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Authors: Conceição, L.; Dias, J.; Pereira, G.V.; Johansen. J.; Buck, B.; Glencross, B.
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GLOSSARY OF ACRONYMS

Acronym	Definition
FM	Fish meal
FO	Fish oil
FPH	Fish Protein hydrolysates
HUFA´s	High unsaturated fatty acids
PAP	Processed Animal Proteins
Salmon	*Salmo salar / Atlantic salmon*
SBM	Soybean meal
Sea bream	*Sparus aurata / gilthead seabream*
SPC	Soy Protein Concentrate
Trout	Oncorhynchus mykiss / rainbow trout
Turbot	Scophthalmus maximus
VPC	Vegetable protein concentrate
YM	Yeast Meal

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1. Executive summary

Green Aquaculture Intensification (GAIN) project aims to develop new generation of sustainable fish feeds specifically designed to facilitate aquaculture eco-intensification through increased circularity and resource utilization, using a set of GAIN-developed candidate ingredients such as algae and by-products of aquaculture activities, together with other emerging commercial ingredients. Therefore, this deliverable consists in develop and design feeds based such ingredients in order to supply a range of key finfish species such as Atlantic salmon (Salmo *salar*), gilthead seabream (*Sparus aurata*), rainbow trout (*Oncorhynchus mykiss*) and turbot (*Scophthalmus maximus*).

The GAIN aquafeed formulation concept starts in replacing - fish meal and fish oil derived from feed-targeted fisheries, together with commodity ingredients derived from vegetables such as soy, wheat and corn, in a drive towards circular economy and zero-waste, and aiming at minimizing sustainability and ethics concerns. Besides the obvious impact of fisheries, using plant protein and oil sources is an increasing concern, due to pressure on farm land resources, resulting deforestation, negative effects on global food security, EU reliance on imports of major crops, and carbon footprint of transportation of some of the raw materials across continents.

New emerging ingredients have been in the formulators' agenda for a while, but have mostly been tested by researchers on a one-by-one basis. Ingredients such as fish protein hydrolysates (FPH), processed animal proteins, yeast meals, insect meals, vegetable meals/protein concentrates of European origin, microalgae, macroalgae, fish oil from trimmings, single cell oil, vegetable oils (e.g., linseed oil, rapeseed oil, sunflower oil, soybean oil), poultry fat, insect oil and DHA-rich algal oils are all suitable alternatives. However, these emerging ingredients have mostly been tested by researchers and on a one-by-one basis. GAIN´s approach is to test different formulations concepts using combinations of these ingredients, rather than one-by-one. Therefore, the aim is not to test individual emerging ingredients, but rather formulation scenarios which may be relevant in the near future for the different species based on consumer acceptance and regulatory trends.

Control diets were formulated for each species to mimic a current standard commercial diet for each species, followed by diets with replacement of FM, FO and other ingredients which may raise sustainability and/or ethical issues, by alternative ingredients in three formulation concepts:

1) A diet rich in processed animal proteins – byproducts from livestock production, in vegetable protein concentrates (VPCs) from European origin (diet PAP) and FPH;
2) A diet resulting from an extend combination of emerging ingredients, allowing the reduction of both fish meal and PAPs inclusion, with small to moderate amounts of insect meal, microbial biomasses, microalgae, macroalgae, FPH and VPCs (diet NoPAP);
3) A diet resulting from an extend combination of emerging ingredients, allowing the reduction of both fish meal, with small to moderate amounts of PAPs, insect meal, microbial biomasses, microalgae, macroalgae, FPH and VPCs (diet MIX).

The diet formulations had to be adjusted for each species, according to its known nutritional requirements and existing knowledge on tolerance to different ingredients. The aim was to maximize the probability of good performances, and reduce fish welfare risks to a minimum. This document contains a first set of formulations of the diets for the four target species that are being currently tested in growth trials.

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2. Introduction

Green Aquaculture Intensification in Europe (GAIN) (www.unive.it/gainh2020_eu) is a project financed by the European Union under the Horizon2020 framework. The project is run by a consortium of 20 partners from a variety of professional backgrounds, spanning across 11 different countries, including Canada and China. The global scope of the consortium is enhanced by the involvement of a US national organization (NOAA) as a third-party member. The composition of GAIN Consortium and the project structure are given in Annex 1. GAIN's primary aim is to assist the ecological intensification of aquaculture in the European Union (EU) and the European Economic Area (EEA), with the dual objectives of increasing production and competitiveness of the industry, while ensuring sustainability and compliance with EU regulations on food safety and environment.

Across the GAIN consortium and elsewhere various approaches have been developed to design feeds based on emerging ingredients in order to supply a range of key finfish species and different farming systems, often aiming at replacement of fish meal and fish oil, in order to improve the environmental and economic sustainability of fish farming. The use of plant protein and oil sources has been the main approach to deal with of fish meal and fish oil replacement so far (e.g., FP7 ARRAINA project, www.arraina.eu). However, the sustainability and ethics of using plants has been an increasing concern of many, due to pressure on farm land resources, resulting deforestation, and food security.

