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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Farm N°	Structure	Fish species and initial mean weight	Initial N° of fish/ structure	Treatment *	Length of trial
1	270 m^3 PVC sheet coated tanks	*Diplodus puntazzo* of g 59.0	30,000	Control BVC HPPA	17 months
2	38 m^3 fiberglass tanks	*Dicentrarchus labrax* of g 1.3	25,000	Control BVC HPPA	9 months
3	700 m^3 floating cages	*Dicentrarchus labrax* of g 18.0	25,000	Control BVC	16 months

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

Table 1. Fish farms where the trial was performed.

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/m^3 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. Ichthyopathological 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.

Farm N°	Fish species	Tank/cage	P (%)*	
			Start	End
Farm 1	*Diplodus puntazzo*	Control	80	80
		BVC	80	25
		HPPA	80	50
Farm 2	*Dicentrarchus labrax*	Control	20	20
		BVC	20	5
		HPPA	20	10
Farm 3	*Dicentrarchus labrax*	Control	/	/
		BVC	/	/

*) P = Prevalence: number of individuals infected / number of fish examined (n=100), rounded to multiples of 5; / = No lesions.

Table 2. Presence of flexibacteriosis in cultured fish at the beginning and the end of the trial.

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,

Farm N°	Fish species	Tank	P (%)*		Mean I **	
			Start	End	Start	End
Farm 1	*Diplodus puntazzo*	Control	80	80	+++	++++
		BVC	80	60	+++	++
		HPPA	80	60	+++	++
Farm 2	*Dicentrarchus labrax*	Control	/	10	/	+
		BVC	/	10	/	+
		HPPA	/	/	/	/
Farm 3	*Dicentrarchus labrax*	Control	/	10	/	+
		BVC	/	10	/	+

*) P = Prevalence: number of individuals infected / number of fish examined (n=100), rounded to multiples of 5; / = No lesions.
**) Mean I = Mean Intensity: mean number of individuals of parasite per infected fish: 1-5 (+), 6-10 (++), 11-25 (+++), 26-50 (++++), 51-100 (+++++), ≥100 (++++++); / = No *Ceratomyxa* sp.

Table 3. Presence of *Ceratomyxa* sp. in cultured fish at the beginning and the end of the trial.

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

Alvarez-Pellitero P, Sitjà-Bobadilla A, Franco-Sierra A & Palenzuela O (1995). Protozoan parasites of gilthead sea bream, *Sparus aurata* L., from different culture systems in Spain. *Journal of Fish Diseases* **18**, 105-115.

Avendaño-Herrera R, Magarinos B, Irgang R & Toranzo AE (2006). Use of hydrogen peroxide against the fish pathogen *Tenacibaculum maritimum* and its effect on infected turbot (*Scophtalmus maximus*). *Aquaculture* **257**, 104-110.

Avendaño-Herrera R, Nuñez S, Barja JL & Toranzo AE (2008). Evolution of drug resistance and minimum inhibitory concentration to enrofloxacin in *Tenacibaculum maritimum* strains isolated in fish farms. *Aquaculture Internationa*l **16**, 1-11.

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.

Cords BR & Dychdala GR (1993). Sanitizers: Halogens, Surface-active agents, and Peroxides. *In* "**Antimicrobials in Food"** (P.M. Davidson and A.L. Branen, Eds.), pp 469-537. 2 Ed. Marcel Dekker, Inc., New York.

Erdal JI, Evensen O, Kaursted OK, Lillehaug A, Solbakken R & Thorud K (1991). Relationship between diet and immune response in Atlantic salmon (*Salmo salar* L.) after feeding various levels of ascorbic acid and omega-3 fatty acids. *Aquaculture* **98**, 363-379.

Fletcher TC (1997). Dietary effects on stress and health. *In* "**Fish Stress and Health in Aquaculture"** (G.K.Iwama, A.D. Pickering, J.P. Sumpter and C.B. Schreck, Eds.), Cambridge Univ. Press, Cambridge.

