

Effect of Fumagillin treatment on sea bass *Dicentrarchus labrax* parasitized by *Sphaerospora testicularis* (Myxosporea: Bivalvulida)

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ABSTRACT: The antibiotic Fumidil-B was tested at a dosage of 0.1 % Fumagillin against sea bass *Dicentrarchus labrax* L. testicular sphaerosporosis in 2 experiments. In Expt 1, fish with naturally acquired advanced sphaerosporosis were treated for 8 wk, whereas in Expt 2, fish experimentally inoculated with *Sphaerospora testicularis* were medicated for 44 d (short treatment, ST) and 91 d (long treatment, LT). Toxic effects consisting of loss of appetite and weight and significant decrease of hemoglobin, hematocrit and red blood cell counts appeared in treated fish in both Expt 1 and LT Expt 2. Efficacy of the drug was not apparent until the following spawning season in Expt 1, whereas in Expt 2 only the data concerning drug toxicity could be evaluated.

INTRODUCTION

Myxosporoses were considered non-treatable fish diseases for a long time, and prophylactic measures were the only way to fight against important economic losses. Nevertheless, in the last decade several drugs have been assayed against myxosporeans, such as Proguanil and Clamoxiquin (Alderman 1986), Toltrazuril (Mehlhorn et al. 1988, Schmahl et al. 1989, Yokoyama et al. 1990) and Fumagillin. The latter is an antibiotic isolated from the fungus *Aspergillus fumigatus*, with a low antibiotic and antifungic action but with a high antibacteriophagic (Mills 1955) and amebicide (McCowen et al. 1951, Killough et al. 1952) activity. First trials were performed against honey bee noseemiasis (Katznelson & Jamieson 1952, Bailey 1953) and, later they were extended to other microsporean diseases of insects (Lewis & Lynch 1970, Lynch & Lewis 1971, Liu 1973) and mammals (Shaddock 1980). Fumidil-B (the bicyclohexyl amine salt of Fumagillin acid) has been successfully used against fish microsporean infections (Takahashi & Egusa 1976, Kano & Fukui 1982, Kano et al. 1982, Hedrick et al. 1991).

Recently, experimental assays have shown the potential use of this drug against several myxosporeans such as *Sphaerospora renicola* in carp (Molnár et al. 1987), PKX in salmonids (Hedrick et al. 1988), *Myxidium giardi* in European eels (Székely et al. 1988), *Hoferellus carassi* in goldfish (Yokoyama et al. 1990) and *Myxobolus cerebralis* in salmonids (El-Matbouli & Hoffman 1991). *Sphaerospora testicularis* has been described as an important parasite of cultured sea bass, which destroys testicular tissue and greatly reduces the fecundity of males (Sitjà-Bobadilla & Alvarez-Pellitero 1990). In the present work, data on the effect of Fumidil-B treatment upon this testicular sphaerosporosis, and its possible side effects on the host are reported.

MATERIALS AND METHODS

Expt 1. Fish: Mature male sea bass *Dicentrarchus labrax* L. naturally infected by *Sphaerospora testicularis*, were obtained from the Instituto de Acuicultura de Torre la Sal (IATS) facilities. They were reared in running sea water (3.78 % salinity) at natural tempera-

ture and photoperiod. Mean weight of fish at the start of the experiment was 800 g.

Experimental conditions: Specimens were divided into 2 groups of 10 fish each, with a similar average level of infection, and kept in 2000 l tanks. One group was fed on a natural diet consisting of trash fish *Boops boops*, containing gelatin capsules with Fumidil-B at a dose of 1 g Fumagillin kg⁻¹ feed. Treatment started on 24 May (= Day 0) and continued for 8 wk. The other group received an unmedicated diet. Both groups were fed at 1.5 % body weight d⁻¹.

Detection of myxosporean and evaluation of toxicity of treatment: As *Sphaerospora testicularis* diagnosis can be performed without necropsy (Sitjà-Bobadilla & Alvarez-Pellitero 1990), fish were periodically anaesthetized with MS-222 (Sigma Chemicals Co., St. Louis, MO, USA) 100 ppm, and stripped or cannulated to obtain seminal fluid or testicular tissue respectively, in which *S. testicularis* stages can be detected. In addition, some fish were necropsied at significant sampling times (Days 20, 250, 309 and 371) and their testes processed for histological studies. At the end of the experiment (Day 371) all fish were killed.