In GAIN WP1, Task 1.2 aims to develop new generation of sustainable fish feeds specifically designed to facilitate aquaculture eco-intensification through increased circularity and resource utilization, including reducing the reliance on traditional marine and vegetable ingredients, using a set of candidate ingredients such as: algae (from Tasks 1.1 and 2.1) and by-products of aquaculture activities (e.g., fish protein hydrolysates, Task 2.2). These ingredients should be complemented by emerging commercial ingredients (e.g., heterotrophic microalgae oils, single cell meals and insect meals, plant protein concentrates of European origin) in compliance with regulatory framework and social acceptance (GAIN Tasks 3.1 and 3.4 respectively). GAIN considers that these changes in feed formulation may impact positively, and eventually negatively, health status, welfare and performance of the fish. Therefore, a set of KPIs such as gene expression, immunological and histological parameters, as well as growth performance, feed convention ratio (FCR), carcass composition and fish mineral balance, needs to be evaluated.

This deliverable describes the results achieved during the first 15 months in Task 1.2, which the main goal was to design feeds based in micro and macroalgae, fish by-products and emerging ingredients for specific species such as salmon (*Salmo salar*), turbot (*Scophthalmus maximus*), seabream (*Sparus aurata*), and trout (*Oncorhynchus mykiss*). Feed design data generated by Task 1.2 will be complemented by the outputs of the growth trials that are being currently undertaken. These results, together with the characterization of ingredients derived from GAIN´s Tasks 1.1 and 2.2, will allow to design and formulate a second set of specific diets for the selected species, to be tested later in the project.

3. Methodology

Here the formulation concept, the ingredient selection and the resulting feeds formulation and production procedures are given.

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3.1 Formulation concept

In line with GAIN consortium approaches, the formulation concept stands on a list of traditional ingredients to avoid or decrease when the main goals are sustainability and a drive towards circular economy and zero-waste. Table 3.1 summarises the main traditional ingredients that were a target for replacement. One of the main concerns in diets for aquaculture is the traditional use of fish meal (FM). Although most sources of FM utilised in the EU are from well-regulated stocks, they are at the limit of sustainable exploitation, meaning that they are increasingly costly and if aquaculture is to grow, alternatives must be found. However, the sustainability and ethics of using plant protein and oil sources is an increasing concern, due to pressure on farm land resources, resulting deforestation, negative effects on food security, and carbon footprint of transportation of some of the raw materials across continents.

New emerging ingredients have been in the formulators agenda for a while, but have mostly been tested by researchers on a one-by-one basis. Ingredients such as fish protein hydrolysates (FPH) from fisheries and aquaculture by-products, processed animal proteins (PAP), and yeast meals (YM), insect meals (IM), and vegetable meals/protein concentrates of European origin (e.g., pea, rapeseed, sunflower), are increasingly available as protein sources.

The use of fish oil (FO) in aquafeeds raises the same concerns as FM. In order to reduce the use of FO from reduction fisheries in aquaculture diets, FO from trimmings, single cell (SC) oil, as well as vegetable oils such as linseed oil, rapeseed oil, sunflower oil, soybean oil or palm oil are suitable alternatives. The last one is not aligned with GAIN project philosophy due to environmental concerns on deforestation. Moreover, poultry fat, insect oil and DHA-rich algal oils are suitable FO replacement options.

Amongst the vegetable meals, soybean meal (SBM) has been currently used in replacing FM, however, the high gut inflammation reported in salmonids (Krogdahl et al., 2015), as well as environmental issues, has led formulators to find other alternatives. Ingredients such as soy protein concentrate (SPC), faba meal, lupins meal, wheat meal, corn meal, sunflower meal, rapeseed meal, yeast meal, and vegetable protein concentrates (VPC), are already used in variable degrees, also depending on fish species, in commercial aquafeeds. However, some of these ingredients, such as wheat and corn products are still considered less sustainable because they can have major contributions to food security, in particular in more vulnerable human populations, and have a very high energy demand to be produced.

Finally, it is important to mention that partial alternative / complement to traditional premixes and feed additives (such as antioxidants), such as algae and yeast products have been raised as nutrient source to be used in feed formulations.

The final list of ingredients selected to be included in GAIN formulations took into consideration a preliminary LCA analysis on conventional aquafeed vegetable ingredients provided by WP4.

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Table 3.1 – List of traditional used ingredient for formulation of aquafeeds and its possible replacement alternatives.

Traditional ingredient	Alternatives
Fish meal	Fish Protein Hydrolysate, Processed Animal Proteins, Yeast meal, Vegetable meals / protein concentrates of European origin (pea, rapeseed, sunflower), Insect meal
Commodity Vegetable meals*	
Fish oil, soybean oil	FO from trimmings, Single cell oil, vegetable oils (linseed, rapeseed, sunflower), Poultry fat, Insect oil
Premixes and additives	Microalgae, macroalgae and yeast products

* Includes soybean meal, Soy Protein Concentrate, Faba meal, Lupins meal, Wheat meal, Corn meal,Pea meal, Sunflower meal, Rapeseed meal.

