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SHORT COMMUNICATION

In-feed trials of fenbendazole and other chemical/natural compounds against *Sparicotyle chrysophrii* (Monogenea) infections in *Sparus aurata* (Osteichthyes)

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The microcotylid *Sparicotyle chrysophrii* (Van Beneden & Hesse, 1863) Mamaev, 1984 (Platyhelminthes, Monogenea) is a gill parasite of the gilthead seabream *Sparus aurata* L., 1758 (Osteichthyes, Sparidae) that has become one of the main harmful threats to its breeding (Sitjà-Bobadilla et al., 2006). Formalin baths against monogeneans are progressively banned, because they are costly, labour intensive, imply health risks to workers, have environmental impact and cause stress to fish (Forwood et al., 2013). In-feed drug administration offers alternatives against fish parasites, avoiding such drawbacks (Williams et al., 2007).

Among chemotherapics, Praziquantel (PZQ) is a synthetic pyrazinoisoquinoline (Bader et al., 2019), and several studies have shown its effectiveness against monogeneans by oral administration (50–200 mg/kg BW/day for 3–6 days) on diverse fish groups, including *S. aurata* (Forwood et al., 2016; Hirazawa et al., 2013; Partridge et al., 2014; Sitjà-Bobadilla et al., 2006; Williams et al., 2007). Fenbendazole (FBZ) is a benzimidazole drug commonly used against nematodes and cestodes (Baeder et al., 1974), scantly used against monogeneans at doses of 20–75 mg/kg BW/day for 3–6 days (Forwood et al., 2013; Gupta et al., 2019; Kimura et al., 2006), and never tried against *S. chrysophrii*.

Among natural compounds, caprylic acid (CAPR) is a medium-chain fatty acid present in milk and in plant oils. Hirazawa et al., (2000), Hirazawa et al., (2001) showed its efficacy against monogeneans at 25–100 mg/kg BW/day for 30–72 days, and Rigos et al., (2013), Rigos et al., (2016) administered 200 mg/kg BW/day for 60 days against *S. chrysophrii*. Finally, the dietary supplementation of garlic *Allium sativum* (GARL) for monogenean control has been tested for diverse host and parasite species, at 400–4000 mg/kg BW/day (Martins et al., 2002; Militz et al., 2013; Fridman et al., 2014; Inoue et al., 2016).

This study aims the evaluation of new in-feed treatments against *S. chrysophrii*, with the goal of developing practical solutions for aquaculture.

To test the efficacy of treatments, five trials were carried out (Table 1). For each trial, a total of 75 fish were collected from local fish farms and transported alive to the Department facilities; an extra sample of ten specimens was collected to evaluate the presence of *S. chrysophrii* and to calculate the host weight.

Fish were split into three 142 L tanks (25 specimens), and tanks had independent recirculation with mechanical/biological filtration. Water quality was maintained between the optimal ranges for the host (temperature 20–23°C; salinity 34–36‰; photoperiod 12:12 h light/dark). Fish were fed 6 days a week, at a rate of 2% of the mean body weight recalculated with the last measurements. Rejected feed was removed 15 minutes after administration and quantified to assess the effective ration and estimate the actual dose administered.

To maintain *S. chrysophrii* under experimental conditions, entangling and hatching of parasite eggs were favoured by placing in

TABLE 1 List of the challenge trials of experimental feeds against *Sparicotyle chrysophrii* on the gills of *Sparus aurata*

Trial	Date	T (°C)	Group 1	Group 2	Group 3	W ± s.d. (g)
01	12/11/18-10/12/18	22.2	Control	GARL	PZQ	35.8±6.8
02	28/01/19-25/02/19	22.1	‘ ‘	CAPR	FBZ	44.7±15.4
03	08/04/19-06/05/19	20.0	‘ ‘	CAPR	PZQ	44.7±14.8
04	17/06/19-15/07/19	23.4	‘ ‘	GARL	FBZ	41.0±12.7
05	30/09/19-28/10/19	20.6	‘ ‘	GARL	CAPR	36.2±8.9

Abbreviation: T, mean temperature in the aquariums; W, mean body weight of fish; s.d., standard deviation.