In order to monitor the possible toxic effects of the treatment, blood was collected from the caudal vessels of anaesthetized fish on Days -20, 20, 61, 98, 128 and 155. Haemoglobin concentration (Hb) (Hemocue Photometer, AB Leo Diagnostics, Sweden), haematocrit level (Hc) and red blood cell counts (RBC) (Neubauer chamber) were determined. Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin content (MCH) and mean cellular volume (MCV) were also calculated. Hematopoietic organs (head kidney and spleen) and liver from necropsied fish were also processed for histology.

Histological procedure: Tissue portions, fixed with Bouin's solution, were embedded in Paraplast, thin sectioned and stained with hematoxylin-eosin (H&E). In some cases, 1 to 3 µm sections were obtained from material fixed with 2.5 % glutaraldehyde, embedded in Historesin (Leica, Spain), and stained with toluidine blue (TB).

Statistics: Student's *t*-test was used to analyze the influence of the treatment on the studied parameters. Statistical significance was estimated at $p < 0.05$ and $p < 0.01$, and is shown in each figure.

Expt 2. Fumagillin treatment was assayed in a second experiment, using sea bass inoculated with sperm containing high quantities of spores and other developmental stages of *Sphaerospora testicularis*, to assess its utility as a preventive method. As experimental transmission was not achieved (unpubl. data), the provided results deal only with the toxic effects of the treatment.

Fish and experimental conditions: Forty male sea bass (age: 2+ yr) free of *Sphaerospora testicularis*,

obtained from an IATS cultured stock, were maintained in 4 experimental tanks (10 fish each) supplied with seawater free of pathogens. Fish from 2 tanks were inoculated intracelomically with stages of *S. testicularis* (20 January = Day 0). One day later, fish began receiving a commercial dry food containing 0.1 % Fumagillin. Treatment was stopped at 44 d post-injection for 1 medicated tank (short treatment, ST) and at 91 d post-injection for the other medicated tank (long treatment, LT). Fish from the other 2 tanks were kept as a control group and received an unmedicated diet.

Detection of the myxosporean, evaluation of toxic effects, histological procedure and statistics applied were the same as in Expt 1.

RESULTS

Efficacy of treatment

All the reported results deal with Expt 1. Fig. 1 shows the evolution of prevalence of infection by *Sphaerospora testicularis*. During most of the experiment the level of infection in both groups was very similar. Nevertheless, in the last sampling (Day 309), which coincided with the subsequent spawning season after treatment, medicated fish showed a clearly lower prevalence of infection than untreated ones, and most of the treated infected fish presented low intensities of infection and harboured no spores. In addition, treated fish started spermiation 2 mo earlier than untreated ones.

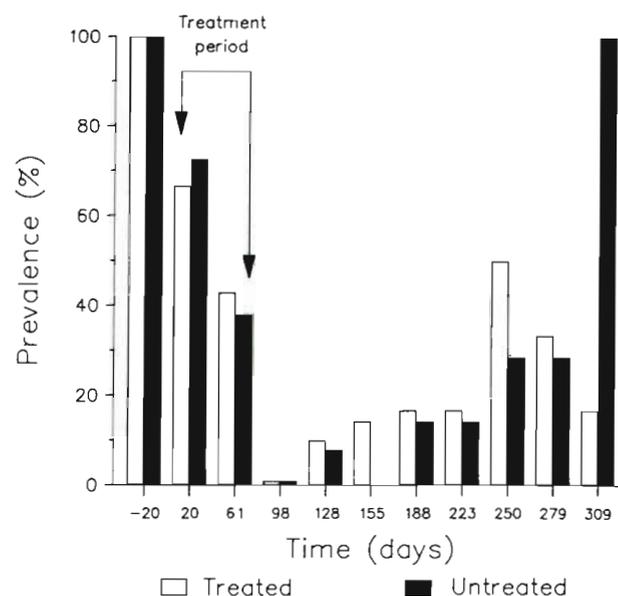


Fig. 1 *Sphaerospora testicularis*. Evolution of the prevalence of infection in sea bass in Expt 1

Histological sections of testes showed a similar testicular damage provoked by the sphaerosporid in both groups throughout most of the experiment. However, at Day 371, in the interbreeding period, testes from control fish appeared with extensive gran-

ulomatous areas and scarce germinative development (Fig. 2A). In contrast, testes from medicated fish had numerous primary spermatogonia (Fig. 2B). Accordingly, at Day 309, during the spawning season, testes from treated fish showed seminiferous tubules filled