These emerging ingredients have mostly been tested by researchers and on a one-by-one basis. GAIN´s approach is to test different formulations concepts using combinations of these ingredients, rather than one-by-one. Therefore, the aim is not to test individual emerging ingredients, but rather formulation scenarios with may be relevant for the different species based on consumer acceptance and regulatory trends. 3.2 Ingredients Selection
According to the literature and other available knowledge in the aquafeeds formulation and based on the GAIN project goal, a list of new emerging ingredients was selected as well as the target inclusion levels were defined (Table 3.2), based on market availability, present and foreseen in the coming 5 years, their nutritional composition, and their foreseen contribution to minimize the Eco-Intensification Sustainability Index (EISI) being developed in WP4. The EISI will be made up of environmental impact data, economic input-output data to assess economic feasibility, and data taken from T3.4 on public perceptions, combined with assessments of labour practices, social capital, and other relevant social impacts identified during the project. In what concerns feed formulation evaluation, the main factors to be considered are expected to be: total emissions of nitrogen and phosphorus into environment, Fish in: Fish out ratios, and LCA data on individual ingredients, and in particular, carbon, water, and land footprints, ozone depletion, photochemical oxidation, eutrophication, and acidification emissions.

3.1 Diets formulation and production

Diets were formulated according to the known nutritional requirements of the target species (NRC 2011) and manufactured by extrusion at SPAROS facilities. All powder ingredients were mixed accordingly to the target formulation in a double-helix mixer (model 500L, TGC Extrusion, France) and ground (below 400 µm) in a micropulverizer hammer mill (model SH1, Hosokawa-Alpine, Germany). Diets (pellet size changed according to species and fish size) were manufactured with a twin-screw extruder (model BC45, Clextral, France) with a screw diameter of 55.5 mm. Extrusion conditions: feeder rate (80-85 kg/h), screw speed (247-266 rpm), water addition in barrel 1 (345 ml/min), temperature barrel 1 (32-34ºC), temperature barrel 3 (111-117°C). Extruded pellets were dried in a vibrating fluid bed dryer (model DR100, TGC Extrusion, France). After cooling, oils were added by vacuum coating (model PG-10VCLAB, Dinnissen, The Netherlands). Coating conditions were: pressure (700 mbar); spraying time under vacuum (approximately 90 seconds), return to atmospheric pressure (120 seconds). Immediately after coating, diets were packed in bags or sealed plastic buckets and shipped to the research site

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where they were stored at room temperature, but in a cool and aerated emplacement. Representative samples of each diet were taken for composition analysis.

Table 3.2 – List of emerging ingredients including source, major benefits and target inclusion for aquafeeds formulation.

Emerging commercial ingredients	Source	Major "benefits"	Alternatives to	Target inclusion levels
Insect meals	Tenebrio Hermetia	Protein Protein	FM, VPC FM, VPC	5-10% 5-10%
Insect oil	Tenebrio Hermetia	Lipids, Lauric acid (antimicrobial)	FO	0-1%
Heterotrophic microalgae	Schizochytrium	Protein, HUFA	FO	1-4%
	Chlorella	Protein	FM	1 - 10%
Autotrophic microalgae	Nannochloropsis	Protein, HUFA´s, antioxidants	FM, FO, Antioxidants	1-3%
	Chlorella	Protein, antioxidants	FM, Antioxidants	1-3%
Single cell protein/meals	bacteria	Protein, nucleotides	FM, nucleotides	5-10%
	yeast	Protein, nucleotides	FM, nucleotides	2-5%
Vegetable meals	Faba, Lupins, Wheat, corn, Sunflower, Rapeseed	Protein	Non European Soya meal	* Depends on the species
European VPC	Pea, potato wheat gluten, corn gluten, Sunflower, lupin, rapeseed	Protein	FM, non European VPC	* Depends on the species
Macroalgae	-	Binders, minerals	Starch rich producs, premixes	1-4%

4. Description of formulated eco-efficient feeds

SPAROS has formulated diets within the project scope and species requirements, with support of SHP, AWI and UoS. This was done using the list of ingredients previously presented (Tables 3.1 and 3.2). The diets for the four selected species using the listed ingredients are described below. These formulations correspond to the first year of trials (Block1) which are being conducted by different project partners. This Block 1 of fish trials aims to evaluate emerging ingredients, which are already commercially available. The final ingredient basket will be defined based of circularity principles, maximizing resource efficiency, while contributing towards zero waste in the agro-

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food value chain, feed cost-effectiveness, and taking into account social acceptance, so to optimize sustainability within the current/predictable regulatory framework, based on work of WP4. This block of work will pave the way for trials to take place in Block 2 of trials, allowing for maximum impact of this second block and task objectives.

Control diets (CTRL) were formulated to mimic a current standard commercial diet for each species, followed by diets with replacement of FM, FO and other ingredients which may raise sustainability and/or ethical issues, by alternative ingredients in three formulation concepts:

1) A diet rich in processed animal proteins – byproducts from livestock production and also in vegetable protein concentrates (VPCs) from European origin (diet PAP);
2) A diet resulting from an extend combination of emerging ingredients, allowing the reduction of both fish meal and PAPs inclusion, with small to moderate amounts of insect meal, microbial biomasses, microalgae, macroalgae, and VPCs (diet NoPAP);
3) A diet resulting from an extend combination of emerging ingredients, allowing the reduction of both fish meal, with small to moderate amounts of PAPs, insect meal, microbial biomasses, microalgae, macroalgae, and VPCs (diet MIX).

Formulation and used ingredients are described on Tables 4.1 to Table 4.4 for the species turbot, seabream, trout and salmon. The diet formulations had to be adjusted for each species, according to its known nutritional requirements and existing knowledge on tolerance to different ingredients. The aim was to maximize the probability of good performances, and reduce fish welfare risks to a minimum.

