Preventive additive strategies for the control of ectoparasites and secondary infections in farmed fish

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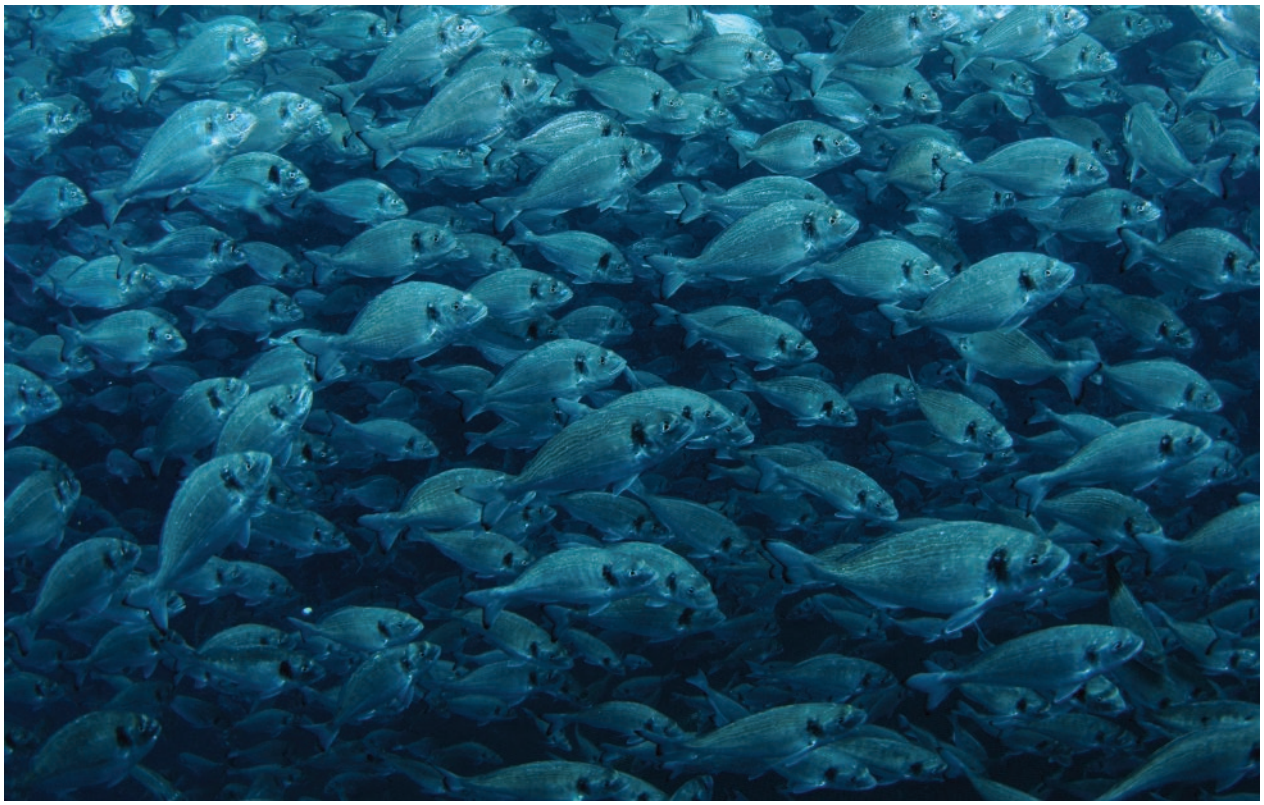

Ectoparasitic infestations have acquired a serious role among the factors limiting the intensive production of cage-reared fish. Poor net hygiene and other environmental upsets may stimulate the occurrence of infestations which can be devastating to caged fish.

Most relevant ectoparasitic infestations in European aquaculture include *Sparicotyle chrysophrii,* a blood-sucking monogenean affecting gilthead sea bream farming (Sitjà-Bobadilla *et al*., 2010), and *Lepeophtheirus salmonis* (sea louse), a caligid copepod feeding on the skin, blood and mucus of Atlantic salmon.

Losses are associated with mortalities and notable growth reduction partly due to emaciated and anemic survivors. Furthermore, parasite attachment to fish skin produce important alterations such as wounds and ulcers, promoting mixed infections with parasites or secondary bacterial infections and thereby increasing stress and mortality (Padros and Crespo, 1995).

