New sustainable GAIN formulations

Nutritionists have been testing new emerging ingredients for aquafeeds, and as alternative for traditional marine and plant meals and oils in a quest for more sustainability and circularity in the use of resources. Ingredients such as fish protein hydrolysates, processed animal proteins, yeast meals, insect meals, emerging vegetable meals/protein concentrates, microalgae, macroalgae, fish oil from trimmings, single cell oil, plant oils (e.g., linseed, rapeseed, sunflower, camellina), poultry fat, insect oil and DHA-rich algal products are all suitable alternatives to ingredients used in conventional formulations. However, these ingredients have mostly been tested by researchers on a one-by-one basis. Moreover, most of them have limitations for their inclusion level (%) due to nutritional or technical constraints, and thereby alternative formulations tend to be more complex. Involving more ingredients, than conventional ones.

![Diagram showing GAIN formulation concepts](image)

GAIN fish trials aimed to evaluate emerging ingredients, which are already commercially available, using different formulation concepts. The GAIN ingredient basket is based on circularity principles, maximizing resource efficiency, while contributing towards zero waste in the agro-food value chain, and looking for feed cost-effectiveness. It also takes into account social acceptance, so to optimize sustainability within the current/predictable regulatory framework. Here we present the main formulation concepts as well as the first results of work already undertaken. The diets for four target species - salmon, turbot, seabream and trout - were formulated and followed the principles outlined in Figure 1, with adjustments for each species based on known nutritional requirements and tolerance to ingredients.
Turbot trial

Turbot (Scophthalmus maximus) experiment was undertaken at AWI - Alfred Wegener Institute’s facilities (Bremerhaven, Germany) (Figure 2) and lasted a total of 12 weeks using fish with initial weigh of 20.3 ± 0.1 (g ± SD). Fish were fed with four different diets in quadruplicate: PAP, NoPAP, MIX and Control diets, in a RAS system. All tested diets performed well (Figure 3), with no differences found in weight gain, feed intake or feed conversion ratio (FCR). Survival for this trial was at 100% in all treatments. However, fish fed with three new formulated diets presented higher hepatosomatic index (HSI) when compared to Control. On the other hand, fish fed MIX diet presented higher condition factor (CF) when compared to fish from Control group.

Rainbow Trout trial

Rainbow trout (Oncorhynchus mykiss) trial was undertaken by FEM - Fondazione Edmund Mach (San Michele all’Adige, Italy) (Figure 4) facilities during 12 weeks in a flow-through system. Fish having initial weight of 62.7 ± 1.6 (g ± SD) were fed with four different formulations in quadruplicate: PAP, NoPAP, MIX and Control diets. All tested diets performed very well, with trout reaching around 345 g at the end of the trial which correspond to 5 times increase of the initial weight, and very good FCR values; around 0.8 (Figure 5). No differences were found in the zootecnical parameters between diets.

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Figure 2: Turbot trial running in AWI - Alfred Wegener Institute’s facilities (Bremerhaven, Germany).

Figure 4: Trout trial running in FEM - Fondazione Edmund Mach (San Michele all’Adige, Italy) facilities.

Figure 3: Final weight of turbot fed GAIN test feeds.

Figure 5: Feed conversion ratio of trout fed GAIN test feeds.
Atlantic salmon trial

GIFAS (Gildeskål, Norway) (Figure 6) was responsible to undertake the salmon (Salmo salar) trial which was performed in 12 sea cages. Fish with initial weight of 152.1 ± 0.5 (g ± SD), fed with three different diets in quadruplicate: PAP, NoPAP and Control diets, reached around 798g after 13 weeks in all 3 diets, which correspond to 5 fold increase on the initial weight. All tested diets performed very well, with fish fed PAP presenting slightly higher FCR when compared to fish fed control diet. Diets had no effect on salmon lice counts performed during the trial, neither in gut health status, welfare indicators or the immune parameters tested (Figure 7).

Gilthead seabream trial

Gilthead seabream (Sparus aurata) trial was undertaken at RIASEARCH/SPAROS facilities (Murtosa, Portugal) facilities. Fish weighing 54.4 ± 2.8 (g ± SD) were fed for 12 weeks with four different diets in quadruplicate: PAP, NoPAP, Mix and Control. There were no differences in fish growth between diets, but fish fed Control and NoPAP diet presented lower FCR than fish fed MIX and PAP diets (Figure 8). It seems that Control and NoPAP formulations can promote a slightly better bioavailability and/or increased absorption of key nutrients.

![Feed conversion ratio (FCR)](chart)

Although, no differences were observed for apparent digestibility of protein and phosphorus (P) among diets, some differences were observed in mineral utilization in this trial. For instance, fish fed PAP presented the higher retention of Zinc (Zn) when compared to control diet.

![Plasmatic values of IgM in Atlantic salmon fed GAIN test feeds](chart)
Gilthead seabream trial

In addition, fish innate immune response was also assessed. Some groups presented higher activity of parameters such as anti-protease activity in NoPAP diet and IgM percentage in Mix diet fed fish. Other parameters tested such as bactericidal activity and protease activity did not present differences among treatments. Gene expression on head kidney have presented a general trend on the PAP group to have a pro-inflammatory profile evidenced by the up regulation of some cytokines and chemokine-related proteins as well as some T-cell markers. NOPAP and MIX fish in a lesser extend showed a more attenuated response on the same genes. These results are indicative that these two alternative formulations could be more appropriate for practical use. Such assumption is supported by current studies in gut microbiota composition.

Overall, the results obtained for the trials for the four fish species suggest that these novel formulations based on sustainability and circularity principles are viable options for turbot, salmon, trout and seabream. A second set of trials using these formulation concepts is underway in GAIN, including ingredients produced by GAIN partners, and aiming to evaluate cost-benefit of the formulations.

The GAIN consortium

More info:
www.unive.it/gain2020_it
Twitter: https://twitter.com/gain2020
Facebook: https://www.facebook.com/gain2020

Coordination:
Roberto Pastres
Email: gain2020@unive.it

Communication and press:
Email: info@gain2020.eu

Coming topics for the Newsletter:
- Consumer acceptance
- Valorisation of secondary products, side streams and by-products of aquaculture

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