In early 2020 a new set of innovative GAIN formulations will be prepared, combining GAIN-developed by-products and specialties with the emerging ingredients commercially available tested in block 1, and eventually other products arising from concurring projects and made available by December 2019. The GAIN-developed by-products and specialties are FPH (Task 2.2) derived from aquaculture side streams (e.g., fish heads, viscera, frames) and fisheries by-catches, microalgae rich in the minerals Selenium and Zinc (Task 1.1), and macroalgae rich in several minerals (task 2.1).

Innovative formulas will be prepared and tested (Block 2 of fish trials) against a commercial-type formula, for each of the 4 target species. The GAIN formulas will have different levels of incorporation of the novel ingredients, and will take into account EISI sustainability index, LCA analysis performed under WP4, and ingredient prices.

4.1 Diets formulation for Turbot

In agreement with nutritional requirements for this species, the diets for turbot (*S. maximus*) were formulated with 50% of high quality fishmeal for control diets, which was replaced by a lower quality by-product fishmeal, together with the use of fish protein hydrolysate (by-products), insect meal, yeast and microbial biomasses for No PAP, PAP and MIX diets. In addition, PAP diet received haemoglobin and poultry meal, and MIX diet received poultry meal. No PAP diet and MIX diet have comprised a combination of microalgae *Spirullina, Chlorella*, and *Tetraselmis*. In addition, fish oil was partially replaced by DHA-Rich algae biomass (Schizochytrium), rapeseed oil, and rapeseed lecithin.

Turbot formulas where by far the most conservative ones, as this species is well known to have a low tolerance for FM replacement.

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4.2 Diets formulation for Seabream

Nutritional requirements and known ingredient tolerance for Seabream (*S. aurata*) allowed formulation with 20% FM and high contents of traditional vegetable protein sources for control diet, following current industry practices. Main replacements of FM and traditional vegetable protein sources were insect meal, fish by-products, microbial and yeast biomasses for NoPAP, PAP and MIX diets. However, PAP and MIX diets comprised poultry meal, feather meal hydrolysate and porcine blood meal additionally. No PAP diet and MIX diet have received the microalgae *Spirullina* and *Chlorella*. Diet MIX contained elevated levels of pea protein concentrate, wheat gluten, corn gluten and rapeseed meal. In addition, in all GAIN alternative formulations fish oil was partially replaced by DHA-Rich algae biomass (*Schizochytrium*), rapeseed oil, and rapeseed lecithin.

4.3 Diets formulation for Trout

The diets for rainbow trout (*O. mykiss*) were formulated with 20% fishmeal (FM) and high level of SPC for control diet, following industry practice. NoPAP, PAP and MIX diets all received insect meal, microbial protein meal and yeast protein meal as FM alternatives. In addition, PAP and MIX diets received feather meal hydrolysate, porcine haemoglobin and poultry meal as protein sources. NoPAP had pea protein concentrate and wheat gluten as additional protein sources. On the other hand, *Spirullina* and *Chlorella* were added in NoPAP and MIX diets to keep the formulation concept.

All new emerging diets received levels of DHA-rich algae (*Schizochytrium*) and salmon oil as fat source. Furthermore, brewer's yeast and macroalgae mix was added to the new formulas.

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Table 4.1 – List of ingredients used for formulation of aquafeeds tested in GAIN project.

Raw materials and feed additives	Commercial name	CP, %	CF, %	Additional information	Supplier	Country
Fishmeal LT70	NORVIK LT	71.9	6.8	Scandinavian	Sopropêche	France
Fishmeal Super Prime	Diamante	66.3	11.5	South America	Pesquera Diamante	Peru
Fishmeal 60 (by-products)	CONRESA 60	61.2	8.4		Conserveros Reunidos S.A.	Spain
Fish hydrolysate (by-products)	-	82.6	9.6		Not disclosed	Not disclosed
Krill meal	-	61.1	17.4		Aker Biomarine	Norway
Insect meal	-	57.8	8.5	*Hermetia illucens*	Not disclosed	Not disclosed
Porcine hemoglobin	-	91.6	1.2		SONAC BV	The Netherlands
Feather meal hydrolysate	-	82.9	11.2		SONAC BV	The Netherlands
Porcine blood meal	-	89.1	0.4		SONAC BV	The Netherlands
Poultry meal 65	-	62.4	14.5		SAVINOR UTS	Portugal
Microbial protein meal	-	68.2	9.8	Methanotrophic bacteria	Not disclosed	Not disclosed
Yeast protein meal	-	68.0	0.9	*Saccharomyces cerevisiae*	Not disclosed	Not disclosed
Brewer's yeast	-	38.9	4.5		Premix Lda	Portugal
Microalgae meal (*Spirullina*)	-	72.1	1.0	*Arthrospira platensis*	Sopropêche	France
Microalgae meal (*Chlorella*)	-	62.5	9.2	*Chlorella vulgaris*	Allmicroalgae	Portugal
Microalgae meal (*Tetraselmis*)	-	23.4	6.2	*Tetraselmis chuii*	Allmicroalgae	Portugal
Microalgae meal (*Scenedesmus*)	-	23.4	6.2	*Scenedesmus obliquus*	Allmicroalgae	Portugal
Soy protein concentrate	Soycomil P	62.2	0.7		ADM	The Netherlands
Pea protein concentrate	Lysamine GPS	78.1	0.9		Roquette Frères	8.00
Wheat gluten	VITAL	80.4	5.6		Roquette Frères	France
Corn gluten meal	-	61.2	6.1		COPAM	Portugal
Soybean meal 48	-	47.4	2.6	Dehulled solvent extracted	CARGILL	Spain
Rapeseed meal	-	34.3	2.1	Defatted	Ribeiro e Sousa Lda	Portugal
Wheat meal	-	11.7	1.6		Casa Lanchinha	Portugal
Yellow peas	-	19.6	2.2		Ribeiro e Sousa Lda	Portugal
Pea starch	NASTAR	0.3	0.1	90% starch	COSUCRA	Belgium
Fish oil	-		98.1	16% EPA; 12% DHA	Sopropêche	France
Salmon oil (by-products)	-		98.3	4.6% EPA; 5.2% DHA	Sopropêche	France
DHA-rich algae (*Schizochytrium*)	ALL-G Rich	10	63.0	16% DHA	Alltech	Ireland
Rapeseed oil			98.2		Henry Lamotte Oils GmbH	Germany
Linseed oil			98.4		Henry Lamotte Oils GmbH	Germany

