Fish-farm application of a direct prophylaxis plan against flexibacteriosis and myxosporidiosis

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Abstract
In order to find a direct prophylaxis plan to control flexibacteriosis and myxosporidiosis, bioflavonoids and vitamin C (BVC) and hydrogen peroxide and peracetic acid (HPPA) based products were used monthly in Diplodus puntazzo and Dicentrarchus labrax rearing aquacultures. BVC treatment significantly limited flexibacteriosis, particularly in D. puntazzo, and induced better growth performances with stronger resistance to pathologies in the two fish species. HPPA product limited flexibacteriosis incidence and the number of Ceratomyxa sp. per fish in D. puntazzo. For its economic price and environmental friendly characteristics, HPPA may substitute formalin to control parasites.

Introduction
Investigations carried out in Sardinia on farmed fish between 1997 and 2002 revealed that the most frequent disease of marine Teleosts is flexibacteriosis (Salati et al., 1999; 2003). Regarding ectoparasites, Amyloodinium sp. and Atrispinum sp. were often responsible for heavy infestations (Merella et al., 2005); whereas regarding endoparasites, a significant and frequent presence of Ceratomyxa sp. was recorded.

Marine flexibacteriosis, caused by Tenacibaculum maritimum, is characterized by a high morbility and a low but constant mortality: fish show typical necrotic lesions on back and fins. Treatment with antibiotics provides uncertain results (Avendaño-Herrera et al., 2008).

Ceratomyxa sp. is an enteric protozoan which debilitates fish, diminishes growth performances and may induce mortality (Alvarez-Pellitero et al., 1995; Rigos et al., 1997; 1999). No therapy seems to be useful against this disease (Rigos et al., 1997; 2000).

Recent environmental policies suggest a careful use of chemotherapeutants and disinfectants to control diseases. This study was therefore performed in order to find a direct prophylaxis schedule, conducted with environmentally friendly products, which could control or reduce these fish diseases.

Materials and methods
Direct prophylaxis products
The first product used in the study contains bioflavonoids and vitamin C (BVC, composition: Bioflavonoids 2.5-3 %, vitamin

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C mg 1,000/Kg product) and must be added to feed (1 L/100 Kg of pellet). The second one contains hydrogen peroxide and peracetic acid (HPPA, composition: hydrogen peroxide 20% and peracetic acid 4.5%) and must be used by immersion (25 mL/m3 of breeding water for 2 or 3 h).

The two products characteristics are different: the first one stimulates fish immune system, whereas the second one is a disinfectant. Both were used to control flexibacteriosis and/or myxosporidiosis.

**Fish farms**
The study was carried out in three Sardinian aquaculture facilities, described in Table 1.

**Treatments**
Monthly, in every farm, a 3 days treatment was performed in the tank chosen for each product; the corresponding control tank, containing fish of the same hatching group, was reared under the same conditions. In the warmer period (May-October), treatments were conducted every 2 weeks.

**Control of health**
The presence of diseases was monitored monthly: 30 fish from each group were randomly sampled. Ichtyopathological examination was performed to reveal the presence of the two diseases and/or of other diseases. In the first and the last sampling, 100 fish from each group (treated and control) were analyzed. The Prevalence of the diseases was calculated as the number of diseased individuals/number of examined fish. However, the Prevalence of each disease was rounded to multiples of 5. For the statistic evaluation, Chi-Square Test was performed.

Microbiological examination was carried out by classical method as described by Viale et al. (2006).

Parasitological examination was carried out at stereo- and light-microscope as described by Merella et al. (2005). The Intensity of *Ceratomyxa* sp. infection was semiquantitatively evaluated following a scale based on the parasites number per microscopic field at 250×: 1-5 (+), 6-10 (++), 11-25 (+++), 26-50 (++++), 51-100 (+++++), ≥ 100 (++++++) (Alvarez-Pellitero et al., 1995).

**Growth**
Monthly, growth performances expressed as body weight mean were measured; final biometries were carried out by the farmers.

