

Functional feed additive for gut integrity and protection against the intestinal myxosporean endoparasite *Enteromyxum leei*

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The importance of gut in fish health and performance

A healthy alimentary canal is a fundamental element for the optimal growth and performance of all cultured fish. Apart from nutrient absorption and digestion, which are its main functions, the gut plays also a major role in sustaining fish health (Kristen *et al.*, 2012; Tarnecki *et al.*, 2017). However, there are several, both non-infectious and infectious factors, that may cause imbalance among the major components of the gut: microbiota, epithelium and intraepithelial leukocytes. The amount of scientific research on gut microbiota has been growing exponentially over the past few years (Egerton *et al.*, 2018; Bozzi *et al.*, 2021), however, the complexity of gut microbiome and the potential interventions that may lead to better fish health and performance has

only scratched the surface (Montalban *et al.*, 2015). The clinical picture of such an imbalanced gut is called enteritis and occurs in the form of generalized intestinal inflammation, triggered by the activation of the intraepithelial immune cells such as macrophages.

The inflammatory response of the intestine is the defensive mechanism of the organism due to an infection or an injury. Although it is rather beneficial in the short term, if prolonged, it can lead to chronic inflammation which undermines both nutrient absorption and immunity functions of the gut. The combination of nutritional factors, such as the use of plant-based ingredients in the feed and gut pathogens, for instance the myxosporean parasite *Enteromyxum leei*, can further cause severe disruption of the gut integrity, undermining the growth and performance of

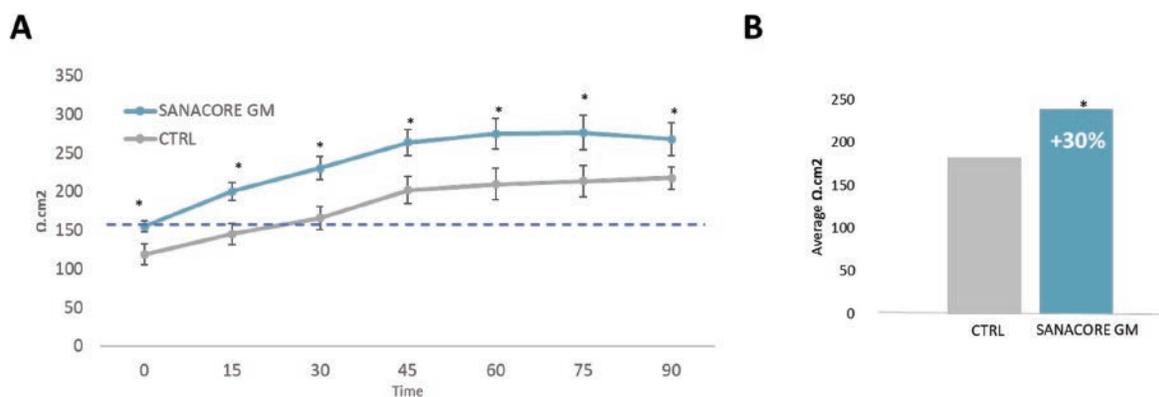


Figure 1. (A) Transepithelial resistance of anterior intestine with and without SANACORE GM® supplementation (0.5%). Tissue resistance in the intestine of healthy seabream juveniles of 100g is expected to be >150 Ω cm², therefore a threshold has been set with dotted line. (B) A statistically significant difference of 30% higher tissue resistance is reported under SANACORE GM® supplementation (0.5%). Statistical significance is stated by *.