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Raw materials and feed additives	Commercial name	CP, %	CF, %	Additional information	Supplier	Country
Rapeseed lecithin	CANOLACITHIN F60		94.0		Novastell	France
Vitamin C (35%)	ROVIMIX Stay C35				DSM Nutritional Products	Switzerland
Vitamin E (50%)	ROVIMIX E50				DSM Nutritional Products	Switzerland
Betaine HCl	Beta-Key 95%				ORFFA	The Netherlands
Macroalgae mix	OceanFeed	11	0.6		Ocean Harvest	Ireland
Antioxidant	VERDILOX				Kemin Europe NV	Belgium
Sodium propionate	-				Disproquímica	Portugal
Sodium phosphate	-			25.8% P, 19.2% Na	Vadequímica	Spain
Monocalcium phosphate	ALIPHOS MONOCAL			22.7% P, 17.5% Ca	ALIPHOS	Belgium
Astaxanthin	Carophyll Pink 10% CWS			10% astaxanthin	DSM Nutritional Products	Switzerland
L-Histidine	-			98% His	Ajinomoto EUROLYSINE S.A.S	France
L-Lysine	-			99% Lys	Ajinomoto EUROLYSINE S.A.S	France
L-Threonine	-			98.5% Thr	Ajinomoto EUROLYSINE S.A.S	France
L-Tryptophan	-			98% Trp	Ajinomoto EUROLYSINE S.A.S	France
DL-Methionine	DL-Met for Aquaculture			99% Met	EVONIK Nutrition & Care GmbH	Germany
L-Taurine	-			98% Tau	ORFFA	The Netherlands
Yttrium oxide	-				Sigma Aldrich	USA

In all diets the inclusion of 1% of the vitamin and mineral premix contributed to an additional supply of the following micronutrients. Vitamins (IU or $mg \cdot kg^{-1}$ diet): DL-alpha tocopherol acetate, 255 mg; sodium menadione bisulphate, 10 mg; retinyl acetate, 26000 IU; DL-cholecalciferol, 2500 IU; thiamine, 2 mg; riboflavin, 9 mg; pyridoxine, 5 mg; cyanocobalamin, 0.5 mg; nicotinic acid, 25 mg; folic acid, 4 mg; L-ascorbic acid monophosphate, 80 mg; inositol, 17.5 mg; biotin, 0.2 mg; calcium panthotenate, 60 mg; choline chloride, 1960 mg. Minerals (g or $mg \cdot kg^{-1}$ diet): copper sulphate, 8.25 mg; ferric sulphate, 68 mg; potassium iodide, 0.7 mg; manganese oxide, 35 mg; organic selenium, 0.01 mg; zinc sulphate, 123 mg; calcium carbonate, 1.5 g; excipient wheat middlings.

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Table 4.2 – Formulated diets for turbot (S. maximus) containing: processed animal protein (PAP), Algae (NoPAP), mixed ingredients (MIX); and Control diet (CTRL).

