A step towards sustainable aquaculture: Multiobjective feed formulation reduces environmental impacts at feed and farm levels for rainbow trout

Aurélie Wilfart, Florence Garcia-Launay, Frederic Terrier, Espoir Soudé, Pierre Aguirre, Sandrine Skiba-Cassy

A step towards sustainable aquaculture: multiobjective feed formulation reduces environmental impacts at feed and farm levels for rainbow trout

Aurélie Wilfart[1]*, Florence Garcia-Launay[2], Frederic Terrier[3], Espoir Soudé[3], Pierre Aguirre[3], Sandrine Skiba-Cassy[3]

Affiliation

[1]INRAE, Institut Agro, SAS, 35000 Rennes, France

[2]INRAE, Institut Agro, PEGASE, 35590 Saint-Gilles, France

[3]INRAE, Univ. Pau & Pays Adour, E2S UPPA, NUMEA, 64310 Saint Pée-sur-Nivelle, France.

*Corresponding author: aurelie.wilfart@inrae.fr (A. Wilfart)

Abstract (400 words)

Aquaculture is growing to meet the increasing demand for aquaculture products but is not free of environmental impacts. One solution is to improve how feed are formulated, limiting the environmental impact of fish production. Multiobjective (MO) formulation, which aims for a compromise between lower cost and lower environmental impacts, appears to be a promising solution to reduce the environmental footprint of aquaculture production. The objectives of this study were to design an eco-friendly trout feed (ECO-diet) using MO formulation and to compare its zootechnical and environmental performances to those of a commercial feed (C-diet) containing 16% fishmeal and 6.5% fish oil. MO formulation changed the composition of the diet greatly, which decreased environmental impacts of the feed as well as its price. It increased the number of ingredients used but reduced the use of fishmeal and fish oil by half. MO formulation also led to the elimination of soy products, faba bean, and gluten in favour of processed animal co-products that have high protein contents and low climate change impact. Rapeseed oil also disappeared from the ECO-diet due to its major contribution to land use, eutrophication, and acidification and, to a lesser extent, climate change. Overall, the ECO-diet had high digestibility, which differed little from that of the C-diet. Mean fish body weight after 12 weeks of growth did not differ significantly from that obtained with the C-diet, but analysis of fish growth curves indicated that the ECO-diet could lead to lower growth in the long term. This observation was consistent with the significantly lower feed intake in fish fed the ECO-diet. The decrease in impacts observed at the feed level was also observed at the farm level, although less so for eutrophication, non-renewable energy use, and climate change calculated per kg of body weight gain. MO formulation is a useful tool to reduce the environmental footprint of aquaculture production without compromising animal performances or necessarily increasing production cost.

Highlights

- Multiobjective (MO) formulation reduced the environmental footprint of fish feed
- Feeding the ECO-diet yielded satisfactory growth performance of trout
- Feeding the ECO-diet decreased environmental impacts of trout production
- MO formulation reduces aquaculture reliance on fishmeal and fish oil

Keywords (4-6): Eco-feed**,** environmental impacts, life cycle assessment, fish, feed formulation

1 Introduction

To meet the growing demand for seafood and the need for protein sources, aquaculture is one of the most dynamic animal production sectors, expanding in volume by 7.7% per year since the 1980s (FAO, 2020) to compensate for the stability of fisheries production in the past decade. Indeed, aquaculture has been the main source of fish for human consumption since 2015. In 2018, it provided 53% of fish, a percentage that is expected to increase over the long term as part of the solution to provide sufficient food and protein to more than nine billion people by 2050.

