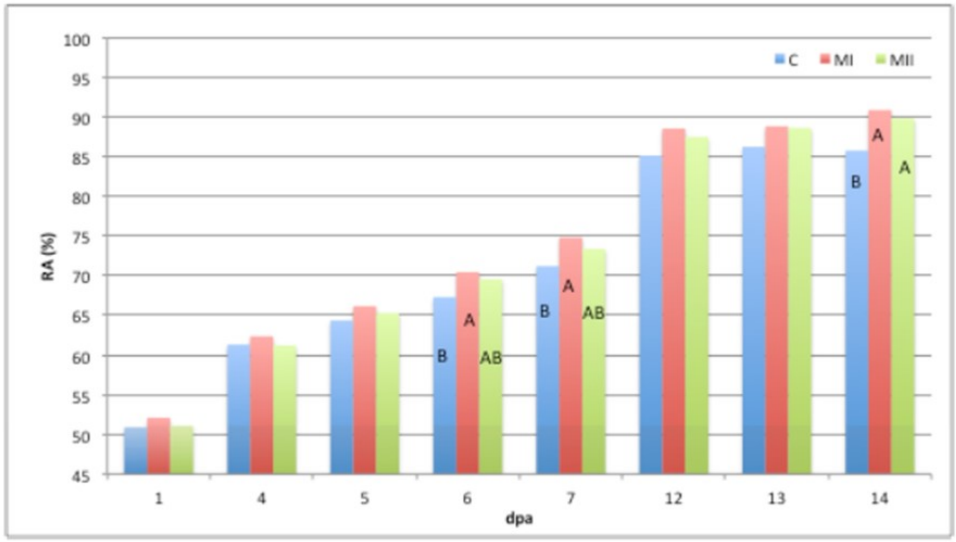

Figure 1. Regenerated area as a function of treatment and time (C – control, MI – MacroGard®, MII – R&D beta-glucan.

On 14 DPA, the RA of MI and MII fish was significantly larger ($P < 0.05$) than that of C fish, but not statistically different from each other.

- **Protocol 2 – skin wound:**

Fish were anesthetized with a 0.2% tricaine solution prior to wounding and wound follow-up. Using a laser, two circular wounds (~2mm diameter) were made on the last black stripe on the cranial right side. Healing area was evaluated on 2, 4, 12, 16, 20, 30, and 32 days post-injury (DPI) using the software Image J®. Evaluation was based on the color

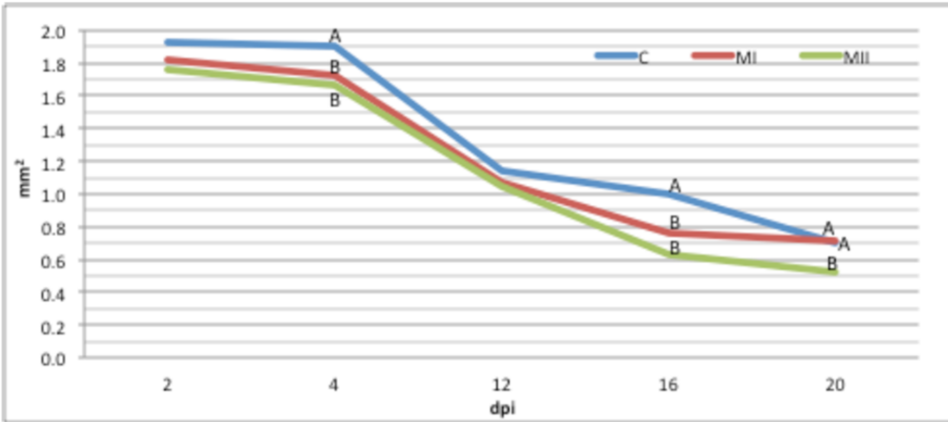

Figure 2. Regenerated area as a function of treatment and time (C – control, MI – MacroGard®, MII – R&D beta-glucan).

difference between the injured area and the intact surrounding tissue.

The wound was considered healed when it was completely regenerated and pigmented, and it was not no longer possible to differentiate it from the surrounding intact tissue. Fish were only considered healed when both wounds were completely healed.

Three treatments were applied: control and two beta-glucan sources: MacroGard® (Biorigin, Brazil) and a "R&D beta-glucan". Beta-glucan feed inclusion dose was calculated based on daily feed intake in order to ensure an intake of 12.5 mg beta-glucans/kg live weight. Each treatment included 3 replicates (tanks). The experimental diets started to be fed 14 days before wounding.

Results of wound healing are shown in Figure 2. On 4 DPI, wounds measured 1.882, 1.725, and 1.665 mm2 in groups C, MI, and MII, respectively. The effects of the dietary supplementation with beta-glucans on wound healing were already apparent on 4 DPI, as shown by the differences ($P < 0.05$) between the supplemented groups (MI and MII) and the controls.

These differences ($P < 0.05$) were observed on 16 DPI (C – 0.994; MI – 0.766; MII – 0.634), whereas on 20 DPI (C – 0.702; MI – 0.713; MII – 0.518), the healed area of the MII group was larger than that of the C and the MI groups, which were not different.

Conclusions:

The results of the studies confirm the beneficial effects of beta-glucans on wound healing in fish through the 2 protocols proposed, and also suggest that beta-glucans are capable of accelerating the beginning of this process.

Moreover, studies open a new opportunity of application of beta-glucans to several aquaculture species, aiming at alleviating the adverse effects of daily management, then promoting an adequate fish development.

More information

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**References available on request.*