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Effect of a functional feed additive on mitigation of experimentally induced gilthead sea bream *Sparus aurata* enteromyxosis

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ABSTRACT: In gilthead sea bream *Sparus aurata*, infection by *Enteromyxum leei* produces a cachectic syndrome with anorexia, weight loss, severe epaxial muscle atrophy and, eventually, death. Currently, there are neither vaccines nor effective prescription medicines to control this infection. Nutraceutical approaches are raising interest in the aquaculture industry, responding to the lack of therapeutic tools for the management of insidious chronic losses due to parasites. In this study, the effect of a commercially available health-promoting feed additive (SANACORE® GM) at 2 different doses was tested in comparison with a basal diet without the additive during a laboratory-controlled challenge with *E. leei*. Group performance and biometrical values were monitored, and an in-depth parasitological diagnosis, quantification of parasite loads and histopathological examination were carried out at the end of the trial. Supplemented diets mitigated the anorexia and growth arrestment observed in challenged fish fed the basal diet. This mitigation was maximum in the highest dose group, whose growth performance was not different from that of unchallenged controls. Treated groups also presented lower prevalence of infection and a lower parasite load, although the differences in the mean intensity of infection were not statistically significant. Although the decrease in parasite levels was similar with both doses of additive tested, the pathogeny of the infection was mostly suppressed with the higher dose, while only mitigated with the lower dose. The mechanisms involved in the effects obtained remain to be investigated, but the results point to a modulation of the immunopathological response to the infection.

KEY WORDS: Parasites · Aquaculture · *Enteromyxum leei* · Functional feed additives · Treatments · *Sparus aurata* · SANACORE · Myxozoa

1. INTRODUCTION

Concern about the impact of parasite infections in aquaculture has increased in recent years. In addition to visible mortality episodes and increased running costs, estimates of the world annual grow-out loss in finfish farming due to parasites range from 1–10% of harvest, with an annual cost of up to US$ 9.58 billion (Shinn et al. 2015a). This impact could be much higher in certain areas and production systems (Shinn et al. 2015b).

In Mediterranean fish farming, one of the major parasitic diseases is enteromyxosis, caused by *Enteromyxum leei* (Palenzuela 2006). This myxozoan parasite infects the intestinal tract of fish and occasionally associated organs like gallbladder and liver. In contrast to a complex, heteroxenous life cycle as is known for about 50 myxozoan species, spontaneous fish-to-fish transmission has been demonstrated for 2 species in the genus: *E. leei* and *E. scophthalmi* (Redondo et al. 2002, Sitjà-Bobadilla et al. 2007). The invasion of the intestine by these parasites often trig-

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gers a catarrhal response, releasing epithelium casts containing developing parasite stages which easily infect other fish per os (Redondo et al. 2004, Sitjà-Bobadilla & Palenzuela 2012). Transmission in aquaculture setups is favoured by fish behaviour such as direct pecking on rectal prolapses (H. Yokoyama pers. comm.) and cannibalism (Padrós et al. 2001).

E. leei has a wide host- and geographical range, including economically important aquacultured species in the Mediterranean, Atlantic, Asian and South American regions (Sitjà-Bobadilla & Palenzuela 2012, O. Palenzula & A. Sitjà-Bobadilla unpubl. data). Outbreaks have also been reported in exhibition aquariums in Europe and North America (Padrós et al. 2001, Katharios et al. 2014, Hyatt et al. 2018). The virulence and mortality in different hosts is variable and is largely affected by species' susceptibility and rearing conditions. In gilthead sea bream *Sparus aurata* (hereafter GSB), enteromyxosis follows a chronic course leading to a cachectic syndrome with anorexia, anaemia, weight loss, severe epaxial muscle atrophy and, eventually, death (Sitjà-Bobadilla & Palenzuela 2012). Direct mortality is most often moderate, whereas the serious economic impact of enteromyxosis in these facilities is largely due to arrested growth and inability of the fish to reach commercial size during late on-growing culture stages. Other species (e.g. sharpsnout sea bream *Diplodus puntazzo* or red sea bream *Pagrus major*) are much more susceptible to enteromyxosis and can suffer mass mortality a few weeks after being seeded in sea cages (Palenzuela 2006).