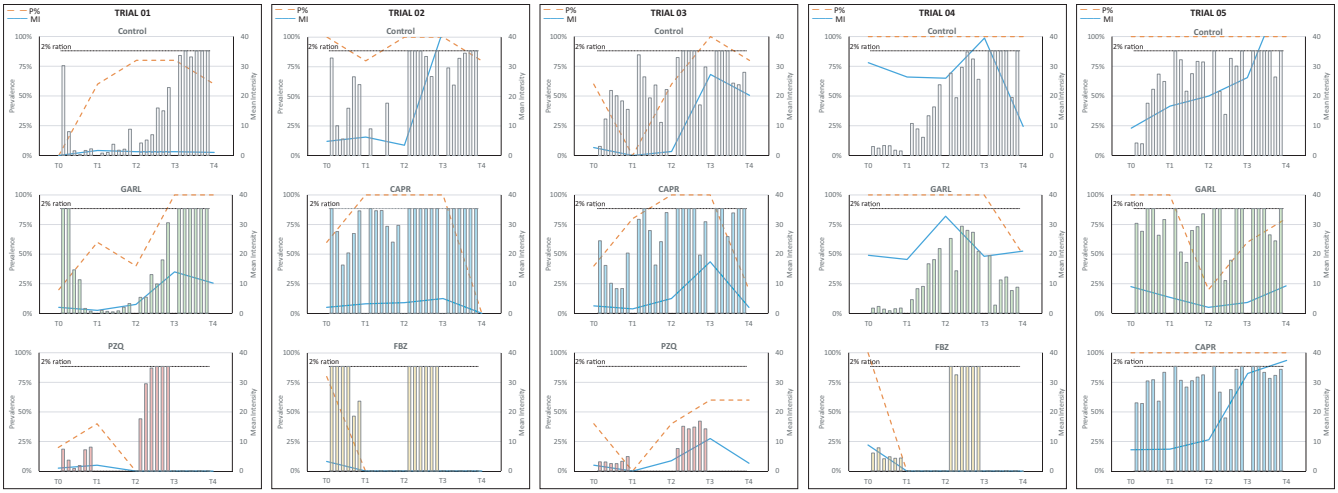

FIGURE 1 Dynamics of the prevalence (dashed orange line) and of the mean intensity (solid blue line) of *Sparicotyle chrysophrii* on the gills of *Sparus aurata* in the five trials of in-feed treatment. Columns represent the effective daily ration of the control group (grey), or of the groups treated with praziquantel (red), fenbendazole (yellow), caprylic acid (blue) and garlic (green)

the aquariums patches of 6 mm nylon mesh, held in a 9 cm floating square frame ballasted on one side.

The trials lasted 28 days, starting 10 days after fish acclimatization. Feeds with PZQ and FBZ were administered during the first week, interspersed by one week with control feed, then the treatment was repeated. Natural compounds were administered throughout 28 days.

Control feed was ‘Vita 2’ (Veronesi, A.I.A. S.p.A, Italy), and experimental feeds were obtained by adding the compounds in aqueous solution, using potato starch as binder; except for the CAPR treatment, for which a commercial feed supplemented with CAPR (2%) ‘Efico Forte’ (BioMar S.A.S, France) was used. Feeds were prepared the day before the first administration and consumed within 6 days. PZQ-medicated feed was obtained by adding 5% of ‘Hadaclean-A’ (5.0% PZQ) (Bayer, Vietnam), obtaining a PZQ concentration of 0.25%, and a dose of 50 mg/kg BW/day. FBZ-medicated feed was prepared by adding 0.60% of crushed ‘Panacur Forte’ tablets (64.1% FBZ) (MSD Animal Health, The Netherlands), obtaining a FBZ concentration of 0.38%, and a dose of 75 mg/kg BW/day. The administration of ‘Efico Forte’ at 2% ration resulted in a dose of 400 mg/kg BW/day of CAPR. The GARL-supplemented feed was prepared at the Veronesi laboratory, adding 2% GARL powder (Roha Italy S.p.a., Italy), resulting in a dose of 400 mg/kg BW/day.

On days 0, 7, 14, 21 and 28, five fish from each group were sacrificed by percussive blow to the head (Directive 2010/63/EU). The experimental protocol was approved by the Italian Ministry of Health (Authorization No. 526/2018-PR of 06/7/2018).

Fish were measured to the nearest millimetre and weighed to the nearest 0.1 g. Gills were examined under a stereomicroscope, and all specimens of *S. chrysophrii* counted and stored in 70% ethanol. Prevalence and mean intensity of infection were calculated (Bush et al., 1997).

The efficacy of treatments was evaluated as a percentage reduction (%R) of mean intensity and prevalence (Sommerville et al., 2016). It was calculated inter-group (a treated group at T4 vs. the control group at T4) and intra-group (a group at T4 vs. the same group at T0). The significance ($p \leq 0.05$) of difference between pairwise comparison of prevalence and mean intensity was evaluated with the unconditional exact test and the bootstrap two-sample t test, respectively (Reiczigel et al., 2019).