Management of ectoparasitic infestations is crucial to limit production losses, maintain acceptable stock and reduce impacts on wild populations (Thorstad *et al*., 2015). Multiple preventative methods can be

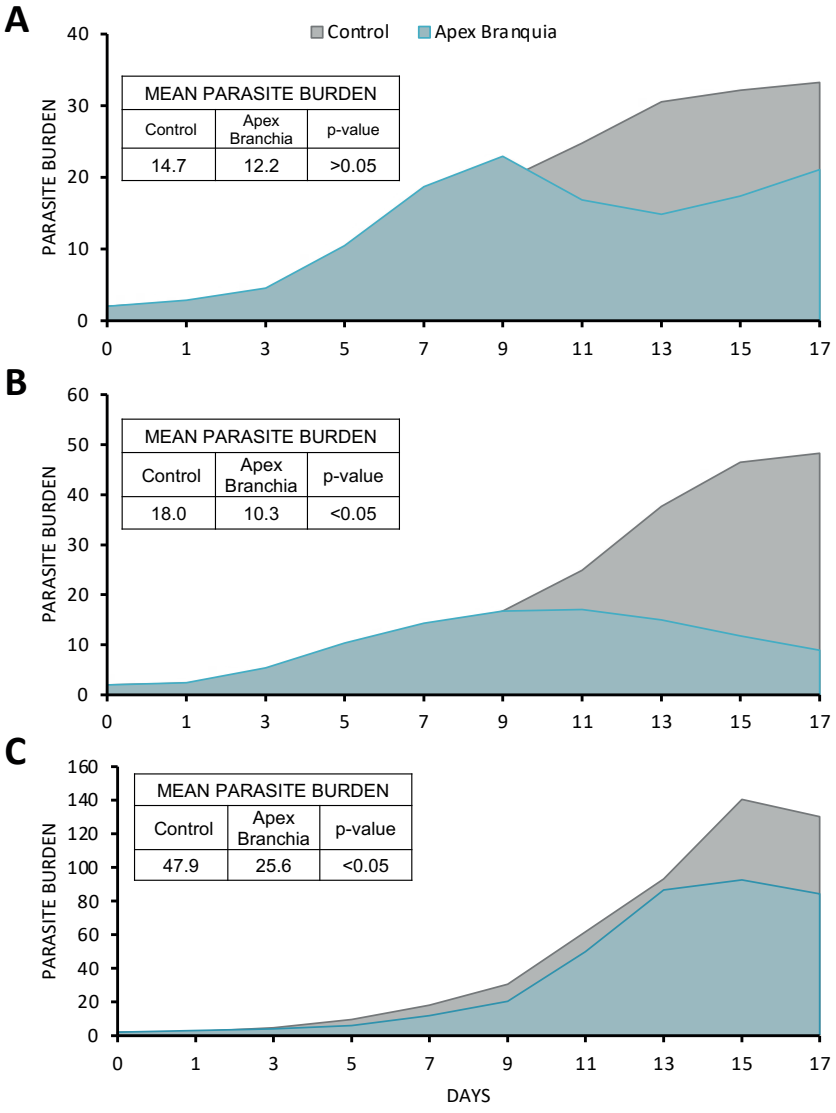

Figure 1. Infection trajectories in guppies (*Poecilia reticulata*, n=18 per treatment) infected with *Gyrodactylus turnbulli*. A) Infection trajectories and mean parasite abundance in juvenile guppies (Trial 1). B) Infection trajectories and mean parasite abundance in adult guppies (Trial 2). C) Infection trajectories and mean parasite abundance in adult guppies (Trial 3).

continuously deployed together to enhance fish resistance traits while simultaneously reducing fish-parasite encounters (Barret *et al.*, 2020). Bolstering of fish resistance traits includes the dietary inclusion of health-promoting functional additives and does not imply any negative impact on fish welfare (Barret *et al.*, 2020). A key attribute for a functional additive to efficiently prevent ectoparasite infestations is the ability to improve the defensiveness properties of blood and skin mucus. This will mainly translate into reduced ectoparasite prevalence and increased protection against secondary bacterial infections.

APEX® BRANCHIA is a health-promoting functional additive based on synergistic combinations of natural components with antiparasitic, antimicrobial and immunostimulant properties. In this article, we present two separate studies that contribute to the proven efficacy of this functional additive to reduce ectoparasite prevalence and to boost the skin mucus defensiveness properties.

Reduction of ectoparasite prevalence as proved by the guppy model

Cohabitation studies are generally used for preliminary assessment of the efficacy of functional additives against ectoparasites. However, this type of challenge is limited by the number of replicates and entails such high variability in infection rates that hinders the interpretation of results. The host-parasite model – the Trinidadian guppy *Poecilia reticulata* and its associated ectoparasite *Gyrodactylus turnbulli* – allows for infections with a known and consistent number of ectoparasites across recipient fish and high replication (Arepi *et al.*, 2019). This model has been standardized and was used to test the efficacy of APEX® BRANCHIA as a preventive strategy against ectoparasitic infestations.