Some anti-coccidial combinations such as amprolium and salinomycin have been proved partially effective against *E. leei* (Golomazon et al. 2006, Hyatt et al. 2018). In addition, promising efficacy of some plant-derived essential oils and compounds against *E. leei* and other myxozoans has been reported (Yiagnisis et al. 2016, reviewed by Wunderlich et al. 2017). However, authorised prescription medicinal products or successful treatment protocols are not available for aquaculture, and control measures are limited to the avoidance of risk factors, early diagnosis and good farm management practices. Therefore, the farming industry needs other solutions to minimise the impact of infection and has recently turned to consider functional feed additives. In livestock farming, health-promoting feed additives are a crucial component of effective disease prevention strategies, and a wide range of products with different modes of actions are currently offered, including yeast extracts, phytobiotics, probiotics, prebiotics, organic acids and their derivatives (Gaggìa et al. 2010, Thormar 2012, Suresh et al. 2017, Patra et al. 2019). In aquaculture, natural compounds with antibiotic activity can work directly on the parasites, whereas immunomodulators and other host-centric approaches can exert multiple beneficial effects (Coutteau et al. 2011, Coutteau & Goossens 2014, Coutteau 2015, Vallejos-Vidal et al. 2016). However, the effectiveness of these strategies for a given disease and production model is difficult to predict and validate under controlled conditions. Since many of these nutraceutical strategies target the gut as a primary focus for health, enteromyxosis in GSB constitutes an excellent model to evaluate their potential.

The objective of this work was to test the effect of a commercially available aquaculture functional feed additive, SANACORE® GM (Adisseo), on an experimental infection trial with *E. leei*. This compound is marketed as a broad-spectrum, health-promoting additive consisting of a mixture of organic acids, inactivated yeast and yeast extracts (*Saccharomyces cerevisiae*) with herbal extracts on a mineral carrier.

2. MATERIALS AND METHODS

2.1. Experimental design and sampling procedure

The trial was run at the indoor experimental facilities of the Institute of Aquaculture Torre de la Sal (IATS; 40° 08′ 16″ N, 0° 09′ 55″ E), using fiberglass tanks in an open flow-through seawater system with natural photoperiod. Water temperature ranged from 18–26.5°C throughout the experiment. Naïve GSB fingerlings were obtained from a local hatchery at 4 g and grown to an average weight of 12.9 g before starting the feeding trial. They were allocated into 90 l tanks (25 fish tank^{-1}) and acclimated to a basal diet for 11 d until feeding with experimental diets started. The experimental design included 3 challenged groups receiving diets differing in the inclusion levels of SANACORE. Group A received a basal control feed, representative of a commercial feed formulation, without additive (Diet A), Group B the same feed with 0.2 % of SANACORE (Diet B) and Group C with 0.4 % SANACORE (Diet C). These groups were distributed in 2 replicate tanks treatment^{-1}. An additional non-challenged control group (CTRL) was stocked in 2 additional tanks and received Diet A throughout the trial. See Appendix for a list of diet ingredients. Fish were fed manually ad libitum twice a day on weekdays and with automatic feeders on weekends during the whole experiment. Daily food intake was recorded and the performance

indexes specific growth rate (SGR), feed conversion ratio (FCR) and Fulton's condition factor (CF = [W / L^3] × 100, where W is weight and L is length) were calculated. After 5 wk on the test diets, fish from groups A, B and C were inoculated with 0.2 ml of a homogenate from intestinal scrapings of *Enteromyxum leei*-infected donor fish via the anal route, as previously described (Estensoro et al. 2010). The non-challenged CTRL fish received the same volume of phosphate-buffered saline (PBS). At 5 wk post-inoculation (p.i.), all fish were sampled for biometrical values after light anaesthesia. A non-lethal rectal swab was gently taken from 30 randomly selected group A fish (15 tank^{-1}), which was then processed for *E. leei* diagnosis by PCR to check the success of the infection after the challenge (Sitjà-Bobadilla & Palenzuela 2012, Fox et al. 2000). The final sampling of all groups was performed 10 wk p.i., when all fish were sacrificed and intestine samples were taken for *E. leei* diagnosis by histology (n = 16–20 group^{-1}) and qPCR (n = 29–30 group^{-1}).

2.2. Diagnosis of the infection

2.2.1. Histology

Portions of anterior (AI), middle (MI) and posterior (PI) intestine were fixed in 10% buffered formalin and embedded in Technovit plastic resin (Heraeus Kulzer), following routine procedures. Slides were stained with Giemsa, and *E. leei* infection intensity was semi-quantitatively evaluated by an experienced pathologist following a conventional scale from 1+ (very low) to 6+ (very high) as previously described (Estensoro et al. 2010). The type of parasitic stages and the histopathological alterations associated with the infection were observed and registered. A fish was considered positive for infection when the parasite was found at least in one intestinal segment.

2.2.1. Parasite quantification by qPCR

E. leei rDNA gene copies were estimated by qPCR as previously described (Piazzon et al. 2017). After necropsy, entire intestines of 30 fish group^{-1} (15 tank^{-1}) were removed and weighed individually. The intestines were homogenized in a laboratory blender (Next Advance) and DNA was extracted from a 200 µl aliquot of each homogenate using an EpMotion 5075 automated robotic system (Eppendorf) and commercial silica-based kits (Macherey-Nagel). Numbers were interpolated from the cycle thresholds (C_T) of the samples using standard curves generated with known numbers of a plasmid containing the target gene (covering 6–7 orders of magnitude), run in the same plates on each assay. Usually 2 dilutions of each DNA sample were run. Samples with C_T < 38 were considered positive. The total number of parasite rDNA copies present in each fish was estimated from these values and the amount of tissue present in the entire homogenate sample. For simplicity, this value was used as a proxy for the number of parasites (i.e. assuming a constant cell number per parasite).