Animal production systems, including aquaculture, are widely criticised for their impacts on the environment (Bohnes et al., 2019; Ottinger et al., 2016; Steinfeld et al., 2006). For its part, aquaculture is responsible for many direct impacts related to eutrophication of aquatic ecosystems due to the emission of nutrients (e.g. nitrogen (N), phosphorus (P), particles) and intensive use of water, land, and energy; but also for indirect impacts related to the production of fish feed (Wilfart et al., 2013), especially for carnivorous fish (Aubin et al., 2009; Pelletier et al., 2009). Feed can represent 65-95% of the environmental impacts of animal products leaving a farm (Wilfart et al., 2018). For rainbow trout, feed production can contribute 73-87% of climate change impacts, 86% of acidification impacts, and 68-96% of net primary production use (NPPU) (Boissy et al., 2011; Papatryphon et al., 2004; Parker and Tyedmers, 2012). Aquaculture is also criticised for its heavy dependence on limited resources due to its massive use of fishmeal and fish oil (Boissy et al., 2011; Edwards, 2015; Papatryphon et al., 2004).

Thus, a major challenge for aquaculture is to find new practices to make its development more environmentally friendly. The main way to decrease environmental impacts of aquaculture is to improve feed efficiency and growth performance. However, as feed contributes greatly to the environmental impacts of aquaculture production, new feed-formulation strategies must be implemented. As environmental impacts of feeds are strongly determined by their ingredients, the potential exists to decrease environmental impacts of aquaculture by formulating feeds with lower environmental impacts.

Currently, feeds are formulated to fulfil fish nutritional requirements (NRC, 2011) while minimising feed cost and meeting incorporation limits for certain feed ingredients, such as fishmeal and fish oil. Several studies have considered environmental impacts in feed formulation (Mackenzie et al., 2016; Nguyen et al., 2012; Pomar et al., 2007). They found that using a single environmental objective leads to pollution swapping between impacts. For example, Boissy et al. (2011) studied how to reduce the NPPU of salmonid production by replacing fish oil and fishmeal with raw plant ingredients, but doing so increased other impacts, especially land occupation. Mackenzie et al. (2016) minimised several environmental impacts of pig feed at the same time but without considering ingredient cost, which led to a drastic increase in feed cost.

Hence, to reduce the environmental footprint of feed, the formulation algorithm should consider the cost and environmental impacts of raw ingredients simultaneously. Recently, Garcia-Launay et al. (2018) developed a multiobjective (MO) formulation that uses the constraints of least-cost formulation (nutrients and feed-ingredient incorporation rates) and calculates a MO function that includes both feed cost and environmental impact indicators obtained by life cycle assessment (LCA) (i.e. climate change, non-renewable energy use, P demand, land occupation). Nevertheless, fish growth is strongly influenced by the type of raw feed ingredients. For example, replacing all fishmeal and fish oil with raw plant ingredients decreased rainbow trout growth by 30% (Lazzarotto et al., 2018).

The objectives of the present study were to design an eco-friendly diet and assess its nutritional effectiveness for rainbow trout. To this end, this diet was designed using MO formulation and compared to a commercial-type diet in a digestibility experiment and a growth experiment. Finally, results of the experiments were used in a life cycle approach to estimate environmental impacts of 1 kg of trout body-weight gain.

2 Materials and methods

Digestibility and growth experiments were performed to compare the nutritional value of the eco-formulated diet (ECO-diet) to that of a commercial-type diet (C-diet), used as a control. The digestibility experiment assessed apparent digestibility coefficients (ADC) of the two diets' protein, lipid, and energy, whereas the growth experiment evaluated effects of the two diets on the growth performance, whole-body composition, and nutrient use of rainbow trout after 12 weeks of feeding.

2.1 Feed formulation

Two isonitrogenous, isolipidic, and isoenergetic diets were designed to fulfil the nutritional requirements of rainbow trout (NRC, 2011). The C-diet was formulated to minimise cost while containing a fixed level of fishmeal and fish oil using Allix® software, whereas the ECO-diet was formulated using the MO formulation algorithm developed by Garcia-Launay et al. (2018) and updated by de Quelen et al. (2021). Information on the nutritional composition of feed ingredients was obtained from the Feed Ingredients Composition database included in the International Aquaculture Feed Formulation Database (IAFFD, 2019). Ingredient prices (as of October 2019) were provided by feed company employees (pers. comm.). See the supplementary materials for details.