Three trials were conducted; one in guppy juveniles (mean standard length ± SE: 8.8 ± 0.6 mm) and two in guppy adults (mean standard length ± SE: 28.9 ± 0.2). Guppy flake feed was supplemented with APEX® BRANCHIA at 0.5%. Guppies (n=18) were maintained into individual 1 L aquariums and fed daily with control and treatment feeds for 14 days. On day 15, each fish was anesthetized and infected on the caudal fin with

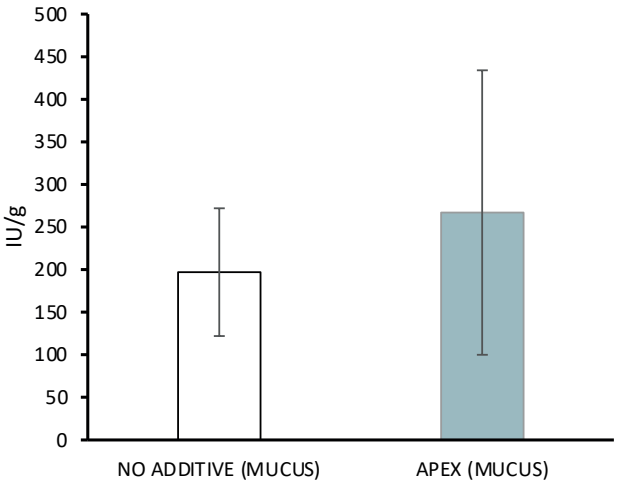

Figure 2. Total protease activity (TPA) in skin mucus following APEX® BRANCHIA supplementation (0.6%) for four weeks. Only numerical difference was observed.

two individuals of *G. turnbulli* from naturally infected guppy donors (Day 0 of infection). After infection, fish were screened again the next day (Day 1) to ensure that infection was successful and
if no parasites were present then fish were re-infected. Fish were fed daily corresponding feed and screened under anesthetic every 48 hours to monitor parasite density over 17 days (until day 31). A Generalised Linear Mixed effect model (GLMM) was constructed and used for statistical analysis.

Results showed that APEX® BRANCHIA consistently lowered the infection trajectories of the ectoparasite across juvenile and adult stages (Fig. 1). In juvenile guppy (Fig. 1A), supplementation numerically reduced the mean parasite burden by 17%. In adult guppies, supplementation significantly reduced the mean parasite burden by 43% and 47% in Trials 2 (Fig. 1B) and 3 (Fig. 1C), respectively. In adult guppies, the infection trajectories and parasite burdens differed between trials; the infection trajectory in Trial 2 did not reach infection rates as high as in Trial 3 and showed a depression after day 11. This can be attributed to a higher proportion of responder and resistant fish in relation to the population of Trial 3, likely composed of more susceptible fish. It was therefore concluded that, under a model testing system with enhanced robustness to evaluate antiparasitic efficacy, APEX® BRANCHIA efficiently reduces infection rates of ectoparasites.

A key attribute for a functional additive to efficiently prevent ectoparasite infestations is the ability to improve the defensiveness properties of blood and skin mucus.

Enhancement of skin mucus defensiveness properties to control secondary infections

Skin mucus is the first barrier by which fish are protected from the attack of pathogens. Skin mucus defensiveness includes biochemical barriers (i.e. antioxidant, detoxifying, bactericidal and immune protection) protecting from the adhesion of secondary bacteria pathogens that worsen the severity of parasitic infestation. The aim of this study was to evaluate the efficacy of APEX® BRANCHIA to enhance the antimicrobial properties of skin mucus in gilthead seabream.

APEX® BRANCHIA was supplemented at 0.6% in commercial-like feed formula and fed to gilthead seabream of 180 g during four weeks in triplicate tanks. At the end of the feeding period, skin mucus samples were collected non-invasively according to Fernández-Alacid *et al.* (2018). Following Sanahuja *et al.* (2019), total alkaline protease activity (TPA), an indicator of proteolytic activity against pathogens, and antibacterial activity against a non-pathogenic (*E. coli*, DSMZ number: 423) and pathogenic (*P. anguilliseptica*, CECT number: 899T) bacteria were measured. An independent t-test was used for statistical analysis.

Results showed that TPA was numerically increased by 30% with the additive supplementation (Fig. 2). Enhanced activity is indicative of enhanced release of proteases into skin mucus and therefore of reduced pathogen adherence to the skin mucus. It must be mentioned that the lack of significant difference can be attributed to the high variability associated with this indicator and to the need for higher replication. The antimicrobial activity of skin mucus was improved by APEX® BRANCHIA supplementation (Fig. 3).