2.3. Data analysis

Quantitative parasitological data variables studied included: prevalence of infection (percentage of infected fish in a group), mean intensity of infection (mean number of parasites per infected fish) and mean parasite abundance (mean number of parasites per fish in a group, including the zero values of uninfected animals). For parasitological data analyses, each individual fish was treated as a replicate and each group included all the fish, i.e. replicate tanks were not treated individually except for the prevalence data. Since previous experiments have shown that individual *E. leei* load data in infected fish is overdispersed and aggregated, quantitative parasite load estimated by qPCR was normalized by logarithmical transformation: $y = \ln(y)$ for intensity data, and $y = \ln(1 + y)$ for abundance data. Biometrical and parasitological data were studied for differences between test groups using software packages Prism (GraphPad Software) and Sigma Plot (Systat Software). Unless specifically stated, results were considered statistically significant when $p < 0.01$.

3. RESULTS

3.1. Food intake and growth performance

A decresse in feed intake was observed 1 wk p.i. in Group A, which continued for the whole period (Fig. 1). A decrease was observed 3 wk p.i. in Group B. No decrease was observed in Group C (with the highest additive dose), which maintained a similar feed intake to that of CTRL fish. The effect of the parasite challenge on biometrical values was clearly evident, particularly by the last sampling at 10 wk p.i. (Fig. 2). Weight, length and CF were significantly reduced in Group A compared to the non-challenged

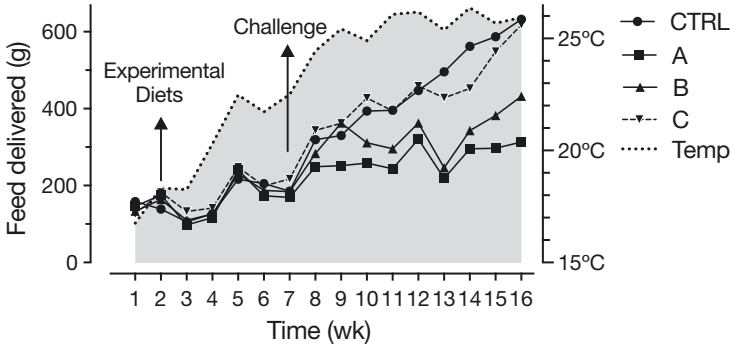

Fig. 1. Weekly feed delivered to each group of gilthead sea bream throughout the experiment assessing effects of the addition of the feed additive SANACORE on fish challenged by infection with *Enteromyxum leei*. CTRL: no infection, received basal diet; Group A: infected with *E. leei*, received basal diet; Group B: infected with *E. leei*, received basal diet plus 0.2% SANACORE; Group C: infected with *E. leei*, received basal diet plus 0.4% SANACORE. Fish were fed the experimental diets for 5 wk before the challenge. The evolution of the average temperature is represented as a dotted line against a secondary *y*-axis (right)

CTRL group. Interestingly, in fish receiving the supplemented diets, the length and weight losses associated with the infection were mitigated. Thus, the size decrease vs. CTRL reached statistical significance in Group B (low dose), but not in Group C (high dose) (Fig. 2) at Wk 5 and Wk 10 p.i. samplings. CF was not significantly reduced in any of the treated groups. As expected, the SGR along the whole trial was lower in all challenged groups (Fig. 2), but the reduction vs. CTRL fish was clearly mitigated in Group C (–10.2%), whereas it reached –21.1 and –25.9% in Groups B and A, respectively (Fig. 3).

3.2. Survival

Lower mortality was detected in challenged groups at 16 d p.i. A total of 14 fish died during the experiment; 6 in Group A and 4 each in Groups B and C. No significant differences among groups were found in survival curves (log-rank and Gehan-Breslow-Wilcoxon tests; data not shown).

3.3. Quantitative parasitological data

No fish were found infected in CTRL, and no significant differences in the variables measured were detected among replicate tanks within each group.