Environmental impacts of feed ingredients were taken from the ECOALIM dataset (v7, accessed 1 Sep 2019) as included in the AGRIBALYSE database (Wilfart et al., 2016), except for NPPU and water dependence, which were calculated for each feed ingredient in the context of this project. The impact categories considered were climate change including land-use change (CC, in kg CO_2-eq) as calculated by the International Reference Life Cycle Data (ILCD) system (JRC, 2012), ILCD acidification potential (AC, in mol H^+-eq), eutrophication potential (EU, in kg PO_4^{3-}-eq) from the Institute of Environmental Sciences (CML), Cumulative Energy Demand 1.8 (CED, in MJ), non-renewable energy demand (NRE, in MJ), CML land occupation (LO, in m^2year), P demand (PD, in kg P) from Wilfart et al. (2016), NPPU (in kg C) from Papatryphon et al. (2004) and water dependence (WD, in m^3) from Boissy et al. (2011). All impacts from the ECOALIM dataset were considered to be those at the storage-organisation gate or factory gate.

The MO algorithm is based on linear programming (Simplex algorithm) using the Python v3.7 programming language (http://www.python.org) and the PulP library for linear programming. The objective function (Eq. 1) included total environmental impacts calculated using LCA (i.e. CC, AC, EU, NRE, LO, PD, NPPU and WD) under a varying ϵ constraint of maximum feed cost (Eq. 2). Constraints were added for the environmental impacts of the ECO-feed to ensure that no impact would increase by more than 5% relative to the same impact of the C-feed (Eq. 3). Constraints were also applied to nutritional composition and the incorporation rates of feed ingredients (Eq. 4).

$$f(x) = \sum_{i \in I} coef_i \frac{Impact_i{}^t x - Min_i}{Ref_{impact_i} - Min_i} \quad \text{Equation 1}$$

$$c^t x \leq \epsilon \qquad \boldsymbol{\epsilon} = \{Ref_{price}, \ldots, Max_{price}\} \quad \text{Equation 2}$$

$$Impact_i{}^t x \leq 1.05 \times Ref_{impact_i} \quad \text{Equation 3}$$

$$\begin{pmatrix} q_{min} \\ n_{min} \\ 1 \end{pmatrix} \leq \begin{pmatrix} Q \\ N \\ 1^t \end{pmatrix} x \leq \begin{pmatrix} q_{max} \\ n_{max} \\ 1 \end{pmatrix} \quad \text{Equation 4}$$

i = [CC, AC, EU, NRE, LO, PD, NPPU , WD]

where $Impact_i$ is the vector of impact i of feed ingredients; c is the matrix of feed-ingredient prices; Max_{price} is the price of feed when formulating without constraint ϵ; Min_i is the level of impact i when formulated at the lowest impact i; x is the matrix of incorporation rates of feed ingredients (decision variables); $Ref_{impacti}$ and Ref_{price} are the impact i and price of least-cost feed formulation, respectively; $coef_i$ is the weighting factor of impact i, with $coef_{AC}$ and $coef_{EU}$ equal to 1, $coef_{LO}$, $coef_{PD}$, $coef_{NRE}$, $coef_{WD}$, and $coef_{NPPU}$ equal to 2, and $coef_{CC}$ equal to 3; q_{min} and q_{max} are the minimum and maximum incorporation constraints on feed ingredients, respectively; and n_{min} and n_{max} are the lower and upper limits, respectively, of the nutritional constraints applied to the feed.

The best feed formulation was the one whose marginal decrease in the environmental index ($Impact_i^{tx}/Ref_{impacti}$) became smaller than the marginal increase in the cost index (c^tx/Ref_{price}).

2.2 Experiments

Both experiments were performed at the French National Research Institute for Agriculture, Food and Environment (INRAE) experimental fish farm facility (IE Numea, 2021), authorised for animal experimentation by the French Veterinary Service (A64-495-1 and A40-228-1). The experiments were conducted in strict accordance with European Union legal frameworks concerning the protection of animals used for scientific research (Directive 2010/63/EU) and according to the National Guidelines for Animal Care of the French Ministry of Research (decree no. 2013-118, 02 Jan 2013). In agreement with the ethical committee (C2EA-73), the experiment did not need approval, as it involved only standard rearing practices, with all diets used formulated to meet the nutritional requirements (NRC2011) of rainbow trout. The staff of the facility received training and personal authorisation to perform the experiments.