Water parameter remained between the optimal ranges for the gilthead seabream, but the fish sometimes refused food, altering the actual dosage of compounds (Figure 1). This happened in trials 01 and 03 for the PZQ group, and trial 04 associated with high infection of *S. chrysophrii* that caused mortality: 3 fish of the control on T2, and 1 and 3 fish of the GARL group on T2 and T3, respectively.

The use of net patches improved the maintenance of the parasite under experimental conditions, and its reproductive success was confirmed by the finding of every developmental stages on the host, and of eggs entangled in the nets.

As shown in Figure 1, FBZ was effective in both trials (02 and 04), with the infection disappearing after the first treatment. PZQ was effective after one treatment in trial 01; however, no reduction of the infection was observed in trial 03, associated with an important food rejection. CAPR showed a possible efficacy in trial 02, but had null efficacy in trials 03 and 05. GARL had generally a null efficacy, and an increase of the infection was observed in trial 01.

Regarding the %R (Table 2), generally it was greater than 50%, but also negative values were recorded, indicating an increase of the infection. The compound with the most important %R was FBZ (100% inter- and intra-group) and with significant differences between prevalences (inter- and intra-group). Oppositely, the compound with the lesser %R was GARL, for which most of %R were negative, and the few positive ranged 20–50%. The %R of PZQ was 100% of in the first experiment although with no significant differences, but not in the third one, where negative %R were recorded. The %R of CAPR group was 100% in trial 02, with significant difference of prevalence inter-group, but in trials 03 and 05, it was lower.

This study is the first trial of FBZ against *S. chrysophrii* on *S. aurata*, showing that a treatment of 75 mg/kg BW/day of FBZ has high efficacy against this parasite. Similar results were reported by Forwood et al., (2013) on the mucophagous monogenean *Lepidotrema bidyana* (Murray, 1931) on *Bidyanus bidyanus* (Mitchell, 1838), and a lower dosage (20 mg/kg BW/day for three alternate days) was experimented by Gupta et al. (2019) against another mucophagous gill parasite *Dactylogyrus* sp. on *Labeo rohita* (Hamilton, 1822). Compared to haematophagous monogeneans, the mucophagous ones involve the need for higher doses to ensure an adequate amount of the compound can be transferred to the mucus and to the parasite (Partridge et al., 2014). Therefore, the haematophagous feeding habit of *S. chrysophrii* could allow treatments with a lower dosage, as reported by Kimura et al., (2006), who used 25 mg/kg BW/day of febantel for 3 days, against the haematophagous *Heterobothrium okamotoi* Ogawa, 1991 on *Takifugu rubripes* (Temminck & Schlegel, 1850). The results associated with PZQ were largely affected by the palatability problems of this compound, and a two-dose treatment of 50 mg/kg BW/day of PZQ had limited efficacy against *S. chrysophrii* due to the feed refusal. Sitjà-Bobadilla et al., (2006) and Williams et al., (2007) reported similar issues with *S. aurata* and *Seriola lalandi* Valenciennes 1833, and proposed 40–50 mg/kg BW/day of PZQ as a possible compromise. Regarding natural compounds, although the dosage of CAPR remained well above 200 mg/kg BW/day (Rigos et al., 2013), the low efficacy achieved suggests that this could be useful as a prevention (Hirazawa et al., 2001; Rigos et al., 2013). On the other hand, 400 mg/kg BW/day of GARL did not result in a reduction of the infection.

In conclusion, further *in vivo* investigations will allow the integration of chemotherapics and functional feeds for the practical control of monogeneans in commercial aquaculture.

TABLE 2 Percentage reduction of the mean intensity (MI) and prevalence (P%) of *Sparicotyle chrysophrii* on the gills of *Sparus aurata* with different in-feed treatments

	N Trial	Inter-Group		Intra-Group	
		MI	P%	MI	P%
PZQ	01	100%	100%	100%	100%
	03	87%	25%	−33%	−50%
FBZ	02	100%	100%*	100%	100%*
	04	100%	100%*	100%	100%*
GARL	01	−920%	−67%	−410%	−400%*
	04	−114%	50%	−7%	50%
	05	84%	20%	−3%	20%
CAPR	02	100%	100%*	100%	100%
	03	90%	75%	20%	50%
	05	36%	0%	−419%	0%

Abbreviation: Column inter-Group, comparison between treated and control groups at T4; Column intra-Group, comparison at T4 and T0 of the same group.

*significant ($p \leq 0.05$) difference between the levels of infection.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design of the study: P.M., F.E.M., G.G; Carrying out of the experiments: P.M., C.B., G.G; Data collection and analysis: P.M., C.B.; Writing of the manuscript: P.M., F.E.M., C.B., G.G.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon reasonable request.

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