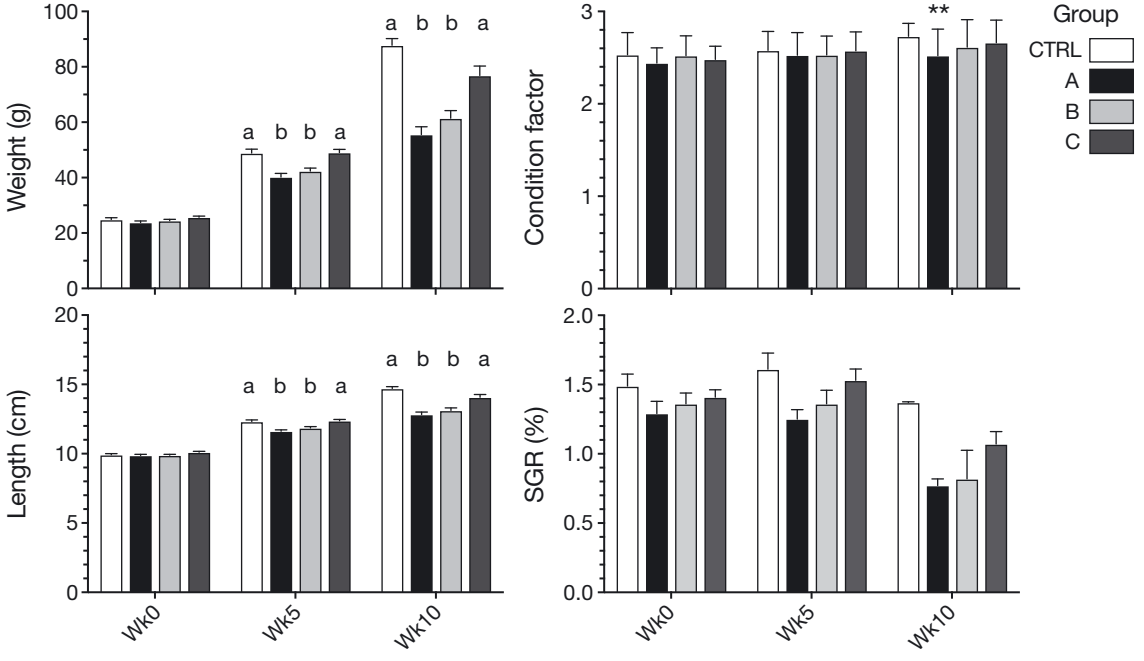

Fig. 2. Biometrical and growth performance data of the experimental groups of gilthead sea bream at the different samplings (weeks post inoculation). See Section 2.1 for explanation of feeding groups. For weigh, length and condition factor, data are represented as the group (all fish from both replicate tanks) mean ± SEM and different letters indicate statistically significant differences between groups at each sampling point ($p < 0.001$ in ANOVA + Tukey's multiple comparison test). For condition factor, asterisks indicate statistically significant differences from the control group at the sampling point ($p < 0.001$ in ANOVA + Dunnett's multiple comparison test). Specific growth rate (SGR) data was calculated per tank and is represented as the mean ± SD of the 2 replicates of each group

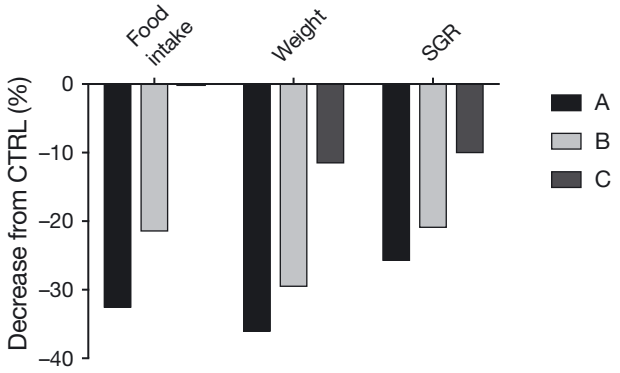

Fig. 3. Percentage of decrease of performance indicators of gilthead sea bream in challenged groups vs. non-challenged control at the end of the experiment (10 wk post-inoculation with *Enteromyxum leei*)

At the intermediate non-lethal PCR check (5 wk p.i.), 50% of Group A fish were found positive for *E. leei*. At the end of the trial, qPCR showed prevalence of infection was significantly higher in Group A (72.4%) than in the supplemented feed groups (Group B: 44.8%; Group C: 46.6%) (Cochran-Armitage contingency test for trend, $p < 0.05$) (Fig. 4A). Furthermore, the differences in the mean prevalence of infection in supplemented tanks (45.8%) vs. the unsupplemented ones (72.4%) was also significant ($p < 0.05$) in a *t*-test (data not shown). The mean and median intensity of infection was lower in both Group B (mean = 12.72; median = 11.32) and Group C (mean = 13.33; median = 12.65) than in the untreated Group A (mean = 14.33; median = 14.67), although these differences did not reach statistical significance (1-way ANOVA; Fig. 4B). The mean parasite abundance values showed the differences between treated and untreated groups very clearly. Both supplemented diets resulted in significantly lower mean abundance values (Group B: 5.70; Group C = 6.22) compared to group A (10.38) (ANOVA, $p = 0.029$). The mean parasite abundance data from both (pooled) treated groups (5.97) was also significantly different from that of Group A (*t*-test, $p = 0.005$). Median abundance values for both Groups B and C were 0, significantly lower than that of Group A (median = 13.85, Kruskal-Wallis test, $p = 0.017$; Fig. 4C).