2.2.1 Digestibility experiment

Six groups of 12 fish (mean body weight of 120 g) were placed in 60 L cylindro-conical tanks supplied with well-aerated water at a regulated flow rate of 4 L/min. Three replicate groups of fish were hand-fed the C-diet or ECO-diet, both of which contained Y_2O_3 as inert marker, twice a day until visual satiation. Fish were allowed to adapt to the diets for 10 days before faeces began to be collected using an automatic, continuous sieving system, as described by Choubert et al. (1982). Rapid recovery of faeces from the water (5-10 sec after excretion) without manipulating the fish limits nutrient loss through leaching. The faeces were collected daily for two weeks, pooled per tank and stored at −20°C. Freeze-dried samples of the pooled faeces were used for further biochemical analyses. The ADC (%) of dry matter, protein, lipids, starch, and energy were calculated as:

$$ADC\ dry\ matter = 100 - \left\lfloor 100 \times \left(\frac{\%\ of\ Y_2O_3\ in\ the\ diet}{\%\ of\ Y_2O_3\ in\ the\ faeces}\right)\right\rfloor \qquad \text{Equation 5}$$

$$ADC\ of\ X\ (\%) = 100 - \left[100 \times \left(\frac{\%\ of\ X\ in\ the\ faeces}{\%\ of X\ in\ the\ diet}\right) \times \left(\frac{\%\ of\ Y_2O_3\ in\ the\ diet}{\%\ of\ Y_2O_3\ in\ the\ faeces}\right)\right]$$ Equation 6

where X is the nutrient considered (i.e. protein, lipids, or starch) or energy.

2.2.3 *In vivo* growth experiment

Rainbow trout (mean initial body weight of 61.23 ± 1.38 g) were randomly distributed among six 120 L tanks (27 fish per tank) supplied with flow-through natural spring freshwater at 17 ± 1°C. Tanks were exposed to the natural photoperiod. Each diet (C-diet or ECO-diet) was allocated to three tanks, and fish were hand-fed twice daily until visual satiety for 12 weeks. Every 3 weeks, fish in each tank were group-weighed and counted, and the amount of feed distributed per tank was recorded to monitor growth performance and feed intake. Mean fish body weight per tank was calculated by dividing total biomass in the tank by the number of fish in the tank. Feed conversion ratio (FCR), specific growth rate (SGR), and daily feed intake (DFI) were calculated as follows:

$$SGR = \frac{(\ln fBW - \ln iBW)}{d} \times 100$$ Equation 7

$$FCR = \frac{WI}{fB - iB}$$ Equation 8

$$DFI = \frac{WI}{\frac{fBW + iBW}{2} \times d}$$ Equation 9

where *iB* and *fB* are initial and final tank biomass (g), respectively; *iBW* and *fBW* are initial and final body weight (g), respectively; and WI is wet intake, the total amount of wet feed distributed during the experiment (g).

At the end of the experiment, 3 fish per tank were sedated by immersing them in a 10 mg/L benzocaine solution and then euthanised by immersing them in a 60 mg/L benzocaine solution (anaesthetic overdose) for 3 minutes. They were then frozen at -20 °C until body composition analysis.

2.2.4 Biochemical composition of diets, whole body and faeces

Proximate analysis of the experimental diets and whole body was determined according to AOAC (2006) as follows: dry matter was analysed by drying samples to constant weight at 105°C for 24 h. Crude protein was determined using the Kjeldahl method after acid digestion and estimated by multiplying the N content by 6.25. Crude lipid was quantified by petroleum diethyl ether extraction using the Soxhlet method. Gross energy content was determined in an adiabatic bomb calorimeter (IKA®). Ash content was calculated after combustion in a muffle furnace at 550°C for 16 h. The total lipid content of feed and whole fish was quantified gravimetrically after extraction by dichloromethane/methanol (2:1, v/v), containing 0.01% of butylated hydroxytoluene as an antioxidant (Folch et al., 1957).