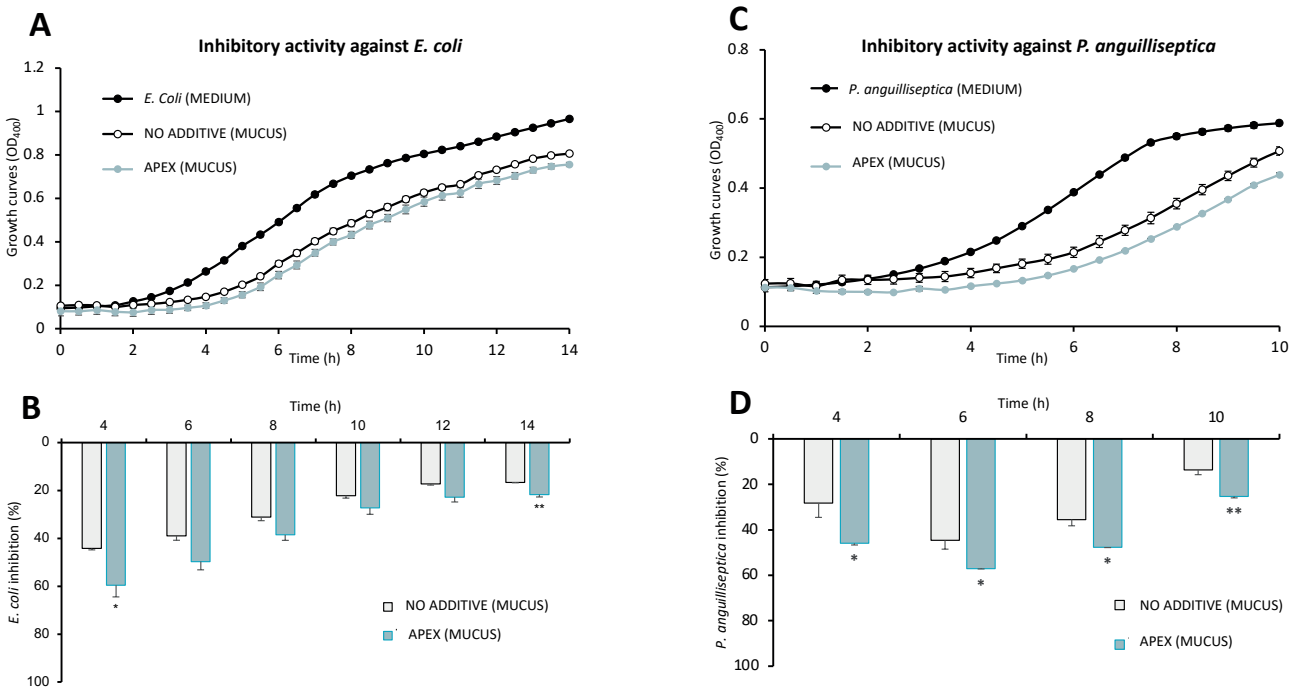

Figure 3. Antimicrobial activity of skin mucus following APEX® BRANCHIA supplementation (0.6%) for four weeks. A) Growth curve of *E. coli* in natural medium (black), mucus of non-supplemented fish (white) and mucus of supplemented fish (blue). B) Percentage of *E. coli* growth inhibition in mucus in relation to growth in medium. C) Growth curve of *P. anguilliseptica* in natural medium (black), mucus of non-supplemented fish (white) and mucus of supplemented fish (blue). D) Percentage of *P. anguilliseptica* growth inhibition in mucus in relation to growth in medium. *Significant differences ($p<0.05$) between control and additive.

The additive showed significant efficacy against both *E. coli* and *P. anguilliseptica*, supporting the numerical effect on total protease activity. Specifically, against the pathogenic *P. anguilliseptica*, growth inhibition was two-fold enhanced with the additive supplementation in relation to the non-supplementation. Altogether, results indicate an optimization of the preventive mechanisms against secondary bacterial infections and specifically on the adherence to the mucosal surface.

Conclusion

Controlling ectoparasitic infestations is one of the biggest challenges in aquaculture farming. Ectoparasites are frequently found in mixed infections with other secondary infections. APEX® BRANCHIA is a functional feed additive based on natural components that has been proven to reduce ectoparasite prevalence and optimize the mucus defensiveness properties that protect against secondary bacterial infections. Feed application is safe, practical and environmentally-friendly. Results here support the use of APEX® BRANCHIA as reinforcement of the preventive strategy against ectoparasitic infestations. Supplementation can compensate for the negative effects associated with farming conditions such as the elimination of skin mucus and increased susceptibility to infections.

References available on request

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