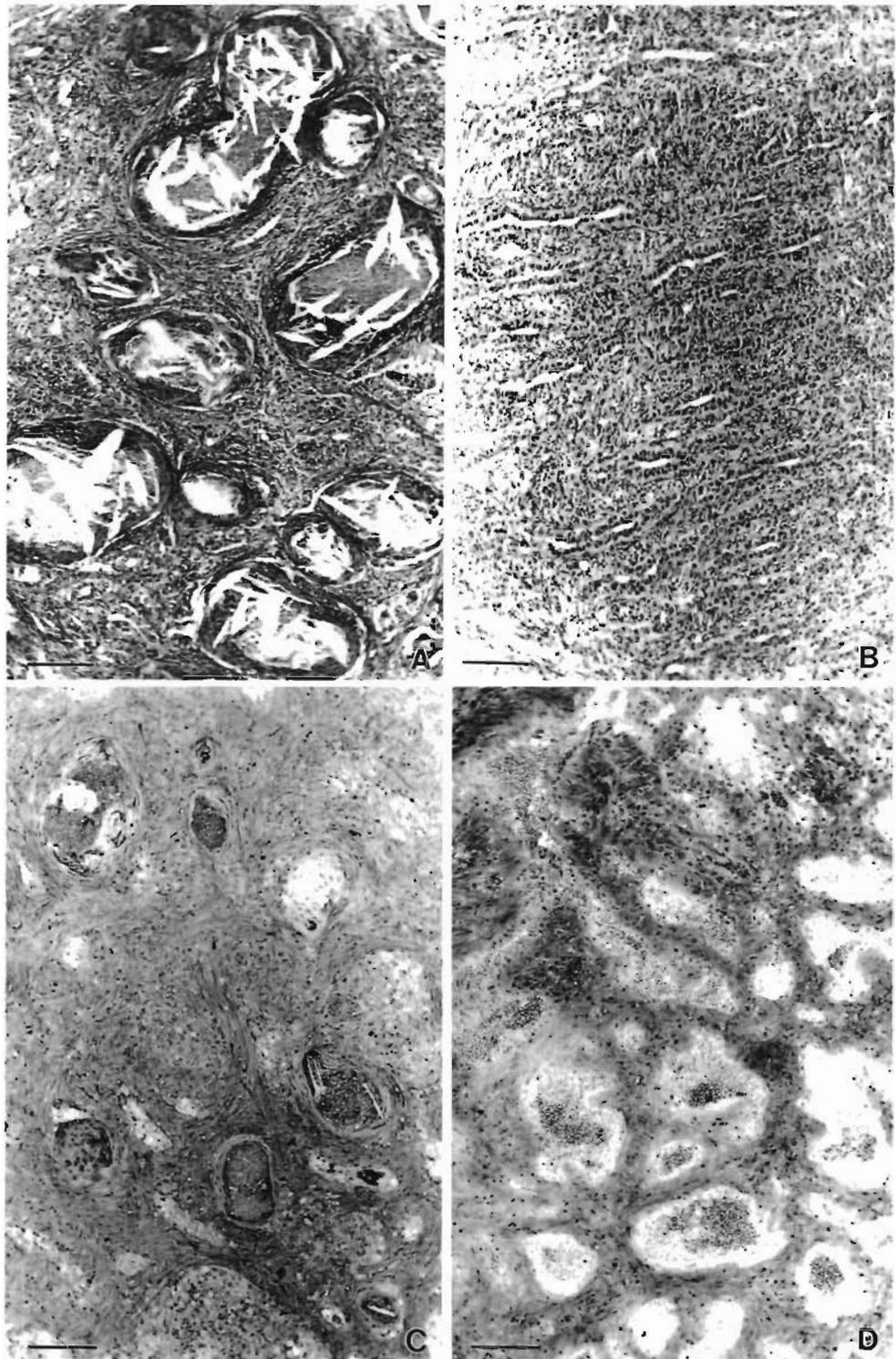


Fig. 2. *Sphaerospora testicularis*, *Dicentrarchus labrax*. Paraplast (A & B; at Day 371, during the interbreeding season) and Histoiresin (C & D; at Day 309, during the spawning season) sections of sea bass testes of Expt 1. (A) Granulomatous view of an untreated fish. (B) Panoramic view of a normal testis with proliferation of spermatogonia from a Fumagillin-medicated fish. (C) Testis with absence of germinative tissue and large areas of necrotic debris, from an untreated male. (D) Testis with spermatozoa from a medicated fish. Stain: (A & B), H&E; (C & D), TB. Scale bars = 100 μ m