2.2.5 Nutrient and energy use

Nutrient and energy intake (g or kJ.kg $BW^{-1}.day^{-1}$), gain (g or kJ.kg $BW^{-1}.day^{-1}$), and retention (%) were calculated as follows:

$$Nutrient\ intake = DFI \times \%\ Nutrient\ feed\ content \qquad \text{Equation 10}$$

$$Nutrient\ gain = \frac{\left(N_{fFish} \times fBW - N_{iFish} \times iBW\right)}{\frac{(fBW + iBW)}{2} \times d \times 1000} \qquad \text{Equation 11}$$

$$Nutrient\ retention = \frac{Nutrient\ gain}{Nutrient\ intake} \times 100 \qquad \text{Equation 12}$$

where *iBW* and *fBW* are initial and final body weight (g), respectively; d is the duration of the experiment (84 days); and N_{fFish} and N_{iFish} are the initial and final nutrient (N) or energy content of the fish, respectively, expressed in g/100 g wet matter.

2.3 Life cycle assessment

Potential environmental impacts were estimated for the two diets from cradle to the experimental facility gate for the 12 weeks of the growth experiment. Feed consumption, water use, oxygen demand, and energy consumption were recorded or calculated during this period. N, P, and solid emissions were estimated as the amount of nutrients provided to fish in the feed minus the amount assimilated as fish weight gain. For each feed ingredient, the distance from the feed plant to the experimental facility was calculated using Google Maps. The energy used to pelletise feed was recorded for both diets.

The potential impacts considered the farm level were the same as those used to assess the feed level. CC including land-use change is the potential impact of greenhouse gas emissions on heat-radiation absorption in the atmosphere. It was calculated according to 100-year-horizon global warming potentials (CC, kg CO_2-equivalents) of the International Panel on Climate Change (IPCC, 2006) as calculated by the ILCD (JRC, 2012). Acidification (AC, expressed in mol H^+-eq/kg) refers to negative effects on soils, ground and surface water, and ecosystems from acidifying pollutants. It was calculated using average European acidification potential factors from ILCD (JRC, 2012). Eutrophication (EU, kg PO_4^{3-}equivalents) refers to potential impacts of high levels of nutrients, especially N and P, in the environment. It was calculated using factors developed by the CML (Guinee et al., 2002) and adding the theoretical oxygen demand caused by solid waste from fish farms. P demand (PD, expressed in kg P) from Wilfart et al. (2016) includes all P and phosphate inputs throughout the life cycle. LO (m^2yr) refers to human use of land that delays natural restoration of land quality during the period of occupation (Guinee et al., 2002). CED (MJ) refers to the consumption of all energy sources used, and non-renewable energy demand (MJ) refers to the consumption of energy from non-renewable sources used. They were obtained from CED v1.8. NPPU (kg carbon) refers to the amount of net primary production (biomass produced by photosynthesis) required as a biotic resource input that cannot be used for other purposes. It was calculated according to Papatryphon et al. (2004). Water dependence (WD, m^3) equals to the evaporative blue water or the volume of water in ground and surface water bodies available for abstraction that is not immediately reusable used by the system (Boissy et al., 2011).

As the study aimed at comparing the two diets in a growth experiment, results were expressed per 1 kg of body-weight gain. Emissions and impacts were calculated using SimaPro® software v8.3.0.0 (PRé Consultants, Amersfoort, The Netherlands), with the attributional database ecoinvent® v3 and AGRIBALYSE® database including the ECOALIM dataset (Wilfart et al., 2016) for background data.

2.5 Statistical analysis

Statistical analyses were performed using R software (v4.01, https://www.R-project.org/). Results were expressed as mean ± 1 standard deviation. The normality of the residuals and homogeneity of the variances were checked using a Shapiro-Wilk test and Bartlett test, respectively. Then, data were tested by one-factor analysis of variance (ANOVA) to measure effects of diets on growth-performance and body-composition parameters, as well as ADC, and environmental impacts per kg of body weight gain. A Kruskal-Wallis test was applied to non-normal data. When a significant difference was observed, Tukey’s range test was applied to compare least-square means. For all statistical analyses, the significance level was set at 0.05.