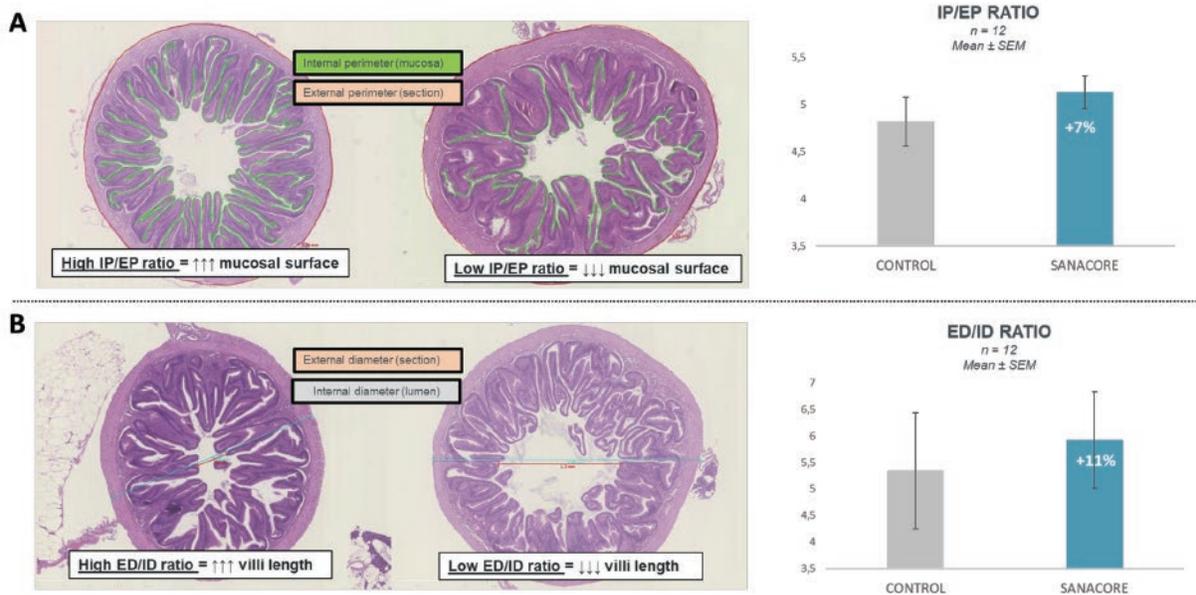


Figure 2. (A) Internal:External perimeter ratio. On the left section (SANACORE GM®) the ratio is higher by 7% compared to the right (CONTROL) and the intestine is presented intact and well-developed with many well-shaped villi. (B) External:Internal diameter ratio. On the left section (SANACORE GM®) the ratio is higher by 11% compared to the right (CONTROL) because the development of the villi is more consistent, leading to a smaller lumen space.

the fish (Sitjà-Bobadilla *et al.*, 2019; Piazzon *et al.*, 2022). It should also be noted that a well-functioning gut is a prerequisite for the avoidance of the subclinical picture of the fish, which may not show direct consequences at once but will do so as soon as any abrupt or steep changes eg. suboptimal temperature, high stocking densities, opportunistic pathogens, vaccination or transfer stress, etc. appear in the environment. Therefore, functional feed additives which are able to enforce and protect gut health may claim today a prominent role in the formula.

The objective of the present article is to assess and present the impact that the functional feed additive under the commercial name SANACORE GM® can render on the host's gut. The documented beneficial effects on gut integrity, villi formation, anti-inflammatory response and consequent protective activity against the myxosporean parasite *E. leei* draw the picture of a healthier gut which not only generates better performance indices but also makes it harder for pathogens to infiltrate and damage the tissue.

SANACORE GM® promotes gut integrity and protects against *E. leei* infestations

SANACORE GM® by Adisseo is a broad-spectrum, health-promoting additive based on components that

deliver gut integrity and anti-inflammatory action. The positive impact of SANACORE GM® on gut integrity and health promotion has been extensively documented in the literature and reported by EU aquaculture projects such as [ParaFishControl](#).

The functionality of the intestine for both digestion and health performance requires gut integrity to be intact. The transepithelial electrical resistance (TER) of the intestine, as an indicator of gut integrity, has been measured in gilthead seabream, *Sparus aurata*, fed a 10% fishmeal diet supplemented with SANACORE GM®. TER was 30% significantly higher under supplementation and in relation to the control group (Fig. 1). The tighter the junctions among the cells, the more difficult for the electrical current to pass, hence, a tangible improvement of gut integrity is capitalized to better nutrient absorption, improved growth performance, and more robust fish health.

Morphometrical studies on the seabream's gut, corroborate the previous result by focusing on the length and surface of intestinal villi. The villi from the hindgut, which is the most sensitive to inflammation part of the gut, indicate that internal:external gut perimeter and external:internal gut diameter fractions are higher by 7% and 11%, respectively, under SANACORE GM® supplementation (Fig. 2). The biological