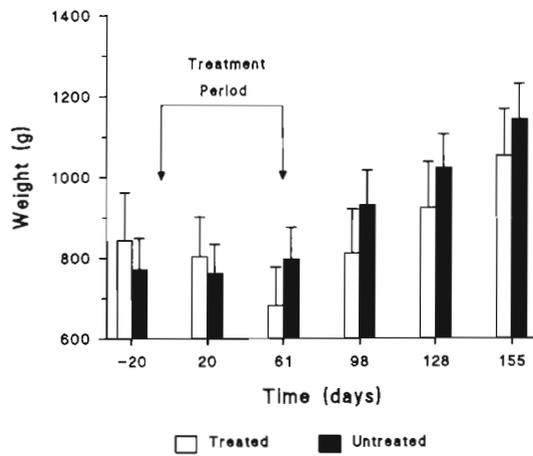


Fig. 3. *Dicentrarchus labrax*. Comparison of sea bass weight (± 1 SE) for treated and control groups in Expt 1

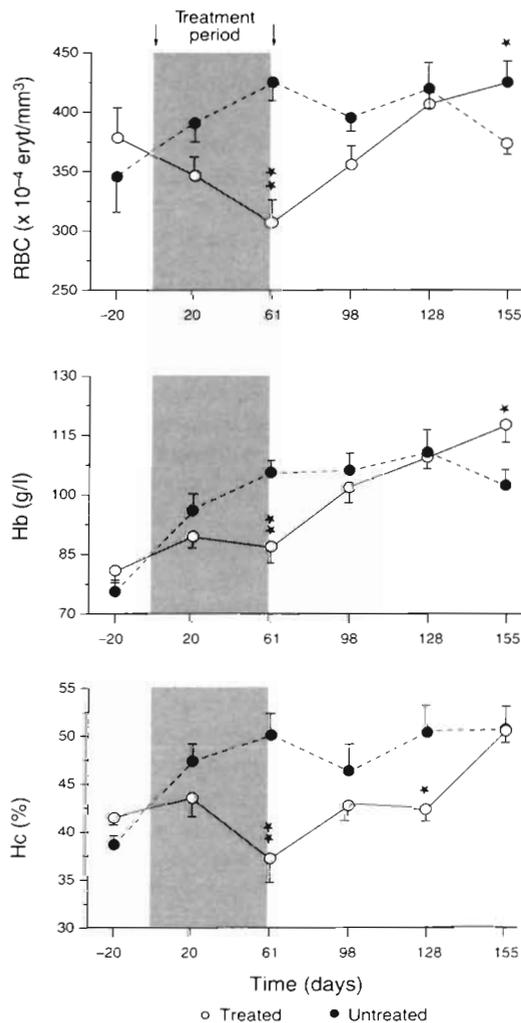


Fig. 4. *Dicentrarchus labrax*. Comparison of red blood cell count (RBC) haemoglobin (Hb) and hematocrit (Hc) (± 1 SE) for treated and control groups in Expt 1. *: $p < 0.05$, **: $p < 0.01$