3.4. Histopathological data

Challenged fish fed the basal diet (Group A) showed typical histopathology induced by the parasite invasion: detachment of the epithelium containing parasites from the lamina propria-submucosae (Fig. 5A) and hyperplasic submucosae infiltrated with numerous eosinophilic granular cells (Fig. 5C). A high number of rodlet cells was commonly present in the epithelium. By contrast, in Group C, the intestine usually presented a lower degree of detachment, even with a high intensity of infection (Fig. 5B). The submucosa was not remarkably hypertrophied but presented abundant lymphocytes, which were also infiltrated at the base of the epithelial layer and in close contact with parasite stages (Fig. 5D). Microscopic examination of the 3 intestinal segments of a subsample of experimental fish also showed differ-

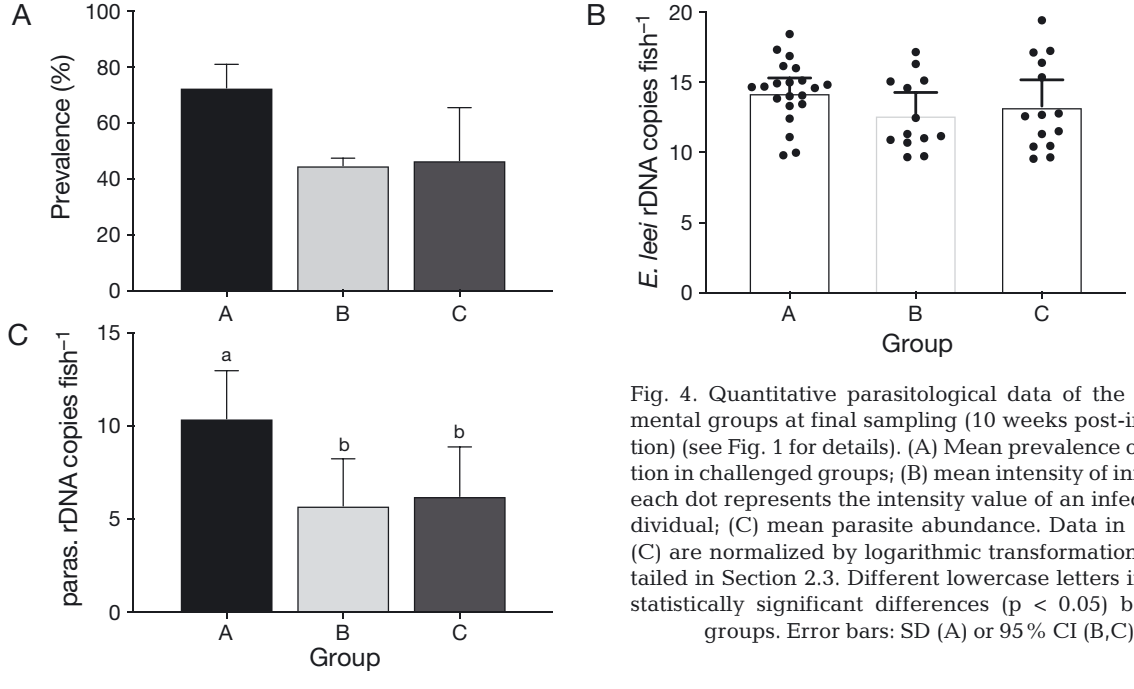

Fig. 4. Quantitative parasitological data of the experimental groups at final sampling (10 weeks post-inoculation) (see Fig. 1 for details). (A) Mean prevalence of infection in challenged groups; (B) mean intensity of infection; each dot represents the intensity value of an infected individual; (C) mean parasite abundance. Data in (B) and (C) are normalized by logarithmic transformation as detailed in Section 2.3. Different lowercase letters indicate statistically significant differences ($p < 0.05$) between groups. Error bars: SD (A) or 95% CI (B,C)