A mixed linear regression model with the tank as the random effect was used to determine effects of the diet, duration of the experiment, and their interaction on trout growth using the *lmer* function of the *lme4* package of R. The regression included mean body weight, duration of the experiment, and the mean initial body weight in each tank following the model:

$$BW_{ij} = (A + a_i) \times Duration_{ij} + B + b_i + e_{ij} \qquad \text{Equation 13}$$

where BW_{ij} is the mean body weight in tank i at time j, $Duration_{ij}$ is the duration of the experiment (in days) in tank I at time i, B is the mean initial body weight (g), b_i is the effect of tank i on BW, A is the mean growth rate, a_i is the effect of tank i on A, and e_{ij} are random errors.

3 Results

3.1 Feed formulas

The mean composition of ingredients, nutritional content, and environmental impacts differed between the two diets (Table 1). The ECO-diet had more ingredients (23) than the C-diet (16). Although the diets were isonitrogenous, isolipidic, and isoenergetic, the ECO-diet contained 20% more starch. Compared to the C-diet, MO formulation halved the percentage of fishmeal and fish oil used in the ECO-diet and introduced poultry by-products (mainly feather meal, poultry blood meal, and poultry oil), soy lecithin, and unconventional ingredients (e.g. yeast, potato protein concentrate, roasted guar meal, dehulled pea flour). The ECO-diet had 8.5 times as much wheat as the C-diet, contained no faba bean or gluten meals, and had its soya bean meal replaced by rapeseed meal. In the ECO-diet, only the inclusion levels of pea protein concentrate and L-lysine. HCl reached one of the constraints. All the other inclusion levels in the ECO-diet, including those of fish meal and fish oil, resulted from the environmental objective under price constraint.

MO formulation changed the contribution of ingredients to the protein, lipid, and starch contents of the feed greatly (Figure 1). While soy protein concentrate (38%), fishmeal (25%), faba bean (11%), and corn gluten (8%) contributed the most to the protein content of the C-diet, pea protein concentrate (36%) became the major protein contributor to the ECO-diet, followed by hydrolysed feather meal (27%), fishmeal (11%), and rapeseed meal (6%). Lipids in the C-diet were provided by rapeseed oil (61%) and fish oil (30%), both of which were halved in the ECO-diet (-32 and -17 percentage points, respectively) to the benefit of soy lecithin (24% of lipids) and poultry oil (16% of lipids). Most of the starch was provided by faba bean (76%) in the C-diet, followed by wheat (13%) and gluten (10%), whereas nearly all of the starch (95%) came from wheat in the ECO-diet.

MO formulation decreased environmental impacts by 21-57% depending on the impact considered: compared to the C-diet, the ECO-diet decreased CC by 46%, NRE by 57%, AC by 30%, EU by 35%, LO by 24%, NPPU by 44%, WD by 44%, and PD by 21%. This reduction in the environmental footprint was associated with a decrease in feed cost of 8%.

Table 1. Composition of the experimental diets (pellets)

	C-diet	ECO-diet
Ingredient, %		
Wheat	2.00	17.31
Faba bean	17.01	-
Fishmeal	16.01	7.24
Fish oil	6.53	3.61
Wheat gluten meal	2.50	-
Corn gluten meal	6.00	-
Soya bean meal	6.10	-
Feather meal, hydrolysed	-	13.99
Rapeseed meal	-	7.94
Rapeseed oil	13.19	6.82
Poultry blood meal	-	1.59
Poultry oil	-	3.55
Soy lecithin	-	5.76
Dehulled pea flour	-	0.5316
Pea protein concentrate	25.01	20.00
Potato protein concentrate	-	0.01
Linseed oil	-	0.02
Roasted guar meal	-	2.97
L-lysine HCl	0.60	0.4
DL-methionine	0.60	0.34
L-threonine	-	0.2
Yeast	-	1.82
Dicalcium phosphate	0.10	1.5
Astaxanthin	0.03	0.03
Shrimp hydrolysate	2.00	2.00
Mineral premix[1]	1.16	1.37
Vitamin premix[2]	1.16	1.00
Number of ingredients	16	23
Chemical composition, g/kg		
Dry matter[3]	966.40	973.40
Crude protein[3,4]	473.70	476.70