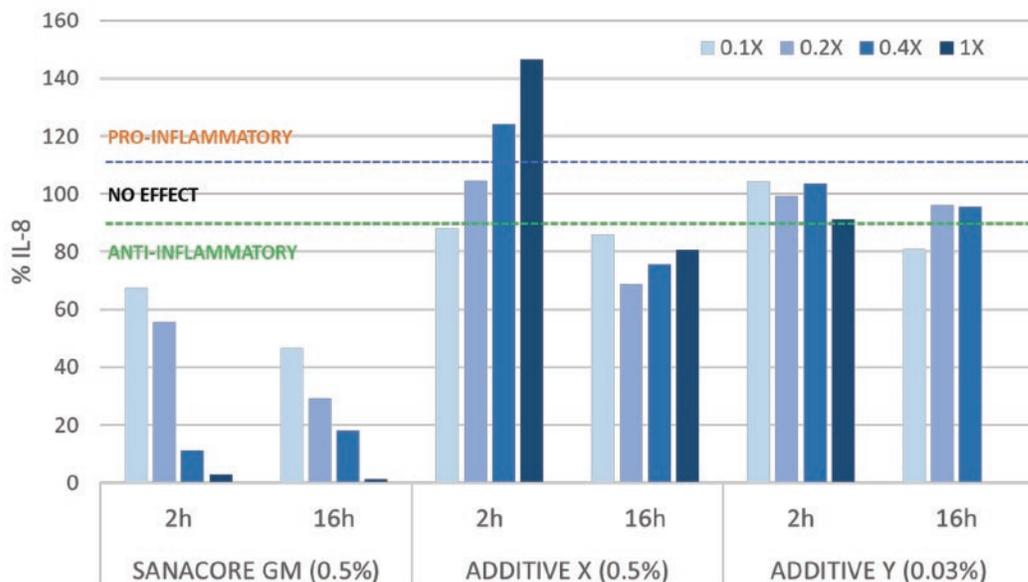


Figure 3. Resilience to inflammation following a cytomix stimulus. The ELISA results showed that in all 4 different concentrations (0.1x, 0.2x, 0.4x and 1x) and both time points (2h and 16h), SANACORE GM® was the most efficient additive in maintaining the inflammatory status below threshold.

meaning of these numbers is translated into more consistently developed villi, less inflamed villi, larger lumen space, bigger mucosal surface and consequently improved gut integrity.

The inflammatory process is mediated by the secretion of proinflammatory cytokines including interleukins (IL). IL-8 has been particularly studied and extensively used in research as a biomarker for the inflammatory cascade acting as a neutrophil leukocyte chemoattractant or activator (Kim *et al.*, 2019; Laing *et al.*, 2002; Laing *et al.*, 2004). The *in vitro* model using the caco-2 cell line (van de Walle *et al.*, 2010), which originates from epithelial cells mimicking a functional intestine barrier, was used in order to assess the inflammatory response to SANACORE GM® and two other commercial feed additives. The cells were exposed to a mixture of proinflammatory cytokines (cytomix) and it was shown that supplementation with SANACORE GM® was the most efficient in maintaining the IL-8 levels below the anti-inflammatory effect threshold of 90%, whereas the other tested additives generated either no effect (90-110%) or proinflammatory effect (>110%) for most concentrations and time points (Fig. 3).

Supporting gut integrity and intestinal health is directly reflected in the successful application of SANACORE GM® as a preventive strategy against pathogenic

infestations. *E. leei* is a myxosporean endoparasite that colonizes the gut and occasionally can also be found in the bile. Enteromyxosis in gilthead seabream is a more chronic, rather than acute, condition since instead of causing immediate mortality, it gradually causes anorexia, weight loss, muscle reduction and eventually death. However, when illustrated in more sensitive fish, such as the sharpsnout seabream (*Diplodus puntazzo*) and the red seabream (*Pagrus major*) mortality can be induced much faster (Palenzuela *et al.*, 2020; Henry *et al.*, 2020). The route of infection for the parasite passes through the already loose junctions among the epithelial cells and further exacerbates the clinical picture of enteritis. The disruption of gut integrity compromises the host's health while rendering it vulnerable to secondary infections as well. *E. leei* is a case in point for the Mediterranean aquaculture, particularly for gilthead and sharpsnout seabream (Palenzuela *et al.*, 2020; Henry *et al.*, 2020). In the former case, SANACORE GM® could mitigate the SGR decrease caused by the infection by up to 15% versus the untreated infected control. A functional feed could also lower the prevalence of the infestation by 30% whereas the mean parasitic abundance per fish was significantly lower compared to untreated control (Palenzuela *et al.*, 2020). In the case of sharpsnout seabream, SANACORE GM® had an

immune-promoting effect on the antibacterial, anti-protease and ceruloplasmin activity compared to the untreated positive control (Henry *et al.*, 2020).

Efforts for sustainable aquaculture and high prices that accompany the ingredients of marine origin have led the industry to constantly increase the inclusion of plant-based components and encourage the development of alternative nutrient sources in aquaculture diets. A recent study has proved that reinforcing a zero fishmeal (FM) diet with SANACORE GM[®] to support fish in dealing with *E. leei* infection is as effective as a 20% FM in avoiding the increased intestinal susceptibility to the parasite (Piazzon *et al.*, 2022).

Conclusion

The economic losses accompanied by hampered productivity along with the increasing research for plant-origin ingredients coupled with the stressors and pathogens to which culture fish are exposed,

highlight the importance and necessity of functional feed additives in the feed formula. Under these circumstances, the beneficial effects SANACORE GM[®] on gut health and fish performance complemented by the protective impact against *E. leei*, enforce its role as a core functional feed additive for the Mediterranean aquaculture.

References available on request.

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