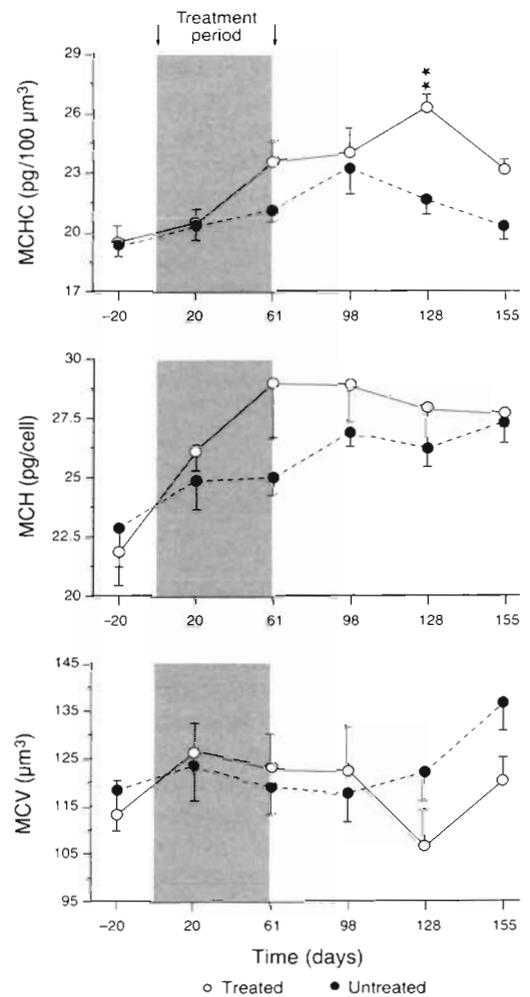


Fig. 5. *Dicentrarchus labrax*. Comparison of mean corpuscular haemoglobin content (MCHC), mean haemoglobin concentration (MCH) and mean corpuscular volume (MCV) (± 1 SE) for treated and control groups in Expt 1. **: $p < 0.01$

with spermatozoa (Fig. 2D), whereas testes from untreated males lacked germinative tissue almost completely, and fibrotic granuloma occupied the seminiferous tubules (Fig. 2C).

Toxic effects

Expt 1

After 8 wk of medication, treated fish showed swimming disorders, loss of appetite and weight (Fig. 3) and statistically significant decreases of Hb, Hc and RBC (Fig. 4). Additionally, treated fish had higher MCHC and MCH than controls, whereas MCV values of the treated group remained slightly lower but similar to those of the untreated group (Fig. 5). Study of sections from the hematopoietic organs did not reveal any noticeable damage or alteration of white and red cells composition.

Table 1 *Dicentrarchus labrax*. Comparison of haemoglobin (Hb), hematocrit (Hc) and red blood cell count (RBC) (all ± 1 SE) in Expt 2. C: control group; ST: short treatment group; LT: long treatment group. Different superscripted letters indicate significant differences ($p < 0.05$) between groups on each sampling day

Sampling day	Type of treatment	Hb (g l^{-1})	Hc (%)	RBC ($\times 10^{-4} \text{ mm}^{-3}$)
-20	C	97.69 \pm 2.92	50.25 \pm 1.73	348.69 \pm 11.69
15	C	108.86 \pm 2.14 ^b	51.95 \pm 1.23 ^{ab}	397.52 \pm 13.84
	ST	98.10 \pm 3.57 ^b	43.70 \pm 1.10 ^b	370.20 \pm 13.40
	LT	130.50 \pm 4.51 ^a	54.37 \pm 1.60 ^a	402.40 \pm 14.50
35	C	83.05 \pm 2.57	36.58 \pm 1.31 ^{ab}	355.32 \pm 11.80 ^a
	ST	82.80 \pm 3.70	40.90 \pm 0.06 ^{ab}	299.30 \pm 13.00 ^{ab}
	LT	91.70 \pm 5.40	46.00 \pm 0.02 ^d	339.20 \pm 22.00 ^{ab}
90	C	98.70 \pm 3.86 ^a	41.20 \pm 1.13 ^d	302.00 \pm 9.86 ^a
	ST	84.70 \pm 4.90 ^{ab}	38.00 \pm 1.50 ^a	332.60 \pm 11.66 ^a
	LT	65.70 \pm 4.90 ^b	29.80 \pm 2.10 ^b	240.10 \pm 19.90 ^b

Table 2 *Dicentrarchus labrax*. Comparison of mean corpuscular haemoglobin content (MCHC), mean haemoglobin concentration (MCH) and mean corpuscular volume (MCV) (all ± 1 SE) in Expt 2. C: control group; ST: short treatment group; LT: long treatment group. Different superscripted letters indicate significant differences ($p < 0.05$) between groups on each sampling day