Ingredients (%)	**CTRL**	**NoPAP**	**PAP**	**MIX**
Fishmeal LT70	50.00			
Fishmeal 60 (by-products)		35.00	35.00	25.00
Fish hydrolysate (by-products)		5.00	5.00	5.00
Insect meal		5.00	5.00	7.50
Porcine hemoblobin			2.50	
Poultry meal			10.20	7.50
Microbial protein meal		2.50	2.50	5.00
Yeast protein meal		2.50	2.50	5.00
Microalgae meal (*Spirullina*)		2.00		3.00
Microalgae meal (*Chlorella*)		0.50		0.60
Microalgae meal (*Tetraselmis*)		0.20		0.20
Soy protein concentrate	10.00			
Pea protein concentrate		12.40	5.00	8.00
Wheat gluten	11.00	11.50	10.00	10.00
Soybean meal 48	4.00			
Wheat meal	8.00			
Pea starch	4.00	8.89	8.99	8.99
Fish oil	11.60	4.64	4.64	4.64
DHA-rich algae (*Schizochytrium*)		1.08	1.08	1.88
Rapeseed oil		4.64	3.44	3.44
Rapeseed lecithin		0.80	0.80	0.80
Vitamin and mineral premix	1.00	1.00	1.00	1.00
Vitamin C (35%)	0.05	0.05	0.05	0.05
Vitamin E (50%)	0.05	0.05	0.05	0.05
Betaine HCl		0.50	0.50	0.50
Macroalgae mix		0.50	0.50	0.50
Antioxidant	0.18	0.18	0.18	0.18
Sodium propionate	0.10	0.10	0.10	0.10
L-Tryptophan		0.15	0.15	0.15
DL-Methionine		0.30	0.30	0.30
L-Taurine		0.50	0.50	0.60
Yttrium oxide	0.02	0.02	0.02	0.02
Total	**100.00**	**100.00**	**100.00**	**100.00**
Theoretical composition (as feed basis)				
Crude protein, % feed	54.0	54.0	54.0	54.0
Crude fat, % feed	16.0	16.0	16.0	16.0
Fiber, % feed	0.9	1.2	0.9	1.4
Ash, % feed	9.0	9.9	10.4	8.9
Gross Energy, MJ/kg feed	21.1	20.9	20.8	21.1
Total P, % feed	1.2	1.3	1.5	1.2

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Table 4.3 – Formulated diets for Seabream (*S. aurata*) containing: processed animal protein (PAP), Algae (NoPAP) and mixed ingredients (MIX); and Control diet (CTRL).

Ingredients (%)	**CTRL**	**PAP**	**NoPAP**	**MIX**
Fishmeal LT70	20.000			
Fishmeal 60 (by-products)			5.000	
Fish hydrolysate (by-products)		5.000	5.000	5.000
Insect meal		5.000	5.000	10.000
Microbial protein meal		5.000	5.000	10.000
Yeast protein meal		2.500	2.500	2.500
Feather meal hydrolysate		5.000		5.000
Porcine blood meal		3.000		3.000
Poultry meal 65	5.000	20.000		10.000
Microalgae meal (*Spirullina*)			5.000	5.000
Microalgae meal (*Chlorella*)			0.500	0.500
Soy protein concentrate	9.000			
Pea protein concentrate			4.100	
Wheat gluten	4.000		4.000	
Corn gluten meal	10.000	4.500	15.000	1.400
Soybean meal 48	12.000			
Rapeseed meal	4.000	5.700	11.500	7.000
Wheat meal	8.470			
Pea starch	3.000	6.000	7.900	9.000
Yellow peas	6.200	14.580	3.000	7.030
Fish oil	6.000			
Salmon oil		3.000	3.000	3.000
DHA-rich algae (*Schizochytrium*)		3.600	3.200	3.700
Rapeseed oil	8.260	6.000	8.500	6.300
Rapeseed lecithin	0.600	1.000	1.000	1.000
Vitamin and mineral premix	1.000	1.000	1.000	1.000
Vitamin C (35%)	0.100	0.100	0.100	0.100
Brewer's yeast		4.000	4.000	4.000
Macroalgae mix		2.000	2.000	2.000
Antioxidant	0.200	0.200	0.200	0.200
Sodium propionate	0.100	0.100	0.100	0.100
Monocalcium phosphate	2.000	1.900	2.500	2.200
L-Tryptophan	0.050	0.150	0.180	0.150
DL-Methionine		0.150	0.200	0.300
L-Taurine		0.500	0.500	0.500
Yttrium oxide	0.020	0.020	0.020	0.020
Total	**100.00**	**100.00**	**100.00**	**100.00**
Theoretical composition (as feed basis)				
Crude protein, % feed	43.5	43.5	43.6	43.5
Crude fat, % feed	17.9	18.0	18.1	18.0
Fiber, % feed	2.0	2.8	3.1	3.0
Ash, % feed	8.0	7.6	7.9	7.9
Gross Energy, MJ/kg feed	20.8	20.9	20.9	21.0
Total P, % feed	1.2	1.2	1.2	1.1

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Table 4.4 – Formulated diets for Trout (*Oncorhynchus mykiss*) containing: processed animal protein (PAP), Algae (NoPAP) and mixed ingredients (MIX); and Control diet (CTRL).