<table>
<thead>
<tr>
<th>Farm N°</th>
<th>Structure</th>
<th>Fish species and initial mean weight</th>
<th>Initial N° of fish/structure</th>
<th>Treatment *</th>
<th>Length of trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>270 m² PVC sheet coated tanks</td>
<td><em>Diplodus puntazzo</em> of g 59.0</td>
<td>30,000</td>
<td>Control BVC HPPA</td>
<td>17 months</td>
</tr>
<tr>
<td>2</td>
<td>38 m³ fiberglass tanks</td>
<td><em>Dicentrarchus labrax</em> of g 1.3</td>
<td>25,000</td>
<td>Control BVC HPPA</td>
<td>9 months</td>
</tr>
<tr>
<td>3</td>
<td>700 m³ floating cages</td>
<td><em>Dicentrarchus labrax</em> of g 18.0</td>
<td>25,000</td>
<td>Control BVC</td>
<td>16 months</td>
</tr>
</tbody>
</table>

*) BVC = bioflavonoids and vitamin C; HPPA = hydrogen peroxide and peracetic acid.

**Table 1.** Fish farms where the trial was performed.
Results

The results obtained in each fish farm are the following:

Fish Farm 1
The temperatures recorded during the trial ranged from 22°C in September to 8°C in March, then to 30°C in August to 8°C in January.

In the warm period, baths with copper sulphate were periodically carried out in all the tanks to prevent *Amyloodinium* sp. blooms.

During the experiment, two mortality episodes occurred: c. 6,000 *D. puntazzo* died in HPPA tank because water pipe was left open. After this, c. 6,000 *D. labrax*, smaller than the remaining fish, were added in the tank. In the second episode, c. 5,000 *D. puntazzo* died in BVC tank for an oxygen lack.

The results of the ichthyopathological survey showed both cutaneous lesions with isolation of *T. maritimum* and the presence of *Ceratomyxa* sp. in the gall bladder, during all the trial period. However, the presence of flexibacteriosis decreased during the trial showing a significant difference (*P* <0.001) from the beginning to the end of over one year treatment (Table 2). Also myxosporidiosis Prevalence decreased during the study (*P* <0.01) as the number of *Ceratomyxa* sp. per fish (Table 3).

At final biometries, Control tank showed the best average weight (but it was greater from the beginning) and a low number of fish (290.0 g and c. 16,000 fish), BVC and HPPA treated fish grew with a parallel trend but with a little advantage in BVC fish (BVC: 270.0 g and c. 20,500 fish; HPPA 260.0 g and c. 14,000 fish).

Fish Farm 2
The temperatures recorded during the trial ranged from 16°C in May, to 30°C in August, then to 8°C in January.

One month after the fish arrival, c. 5,000 fish in Control tank, c. 700 fish in BVC and c. 6,000 fish in HPPA tank died due to the contemporary presence of vibriosis, against which fingerlings were not immunized, flexibacteriosis and oodiniasis.

The high number and great variety of ectoparasites observed in this fish farm, which water was pumped from a semi-closed natural basin, confirmed that poor water quality can affect fish health and breeding. Nevertheless,
very few endoparasites were found in these fish.

The results of the ichthyopathological survey showed that flexibacteriosis presence decreased during the trial with a significant difference (\( P < 0.01 \)) from the beginning to the end of BVC nine months’ treatment; whereas HPPA showed less efficacy (Table 2). However, it is likely that in this farm BVC treatment against myxosporidiosis was not so effective (Table 3), taking into account that Ceratomyxa sp. infection was very low.

At final biometries, HPPA tank showed the best growth performances, but the lowest number of fish (mean weight 55.0 g and c. 17,310 fish), followed by Control (45.5 g and c. 18,760 fish) and BVC (45.3 g and c. 21,640 fish) tanks.

**Fish Farm 3**

Temperatures ranged from 25°C in October to 10°C in March, then to 27°C in August to 14°C in December.