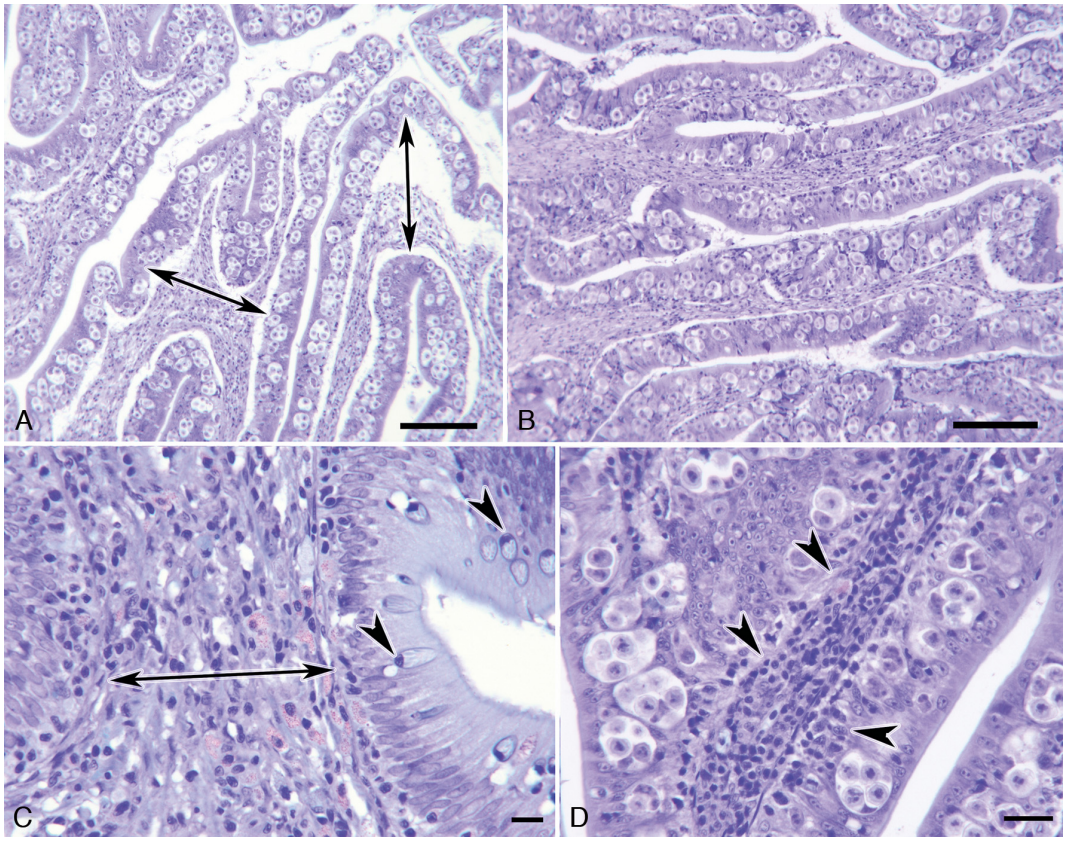

Fig. 5. Giemsa-stained photomicrographs from histological resin sections of intestines of gilthead sea bream challenged with *Enteromyxum leei* and fed (A,C) the basal diet and (B,D) the highest dose of SANACORE-supplemented diets. Note the severe detachment of the submucosae from the epithelial layer; double headed arrows in (A), in contrast with the less altered tissue in (B). (C) Typical hyperplasia of the lamina propia-submucosae (double-headed arrow) with abundant eosinophilic granular cells and rodlets cells in the epithelium (arrowheads). (D) Abundant lymphocytes at the lamina propria-submucosae and at the base of the epithelium (arrowheads) close to parasite stages. Scale bars = (A,B) 100 µm, (C) 10 µm, (D) 20 µm

ences in the extent of infection among the groups. Significant differences in the prevalence of infection were found between Groups C (31.3%) and A (64.7%) (chi-squared test, p = 0.05), whereas no differences were found for Group B (68.8%). The prevalence was highest at the PI in all challenged groups, which corresponds with the usual *E. leei* spatial distribution in GSB. Group C had the lowest prevalence and intensity at the 3 intestinal segments, and the percentage of fish with more than one infected portion was signif-

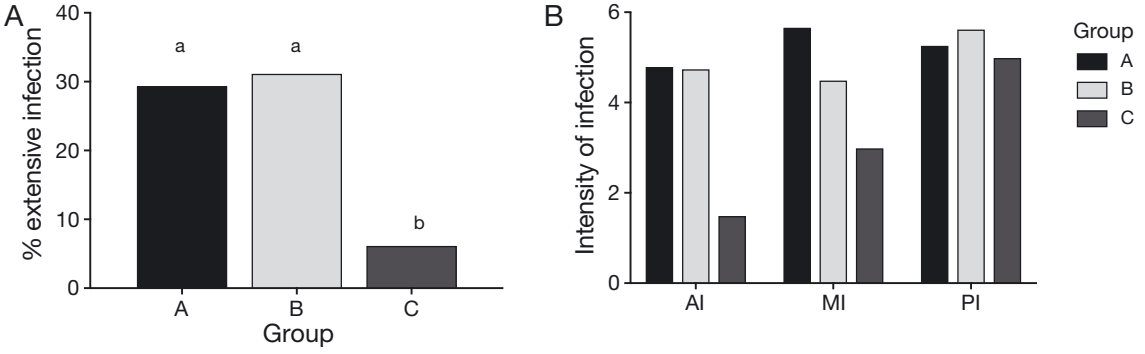

Fig. 6. Parasitological data from histopathological examination of gilthead sea bream challenged with *Enteromyxum leei* and fed a diet differing in the feed additive SANACORE (see Section 2. 1 for details). (A) Percentage of fish with parasites in more than 1 infected intestinal segment. Different letters indicate statistically significant differences between groups; (B) mean intensity of infection at the different intestinal segments; AI: anterior; MI: middle; PI: posterior

icantly lower in Group C (chi-squared test, $p < 0.05$), but not in Group B (Fig. 6A). The mean intensity of infection by intestinal segment was lowest in Group C, especially in the AI, though these differences were not statistically significant (Fig. 6B).