Crude lipid[3,4]	237.00	237.85
Starch[3,4]	91.50	111.10
Ash[3,4]	61.65	71.85
GE, kJ/g dry matter[3,4]	25.72	24.59
Environmental impacts of diets per kg of feed[5]		
CC (kg CO_2-eq)	1.387	0.751
NRE (MJ)	14.851	8.547
AC (molc H^+-eq)	0.017	0.012
EU (kg PO_4^{3-}-eq)	0.007	0.00458
LO (m^2year)	1.625	1.240
NPPU (kg C)	21.593	12.150
WD (m^3)	10.321	5.759
PD (kg P)	0.007	0.00556
Price (€/t)	1276.90	1171.50

[1]Provided per 100 g of premix: calcium hydrogen phosphate 49 478 mg, calcium carbonate 21 500 mg, sodium chloride 4000 mg, potassium chloride 9000 mg, magnesium oxide 12 400 mg, iron sulphate 2000 mg, zinc sulphate 900 mg, manganese sulphate 300 mg, copper sulphate 300 mg, cobalt chloride 2 mg, potassium iodide 15 mg, selenite sodium 5 mg, fluoride sodium 100 mg.

[2]Provided per 100 g of premix: vitamin A 500 000 IU, vitamin D3 250000 IU, vitamin E 5 00 mg, vitamin C 1429 mg, vitamin B1 10 mg, vitamin B2 50 mg, vitamin B3 100 mg, vitamin B5 200 mg, vitamin B6 30 mg, vitamin B7 3000 mg, vitamin B8 100 mg, vitamin B9 10 mg, vitamin B12 100 mg, vitamin K3 200 mg, folic acid 10 mg, biotin 100 mg, choline chloride 16 700 mg, cellulose 76 921mg.

[3]Analysed values

[4]Expressed per kg of dry matter

[5]CC = climate change, NRE = non-renewable energy demand, AC = acidification, EU = eutrophication, LO = land occupation, NPPU = net primary production demand, WD = water dependence, PD = P demand.

3.2 Animal performance

3.2.1 Feed digestibility

For the ECO-diet, MO formulation significantly decreased the ADC of lipid, energy, and ash (by 1.6%, 2%, and 13.6%, respectively) but increased the ADC of starch by 5.5% (Table 2).

Table 2. Apparent digestibility coefficients (%) of components of the experimental diets

	C-diet		ECO-diet		
Component	*Mean*	*SD*	*Mean*	*SD*	*P*
Protein	91.7	0.23	91.0	0.17	0.08
Lipid	95.6	0.27	94.0	0.08	0.0003
Starch	92.5	0.48	97.7	0.32	0.0003
Energy	89.1	0.34	87.3	0.29	0.02
Ash	44.9	1.36	38.8	0.30	0.04

3.2.2 Growth performance

Only certain indicators of growth performance and nutrient use by the rainbow trout after 12 weeks of feeding differed by feed (Table 3). At the end of the growth experiment, body weight and SGR did not differ significantly between diets, but analysis of the growth curves revealed a significant interaction between diet and time ($p = 0.0005$) (Figure 2). FCR were similar, but DFI was significantly higher (7%) for fish fed the C-diet. Feed formulation did not influence the body composition of fish (Table 4). Fish fed the ECO-diet showed lower protein gain due to lower protein intake, as the two diets had similar protein retention. Feed formulation did not influence lipid or energy use.