No flexibacteriosis was observed, whereas few Ceratomyxa sp. were found in both groups (Table 2 and 3): therefore, it was not possible to suggest anything about the BVC effect on fish bred in floating cages, probably for the positive influence of the good environmental conditions. At the end of the trial, fish showed similar growth without remarkable differences between the groups.

**Prevalence of flexibacteriosis and myxosporidiosis in treated fish**

The results obtained regarding the two diseases are summarized in Table 2 and 3. Particularly, against flexibacteriosis, BVC treatment was effective in *D. puntazzo* (\( P < 0.001 \)) and *D. labrax* (\( P < 0.01 \)). While, against myxosporidiosis both BVC and HPPA treatments showed interesting results in *D. puntazzo* only.

**Discussion**

The goal of this study was to demonstrate to fish farmers that prophylaxis plans, carried on during the whole production cycle, may limit or avoid pathologies and losses.

Formalin and iodophors are the most widely used disinfectants in aquaculture. Therefore, the prophylaxis in our study was performed using environmentally friendly products.
Peracetic acid is a strong oxidant and disinfectant (Cords and Dychdala, 1993; Sanchez-Ruiz et al., 1995), which reaction products are easily biodegradable without toxic residues. Hydrogen peroxide is completely biodegradable in water and oxygen and has been used to reduce T. maritimum infection in turbot (Scophthalmus maximus) (Avendaño-Herrera et al., 2006). Vitamin C biosynthesis does not occur in fish due to an enzyme lack: it must therefore be supplied by the feed. It has been observed that fish fed with high doses of vitamin C have protective immune responses (Erdal et al., 1991; Hardie et al., 1991; Thompson et al., 1993; Roberts et al., 1995). In marine fish, Hemre et al. (1991) demonstrated that production of cortisol, released in case of stress, leads to vitamin C depletion. Therefore, Fletcher (1997) suggested that vitamin C has a positive role in the amelioration of stress and Lall (2000) showed that it is effective in reducing stress and enhancing disease resistance. Bioflavonoids are water-soluble plant pigments and they help to modify body’s reactions to allergens, viruses and carcinogens; they work in conjunction with vitamin C, preventing its destruction by oxidation (Vinson & Bose, 1988). Nevertheless, few studies on the effect of bioflavonoids on fish have been published (Bai & Gatlin III, 1992; Jenkins & Barker, 2004).

Nowadays, the use of prophylactic methods in aquaculture may contribute to substitute antibiotics, which use often induces more problems than advantages. Moreover, little bibliography regarding the control of flexibacteriosis and myxosporidiosis on fish is available.

Mortality related to external flexibacteriosis in Walleye and Channel catfish fingerlings has been proven to be significantly reduced when fish are treated with baths of hydrogen peroxide (Rach et al., 2003). In our study, good results have been obtained against flexibacteriosis of D. puntazzo treated with HPPA; nevertheless, less clear effects on myxosporidiosis were recorded.

Fish treated with BVC showed significantly higher resistance to diseases and better survival rates than controls. Particularly, BVC treated fish showed significantly less flexibacteriosis lesions, particularly on D. puntazzo when compared to controls, good growth performances and stronger resistance to pathologies in the two fish species tested. HPPA treated fish showed reduced incidence of flexibacteriosis, particularly on D. puntazzo and reduced number of Ceratomyxa sp. in D. puntazzo. Moreover, HPPA may substitute formalin due to its good efficacy and economic price. The choice of these two direct prophylaxis products was verified for their easy availability and application in fish farms, for dosage calculation and administration procedures.

In conclusion, the results obtained in this trial confirm the importance, particularly in land-based aquacultures, of direct prophylaxis plans, necessarily supported by a correct zootechnical hygiene and, when possible, by vaccination plans.

References


Bai JJ & Gatlin III DM (1992). Dietary rutin has limited synergistic effects on vitamin C nutrition of fingerling channel catfish (Ictalurus punctatus). *Fish Physiology and Biochemistry, 10*, 183-188.