Sampling day	Type of treatment	MCHC ($\text{pg } 100 \mu\text{m}^{-3}$)	MCH (pg cell^{-1})	MCV (μm^3)
-20	C	19.70 \pm 0.76	28.52 \pm 1.38	146.75 \pm 7.53
15	C	21.11 \pm 0.50 ^b	27.99 \pm 1.01	132.99 \pm 4.34 ^{ab}
	ST	22.50 \pm 0.73 ^{ab}	26.71 \pm 1.08	119.43 \pm 4.91 ^b
	LT	23.20 \pm 3.00 ^a	30.62 \pm 2.06	131.97 \pm 8.03 ^a
35	C	22.90 \pm 6.60 ^a	25.02 \pm 0.06	110.09 \pm 3.73 ^b
	ST	20.20 \pm 2.70 ^{ab}	27.63 \pm 1.01	136.72 \pm 3.49 ^{ab}
	LT	20.00 \pm 6.00 ^b	27.23 \pm 1.01	136.17 \pm 3.09 ^a
90	C	23.90 \pm 0.80	32.90 \pm 1.48 ^a	137.22 \pm 4.21 ^a
	ST	22.30 \pm 0.80	24.58 \pm 1.78 ^b	110.61 \pm 7.00 ^b
	LT	22.00 \pm 0.60	27.78 \pm 1.52 ^b	126.61 \pm 7.15 ^{ab}

Two months following cessation of treatment (Day 128), Hb, RBC and MCH of medicated fish recovered to normal values, though Hc remained significantly lower than in control fish. Furthermore, MCHC increased significantly in treated fish, while MCV was lower than in controls. Later on (Day 155), a rebound effect was observed in Hb and RBC, whereas Hc, MCHC and MCH tended to reach control values.

Expt 2

In Tables 1 & 2, the values of the studied hematological parameters in ST, LT and control groups are expressed. A statistically significant decrease of Hb, Hc and RBC was observed in the LT group at 90 d post-injection, similar to that observed in Expt 1. Nevertheless, this decrease was lower in the ST group, and without statistical significance.

The histological study did not reveal significant changes in hematopoietic organs of treated fish. Nevertheless, the liver of medicated fish exhibited a certain degree of steatosis at 15 d post-injection (Fig. 6), which was more evident at Day 35.

DISCUSSION

The reported results revealed the potential use of Fumagillin against *Sphaerospora testicularis*. It was shown in Expt 1, by the lower prevalence of infection of treated fish than that of untreated ones in the spawning season following Fumagillin medication, the testicular recovery of some medicated fish and their earlier spermiation time. Fumagillin has been reported to stop sporogenesis of *Myxidium giardi* from *Anguilla anguilla* (Székely et al. 1988), *S. renicola* from *Cyprinus carpio* (Molnár et al. 1987), PKX

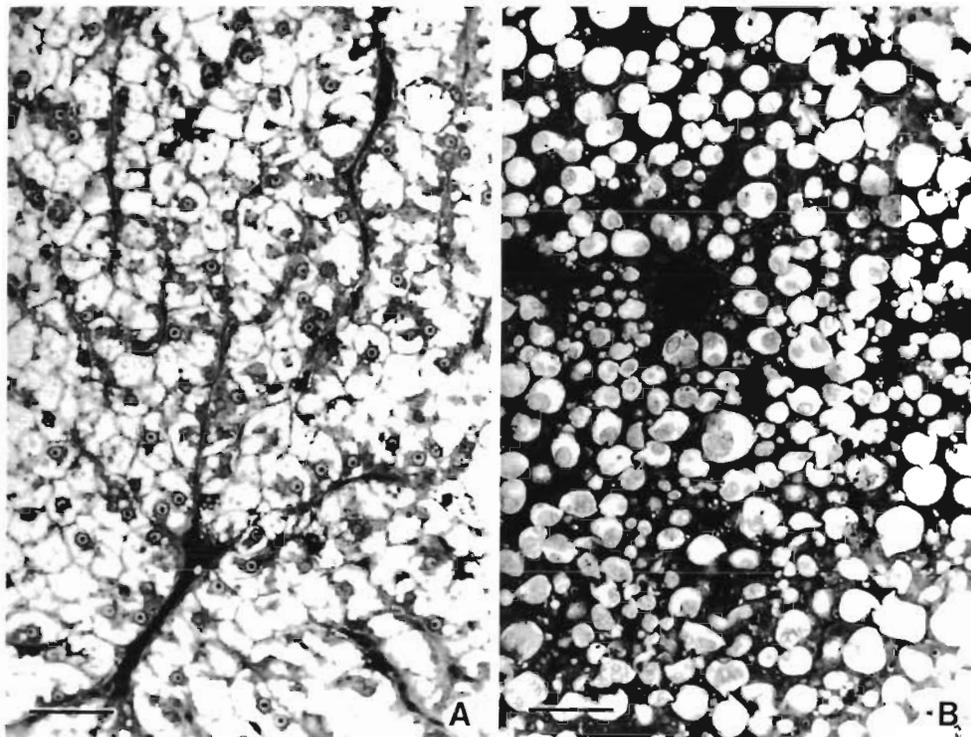


Fig. 6. *Dicontrarchus labrax*. Paraffin sections of liver from sea bass in Expt 2 at 15 d post-injection. (A) Untreated and (B) treated fish. Stain: H&E. Scale bars = 50 μ m