Ingredients, %	**CTRL**	**NoPAP**	**PAP**	**MIX**
Fishmeal LT70	20.000	5.000	5.000	
Fish hydrolysate (by-products)	3.000	3.000	3.000	3.000
Insect meal		5.000	5.000	10.000
Microbial protein meal		5.000	5.000	10.000
Yeast protein meal		3.000	3.000	3.000
Feather meal hydrolysate			5.000	5.000
Porcine hemoglobin			2.500	2.500
Poultry meal 65			20.000	10.000
Microalgae meal (*Spirullina*)		5.000		5.000
Microalgae meal (*Chlorella*)		0.500		0.500
Pea protein concentrate		6.000		
Wheat gluten	8.000	8.500		
Corn gluten meal	5.000	5.000	5.000	4.500
Soy protein concentrate	18.000	5.000		
Soybean meal 48	5.000			
Wheat meal	10.000	9.250	11.950	9.750
Pea starch	5.000	5.000	5.000	5.000
Fish oil	7.400	3.700	3.700	3.700
Salmon oil (by-products)		8.000	8.000	8.000
DHA-rich algae (*Schizochytrium*)		3.200	3.200	3.200
Rapeseed oil	9.700	2.800		0.600
Linseed oil	4.100	4.100	4.100	4.100
Rapeseed lecithin	0.500	1.000	1.000	1.000
Vitamin and mineral premix	1.000	1.000	1.000	1.000
Vitamin C (35%)	0.100	0.100	0.100	0.100
Betaine HCl	0.280	0.280	0.280	0.280
Brewer's yeast		4.000	4.000	4.000
Macroalgae mix		1.000	1.000	1.000
Antioxidant	0.350	0.350	0.350	0.350
Sodium propionate	0.100	0.100	0.100	0.100
Monocalcium phosphate	1.900	2.850	1.300	2.200
L-Lysine	0.300	1.000	0.500	0.950
L-Tryptophan	0.100	0.300	0.200	0.250
DL-Methionine	0.150	0.550	0.400	0.600
L-Taurine		0.400	0.300	0.300
Yttrium oxide	0.020	0.020	0.020	0.020
Total	**100.00**	**100.00**	**100.00**	**100.00**
Theoretical composition (as feed basis)				
Crude protein, % feed	42.2	42.2	42.2	42.2
Crude fat, % feed	24.1	24.1	24.1	24.1
Fiber, % feed	1.4	1.9	1.5	1.9
Ash, % feed	8.0	7.3	6.9	6.9
Gross Energy, MJ/kg feed	22.3	22.3	22.4	22.4
Total P, % feed	1.1	1.1	1.1	1.1

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4.4 Diets formulation for Salmon

A total of three different diets were formulated for salmon (*S. salar*). Control diet (CTRL) was designed using 15% of fishmeal (FM) according to this industry practice. In replacement of FM, NoPAP and PAP diets received fish protein hydrolyse as well as insect meal, and microbial biomass. In addition, PAP diet comprised several processed animal proteins such as feather meal hydrolysate, haemoglobin powder and poultry meal. NoPAP contained elevated levels of pea protein concentrate.

While fish oil was used in CTRL diet, a combination of fish oil, salmon oil and DHA-Rich algae biomass (Schizochytrium) and rapeseed oil were used in PAP and NoPAP diets in order to keep fat levels comparable to CTRL diet. Moreover, NoPAP received *Scenedesmus* and *Chlorella* microalgae. Both new formulated (NoPAP and PAP) diets contained brewer's yeast and macroalgae mix.

Table 4.5 – Formulated diets for Salmon (A. salmon) containing: processed animal protein (PAP), Algae (NoPAP) and mixed ingredients (MIX); and Control diet (CTRL).

Ingredients (%)	**CTRL**	**NoPAP**	**PAP**
Fishmeal LT70	5.000		
Fishmeal Super Prime	10.000		
Fishmeal 60 (by-products)		2.500	2.500
Fish hydrolysate (by-products)		5.000	5.000
Krill meal	4.000		
Feather meal hydrolysate			5.000
Hemoblobin powder			5.000
Poultry meal 65			9.000
Insect meal		10.000	10.000
Microbial portein meal		10.000	10.000
Microalgae meal (*Scenedesmus*)		0.900	
Microalgae meal (*Chlorella*)		1.100	
Soy protein concentrate	15.000		
Pea protein concentrate	10.000	11.500	
Wheat gluten	10.000	11.500	10.000
Corn gluten meal	4.500	2.500	0.000
Wheat meal	10.575	9.925	7.165
Pea starch			3.000
Fish oil	6.500	3.250	3.250
Salmon oil (by-products)		6.000	6.000
DHA-rich algae (*Schizochytrium*)		3.000	3.000
Rapeseed oil	18.500	13.400	11.900
Vitamin and mineral premix	1.000	1.000	1.000
Vitamin C (35%)	0.100	0.100	0.100
Betaine HCl	0.150	0.150	0.150
Brewer's yeast		2.000	2.000
Macroalgae mix		1.000	2.000
Antioxidant	0.200	0.200	0.200
Sodium phosphate	3.000	3.000	2.700

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Ingredients (%)	**CTRL**	**NoPAP**	**PAP**
Astaxanthin (10%)	0.055	0.055	0.055
L-Histidine	0.700	0.800	0.300
L-Lysine	0.200	0.350	0.200
L-Threonine	0.000	0.100	0.000
L-Tryptophan	0.150	0.150	0.060
DL-Methionine	0.200	0.300	0.250
L-Taurine	0.150	0.200	0.150
Yttrium oxide	0.020	0.020	0.020
Total	**100.00**	**100.00**	**100.00**
Theoretical composition (as feed basis)			
Crude protein, % feed	44.5	44.5	44.5
Crude fat, % feed	28.0	28.0	28.0
Fiber, % feed	1.2	1.6	1.0
Ash, % feed	7.2	6.2	6.9
Gross Energy, MJ/kg feed	23.2	23.3	23.4
Total P, % feed	1.3	1.3	1.3

5. Conclusions

GAIN´s Task 1.2 aim for the first project year was achieved. New diet formulations using emerging ingredients were designed and formulated, in order to facilitate aquaculture eco-intensification. These formulations are based on circularity principles, aim to maximizing resource efficiency, while contributing towards zero waste in the agro-food value chain, feed cost-effectiveness, and are expected to have a good social acceptance, so to optimize sustainability within the current/predictable European regulatory framework.