Hardie LJ, Fletcher TC & Secombes CJ (1991). The effect of dietary vitamin C on the immune response of Atlantic salmon (*Salmo salar*). *Aquaculture* **95**, 201-214.

Hemre GI, Lambertsen G & Lie O (1991). The effect of dietary carbohydrates on the stress response in cod (*Gadus morhua*). *Aquaculture* **95**, 319-328.

Jenkins CS & Barker DE (2004). The efficacy of oral immunostimulants in enhancing resistance against microsporidiosis in juvenile Atlantic cod (*Gadus morhua* L.) *Aquaculture Association of Canada*, Special Publication, **9**, 97-100.

Lall SP (2000). Nutrition and health of fish. *In* "**Avances en Nutrición Acuícola V. Memorias del V Simposium Internacional de Nutrición Acuícola**" (L.E. Cruz -Suárez, D. Ricque-Marie, M. Tapia-Salazar, M.A. Olvera-Novoa and R. y Civera-Cerecedo, Eds.). 19-22 Noviembre, 2000. Mérida, Yucatán, Mexico.

Merella P, Cherchi A, Salati F & Garippa G (2005). Parasitological survey of Snarpsnout seabream, *Diplodus puntazzo* Cetti 1777, reared in sea cages in Sardinia (western Mediterranean). *Bulletin of the European Association of Fish Pathologists* **25**, 140-147.

Rach JJ, Schleis SM, Gaikowski MP & Johnson A (2003). Efficacy of hydrogen peroxide to control mortality associated with columnaris infections on walleye and channel catfish fingerlings. *Journal of North American Aquaculture*, **65**, 300-305.

Rigos G, Christophilogiannis P, Yiagnisi M, Andriopoulou A, Koutsodimou M, Nengas I & Alexis M (1999). Myxosporean infections in Greek mariculture. *Aquaculture International* **7**, 361-364.

Rigos G, Kotzamanis I, Gialamas I, Nengas I & Alexis M (2000). Toxicity and digestibility of fumagallin DHC in gilthead sea bream, *Sparus aurata* L. *Journal of Fish Diseases* **23**, 161-164.

Rigos G, Grigorakis K, Christophilogiannis P, Nengas I & Alexis M (1997). *Ceratomyxa* spp. (Myxosporea) infections in cultured common dentex from Greece. *Bulletin of the European Association of Fish Pathologists* **17**, 174-176.

Roberts ML, Davies SJ & Pulsford AL (1995). The influence of ascorbic acid (vitamin C) on non-specific immunity in the turbot (*Scophtalmus maximus* L.). *Fish and Shellfish Immunology* **5**, 27-38.

Salati F, Meloni A & Viale I (1999). L'acquacoltura e lo stato sanitario dei pesci allevati in Sardegna. *Bollettino della Società italiana di Patologia ittica* **26**, 3-9.

Salati F, Meloni A, Cubadda C & Viale I (2003). Prove di allevamento e patologie del dentice (*Dentex dentex*, L.) in Sardegna. *Bollettino della Società italiana di Patologia ittica* **38**, 12-26.

Sanchez-Ruiz C, Martinez-Royana S & Tejero-Monzon I (1995). An evaluation of the efficiency and impact of raw wastewater disinfection with peracetic acid prior to ocean discharge. *Water Science Technology* **32**, 159-166.

Thompson I, White A, Fletcher TC, Houlihan DF & Secombers CJ (1993). The effect of stress on the immune response of Atlantic salmon (*Salmo salar* L.) fed diets containing different amounts of vitamin C. *Aquaculture* **114**, 1-18.

Viale I, Angelucci G, Kusuda R & Salati F (2006). Immunization of European sea bass, *Dicentrarchus labrax* L., fingerlings with a commercial vaccine against vibriosis: an one year survey on antibody level, diseases and growth. *Journal of Applied Aquaculture* **18**, 53-67.

Vinson JA & Bose P (1988). Comparative bioavailability to humans of ascorbic acid alone or in a citrus extract. *American Journal of Clinical Nutrition* **48**, 601–4.