in salmonids (Hedrick et al. 1988), early intracellular trophozoites and more developed plasmodia of *Hoferellus carassi* (Yokoyama et al. 1990), and *Myxobolus cerebralis* in rainbow trout (El-Matbouli & Hoffman 1991).

Nevertheless, our treatment did not appear to be capable of stopping a well established sphaerosporosis with spores and other developmental stages, as the infection persisted in treated fish throughout most of the experimental period, until the following spawning season. The seasonal fluctuations and the testicular damage observed in parasitized fish were similar to those reported previously in other sea bass stocks (Sitjà-Bobadilla & Alvarez-Pellitero 1990, 1992). The inability of Fumagillin to stop advanced myxosporean infections has been pointed out by other authors (Molnár et al. 1987, Székely et al. 1988), though it might alter spore viability of *Myxobolus cerebralis* (El-Matbouli & Hoffman 1991). On the other hand, the real efficacy of Fumagillin as a preventive treatment of testicular sphaerosporosis remains to be elucidated, due to the lack of an experimental model.

Toxic effects of Fumagillin treatment have been documented by several researchers. Effects ranged from the dizziness and loss of appetite of human patients treated for amebiasis (Killough et al. 1952), to the mortality provoked by an 8 wk dosage of 1.0 g kg^{-1} feed in PKD-infected rainbow trout (Hedrick et al. 1988). However, moderate side effects are most

commonly reported. Hedrick & McDowell (1987) and Hedrick et al. (1988) described certain toxicity in chinook salmon *Oncorhynchus tshawytscha*, experimentally infected by PKX, with a Fumagillin-DCH treatment similar to ours. They also observed a depletion of haematopoietic cells in the kidney interstitium and vacuolation of the epithelium of the renal tubules. In contrast, we did not observe any histopathological sign in haematopoietic tissues of medicated sea bass. A slighter treatment (4 wk dosage of 0.01 %) applied against chinook salmon experimentally infected by the microsporean *Enterocytozoon salmonis*, was less toxic but it also induced a decrease of Hc and haematopoietic renal tissue (Hedrick et al. 1991).

Our treatment assays had toxic side effects, mainly consisting of a decrease in Hc, Hb and RBC values. These decreases were probably due to a destruction of erythrocytes, which provoked the compensatory increase of MCHC and MCH. In Expt 1, the progressive recovery of haematologic values after the withdrawal of the drug indicates the reversible nature of the toxicity, as it has been reported by other authors (Lauren et al. 1989, Wishkovsky et al. 1990, Hedrick et al. 1991). The slower recovery of the Hc values than those of RBC and Hb could be explained by the decrease of MCV. However, these blood changes were not correlated with histological anomalies of the haematopoietic tissues in any experiment. In the opinion of Lauren et al. (1989), the decrease in hemato-

crit combined with hypocellularity of the head kidney and spleen suggests that the treatment induces aplastic anemia.

Steatosis has been described as a physiological process in wild sea bass, and as a consequence of unbalanced artificial diets in cultured fish as well (Lemaire et al. 1991). In Expt 2, the vacuolation of hepatic tissue due to an excessive lipid storage in treated individuals cannot be attributed to the diet, since untreated fish, fed with the same diet, did not exhibit such a hepatic degeneration. Probably, Fumagillin medication affected lipidic metabolism. Lauren et al. (1989) have also reported hepatic changes (hemorrhages, congestion and focal perisinusoidal extravasation of blood) in rainbow trout injected via aorta with 60 and 30 mg Fumagillin-DCH kg⁻¹ body wt. Nevertheless, hepatic toxicity was not apparent when F-DCH was administered in the diet over a long period of time.

In conclusion, the toxic side effects of this drug seem to depend on the dose and duration of treatment. The efficacy of Fumagillin appears to be related to the degree of establishment of the parasite in the host, at the time of treatment and thus Fumagillin should be considered more as a parasitostatic than as a parasiticide.

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