A first set of *in vivo* growth trials are being undertaken in order to test these novel sustainable fish feed formulations in turbot, seabream, trout and salmon. Furthermore, health status of the fish will be evaluated using KPIs such as gene expression, immunological and histological parameters, as well as growth performance, feed convention ratio (FCR), carcass composition and fish mineral balance.

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ANNEX 1 - GAIN Consortium and project structure

Table A 1 - GAIN Consortium

Participant № (leadership role)	Participant legal name	Country	Type
1 (Coordinator; WP5; WP7)	Università Ca' Foscari Venezia (UNIVE)	Italy	RTD
2 (WP3)	The University of Stirling (UoS)	UK	RTD
3 (WP1)	Alfred-Wegener-Institut Helmholtz- Zentrum für Polar- und Meeresforschung (AWI)	Germany	RTD
4	IBM Ireland Limited (IBM)	Ireland	CORP[1]
5 (WP2)	Agencia Estatal Consejo Superior de Investigaciones Cientificas (CSIC)	Spain	RTD
6 (WP4)	Longline Environment Limited (LLE)	Ireland	SME
7 (WP6)	Sparos Lda (SPAROS)	Portugal	SME
8	Salten Havbrukspark (SHP)	Norway	SME
9	Wageningen University (WU)	Netherlands	RTD
10	Johann Heinrich von Thuenen-Institut, Bundesforschungsinstitut Fuer Laendliche Raeume, Wald Und Fischerei (TI)	Germany	RTD
11	Agrifood and Biosciences Institute (AFBI)	UK	RTD
12	Zachodniopomorski Uniwersytet Technologiczny W Szczecinie (ZUT)	Poland	RTD
13	Asociacion Nacional de Fabricantes de Conservas de Pescados y Mariscos-Centro Tecnico Nacional de Conservacion de Productos de la Pesca (ANFACO)	Spain	NPO[2]
14	WAISTER AS (WAS)	Norway	SME
15	Gildeskal Forskningsstasjon AS (GIFAS)	Norway	SME
16	Lebeche (LEBCH)	Spain	CORP[1]
17	Sagremarisco-Viveiros de Marisco Lda (SGM)	Portugal	SME
18	Fondazione Edmund Mach (FEM)	Italy	NPO[2]
19	Dalhousie University (DAL)	Canada	RTD
20	South China Sea Fisheries Research Institute (SCSFRI)	China	RTD

GAIN is structured in 7 Work Packages, plus an Ethics Work Package, which was added by the EC during the negotiation (see Fig. A1). WP leaders are indicated in Table A1. The main objects of each WP are listed below.

WP1 - Production and Environment: will develop novel sustainable feeds and tools for enhancing aquaculture sustainable management of aquafarm based on Big Data analytics.

WP2 - Secondary products: will develop new co-products, in order to enhance circularity, sustainability and profitability of aquaculture supply chains.

WP3 - Policy and markets will analyse the state-of-the-art of EU and national legislations with respect to the valorisation and marketing of innovative GAIN products and co-products and provide suggestions to policy makers.

WP4 - Eco-intensification: will develop new approaches and tools for assessing the level of eco-intensification of GAIN innovative solutions, in comparison with standard

[1] Corporation (Not SME)

[2] Non-profit Organisation

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practices.

WP5 - Professional development: will deliver both on-line and in presence courses, in order to facilitate the adoption of GAIN innovative solutions by aquafarm operators.

WP6 - Dissemination, Exploitation, Communication: will maximize GAIN impact, by careful matching communication&dissemination tools to targeted audiences and developing platforms for exploiting GAIN results beyond its life time.

WP7 - Coordination: will ensure the timely delivery of all GAIN contractual items.

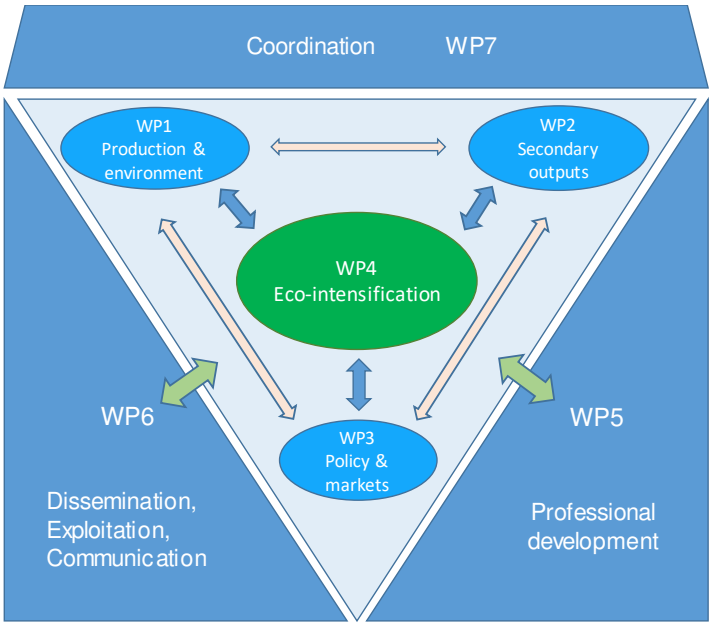

Figure 1 GAIN structure

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