4. DISCUSSION

Enteromyxum leei infections can cause severe mortality in several cultured fish species such as sharpsnout sea bream, red sea bream, fugu *Takifugu rubripes* and Japanese flounder *Paralichthys olivaceus* (Yasuda et al. 2005, Palenzuela 2006, Rigos & Katharios 2010). The most obvious effects of enteromyxosis in GSB include anorexia, weight loss and arrested growth (Palenzuela 2006, Sitjà-Bobadilla & Palenzuela 2012). In the current laboratory challenge with *E. leei*, the parasite effectively induced these clinical signs in GSB fed a standard commercial diet (Group A). This group exhibited a dramatic decrease in feed intake (–32.7%), weight (–36.2%) and SGR (–25.9%) compared to the unchallenged CTRL group after 10 wk p.i. At 5 wk p.i., 50% of Group A tested positive for the parasite in a nonlethal PCR, and 72.4% were infected at the final sampling at wk 10 wk p.i. This indicates exposure to a high infection pressure, further magnified by the high water temperatures sustained during the trial.

Anorexia is a complex pathophysiological response to some parasitic infections, and it is widespread among livestock including fish (Symons 1985, Bernier 2010). While it may seem counterproductive for the energetic demands of a successful fight against infection, there are several hypotheses to explain this phenomenon as a conciliation of immune and metabolic needs (Exton 1997, Kyriazakis et al. 1998). In gastrointestinal infections, direct alterations of the enteric nervous system also needs to be considered (Halliez & Buret 2015). In turbot *Psetta maxima* with enteromyxosis, down-regulation of the neuropeptide Y-receptor NPY2R, a main regulator of appetite (Zou & Secombes 2011), has been reported in early stages of the infection (Ronza et al. 2016). This observation is in agreement with the early onset of anorexia in Group A in the current trial, which was observed as early as 1 wk p.i. and persisted until the the end of the experiment, reaching 32.7% reduction in food intake compared to the CTRL group. Diets supplemented with SANACORE mitigated this decrease to the extent that Group C (high dose) did not differ significantly from the CTRL group with respect to food intake. In Group B, anorexia started later and was not as acute as in Group A (–21.63%). It remains to be investigated if this reduction in the primary pathophysiological response to infection is related to a protective role of the additive at the early stages of parasite invasion or to other factors. In a previous *E. leei* infection using parasite-containing effluent water, food intake decrease ranged between 22.1 and 15.4% in groups fed different diets (Estensoro et al. 2011). Although these values are not as low as in the untreated group in the current experiment, this is most likely related to differences in infection routes, temperatures and infective pressure between both studies.

Weight loss and arrested growth are obvious consequences of anorexia and of the diversion of energy invested in mounting an immune response, but they are also largely caused by direct structural damage to the intestine epithelium and interference of the parasites with gastrointestinal function (Estensoro et al. 2011, Sitjà-Bobadilla & Palenzuela 2012). In *E. leei*-infected fugu, dehydration and osmoregulatory failure, together with impaired intestinal absorption, were the main contributors to a rapid weight loss observed in infected fish (Ishimatsu et al. 2007). In the current experiment, the evolution of biometrical data clearly evidenced the clinical effect of the infection in challenged groups, in consonance with field studies on *E. leei*-infected stocks and with the results of previous laboratory challenge studies with this parasite (e.g. Estensoro et al. 2011, Piazzon et al. 2017). As deduced from the results of this trial, supplemented diets can partially alleviate these effects, as Group C reached similar performance levels as CTRL. The effect in Group B, however, was moderate and fish still performed worse than in the CTRL group, reaching statistical significance in all the biometrical variables measured except CF. Interestingly, the PCR results showed that both treated groups presented similar and lower prevalence values than Group A. Thus, the different pathological effects between treated groups cannot be attributed exclusively to prevalence; other variables, like the intensity of infection, must be considered. However, the differences in the mean number of parasites per infected fish in the different groups did not reach statistical significance. Although mean and median intensity values in Group A were higher than in both treated groups, the differences between the latter were modest (indeed, Group B presented values slightly lower than Group C).

Regarding the prevalence of infection and the mean intensity of infection, neither parameter could directly explain the differences in the pathological effect of the challenge found between both treated groups. The observed differences in the mean intensity of in-

fection and in the extent of infection along the intestinal tract could suggest delayed invasion or variable degrees of 'resistance' to the multiplication of the parasite once it breached the host barriers. Since all fish were inoculated simultaneously with a single dose, these differences could indicate slightly improved ability to constrain parasite development or to eliminate parasites more efficiently. It must be noted, however, that the overdispersion of intensity values makes it very difficult to compare this variable between groups, even with normalized data. This is a common phenomenon in quantitative parasitology (Rózsa et al. 2000, Klar et al. 2010), further exacerbated by the type of parasite studied in this work (i.e. a rapidly multiplying pluricellular microorganism).

Mean parasite abundance data takes into consideration the mean parasite quantities in all the fish from the group, including uninfected animals, thus merging prevalence and intensity data in a single variable. This parameter showed very significant differences between treated and non-treated groups, but failed to prove substantial differences between both treated groups or to correlate with the differential pathogeny observed with the 2 doses of additive. The observation that fish receiving the higher dose could cope with relatively high individual parasite loads, which generated more severe pathophysiological effects in other groups, is remarkable. Variability of infection-associated immunopathology has been widely recognized in many serious myxozoan infections, including *Enteromyxum* spp., *Ceratonova shasta* and *Tetracapsuloides bryosalmonae* (Gorgoglione et al. 2013, Bjork et al. 2014, Sitjà-Bobadilla et al. 2015). In this scenario, the benefits of the additive at the higher dose could be interpreted as being due in part to an immunomodulatory action. This interpretation is further supported by the differences in the pathological alterations and cellular immune response observed in the intestine of the experimental groups. A conspicuous hyperplasia and eosinophilic infiltration of the submucosae, as well as abundant rodlet cells were commonly observed in Group A fish, which is normally associated with extensive detachment of the epithelial layer from the submucosae. In contrast, even with comparable levels of parasite intensity, fish from Group C generally displayed better overall epithelium topology and a more moderate cellular immune response, which mainly consisted of lymphocyte infiltration.

The effect of feed supplements on growth performance, immune response and disease resistance has been previously studied in fish, but mainly in relation to bacterial pathogens (Newaj-Fyzul & Austin 2015, Hoseinifar et al. 2015, Ringø & Song 2016). In GSB, different prebiotic and probiotic ingredients have been shown to enhance immune status, change intestinal morphology (Cerezuela et al. 2013) and gut microbiota (Cordero et al. 2015, Piazzon et al. 2017). However, the efficacy of probiotics against ecto- and endo-parasites seems very limited (e.g. reviewed by Banerjee & Ray 2017). Although the commercial interest of dietary additives for fish parasite control is high, their efficacy remains largely unexplored, and the benefits for a given fish disease model are difficult to predict. In a recent study, the effect of sodium butyrate on the infection and growth performance of GSB was studied with experimentally induced enteromyxosis (Piazzon et al. 2017). Interestingly, the histopathological study also showed a statistically significant influence of diet on the prevalence of infection in the AI. Similar to the current study, the number of fish with more than one infected intestinal segment was significantly higher in the diet group showing the worst growth performance. Butyrate did not decrease the infection levels significantly, but it mitigated the growth arrestment and alleviated the disease outcome.

5. CONCLUSIONS

Quantitative parasitological data on prevalence, intensity and abundance, as well as histopathological studies on the extent of infection, confirmed the effect of SANACORE-supplemented diets in reducing infection level and severity. Both GSB groups receiving the functional feed additive showed lower prevalence and intensity of infection compared to the challenged group not receiving the feed additive. Although the additive proved to have a significant effect on the reduction of infection prevalence and parasite abundance at both doses tested, the maximum benefits in terms of pathogenesis reduction were obtained in the high-dose group. Since the negative impact in GSB production through enteromyxosis is mostly related to the symptons of the disease, this additive appears to be a promising tool for management of the disease in GSB aquaculture.

It cannot be ignored that the current trial was based on a laboratory-controlled experimental infection with homogeneous and time-controlled exposure to the parasite and under stocking and feeding regimens unlike those of farmed fish. Further studies in more complex farming settings are necessary to determine the feeding regime and dosage of the supplement that could offer these benefits.

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Appendix. Crude composition of the experimental feeds (ingredients in %) provided to gilthead sea bream; different groups received diets differing in amount of the additive SANACORE

	Diet A (no additive)	Diet B (low dose)	Diet C (high dose)
Crude protein/ fat proportion	45/20	45/20	45/20
LT fishmeal	15	15	15
Poultry by-product meal	12	12	12
Soybean meal	25	25	25
Soybean protein concentrate	10	10	10
Corn gluten	8	8	8
Wheat gluten	3.4	3.4	3.4
Wheat flour	10.5	10.5	10.5
Soybean oil	9.7	9.7	9.7
Fish oil	6	6	6
Amino acids	–	–	–
Vitamins and mineral premix	–	–	–
SANACORE	0	+0.2	+0.4

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