299 Table 3. Growth performance of rainbow trout fed the experimental diets

Characteristic[1]	C-diet Mean	C-diet SD	ECO-diet Mean	ECO-diet SD	*P*
Initial BW, g	61.7	1.54	61.2	1.54	0.71
Final BW, g	240.7	17.32	210.4	13.72	0.08
SGR, %	1.6	0.06	1.5	0.08	0.07
DFI, g kg^{-1} day^{-1}	16.2	0.03	15.0	0.02	0.009
FCR	1.2	0.02	1.2	0.05	0.93
Nutrient intake					
Protein, g kg^{-1} day^{-1}	7.4	0.08	6.9	0.06	0.01
Lipid, g kg^{-1} day^{-1}	3.6	0.04	3.5	0.03	0.09
Energy, kJ kg^{-1} day^{-1}	4.0	0.04	3.6	0.03	0.003
Nutrient gain					
Protein, g kg^{-1} day^{-1}	2.4	0.07	2.1	0.06	0.04
Lipid, g kg^{-1} day^{-1}	2.7	0.06	2.6	0.06	0.60
Energy, kJ kg^{-1} day^{-1}	1.6	0.03	1.6	0.03	0.17
Nutrient retention					
Protein, %	32.1	0.93	30.4	1.09	0.31
Lipid, %	74.3	0.81	75.4	2.21	0.67
Energy, %	41.1	0.47	43.5	1.25	0.20

300 [1]BW= body weight, SGR= specific growth rate, DFI= daily feed intake, FCR= feed conversion ratio

301

302 Table 4. Body composition at the end of the experiment of rainbow trout fed the experimental diets

Body composition[1]	C-diet Mean	C-diet SD	ECO-diet Mean	ECO-diet SD	*P*
Dry matter	34.0	0.42	34.1	0.14	0.66
Protein, % BW	16.6	0.36	16.0	0.21	0.09
Lipid, % BW	15.5	0.45	15.8	0.30	0.39
Ash, % BW	2. 19	0.02	2.2	0.03	0.05
Energy, kJ.g^{-1}	10.2	0.15	10.2	0.18	0.86

303 [1]BW= body weight

3.3 LCA impacts per kg of fish body weight gain

The environmental impacts calculated from the growth experiment and expressed per kg of body weight gain (Table 5) or as a percentage of the highest impact by category (Figure 3) varied. Except for EU (mean of both diets: 0.049 kg PO_4^{3-}-eq), compared to the C-diet, the ECO-diet decreased CC, NPPU, and WD by 44%; NRE by 38%; AC by 27%; LO by 23%; and PD by 24% per kg of body weight gain.

Table 5. Environmental impacts per kg of fish body weight gain of rainbow trout fed the experimental diets

	C-diet		**ECO-diet**		
Impact category[1]	*Mean*	*SD*	*Mean*	*SD*	*P*
CC (kg CO_2-eq)	1.76	0.039	0.99	0.058	0.0004
NRE (MJ)	21.24	0.467	13.09	0.0762	0.0001
AC (molc H^+-eq)	0.021	0.0004	0.015	0.0008	0.0003
EU (kg PO_4^{3-}-eq)	0.051	0.0014	0.047	0.0034	0.16
LO (m^2year)	1.88	0.04	1.44	0.08	0.001
NPPU (kg C)	24.81	0.535	14.01	0.807	0.05
WD (m^3)	11.86	0.256	6.64	0.383	0.05
PD (g P)	0.0084	0.0002	0.0064	0.0004	0.001

[1]CC = climate change, NRE = non-renewable energy demand, AC = acidification, EU = eutrophication, LO = land occupation, NPPU = net primary production demand, WD = water dependence, PD = P demand.

Feeds contributed the most to all environmental impacts at the experimental facility gate (Figure 4), except for EU, for which rearing activities predominated (85% and 90% for the C-diet and ECO-diet, respectively). Transportation of feed ingredients to the experimental facility contributed 19% and 23% of NRE, 8% and 10% of CC, and 3% and 3.5% of AC for the C-diet and ECO-diet, respectively. Feed was the only contributor to NPPU, PD, and WD.

4 Discussion

The objectives of the study were to evaluate the nutritional effectiveness of eco-formulated diets for juvenile rainbow trout and to estimate consequences of using these diets on the environmental footprint of increasing body weight by 1 kg.

4.1 Consequences of MO formulation on the composition and environmental impacts of diets

Unlike current commercial aquaculture feed-formulation practices, which aim for trade-offs among nutritional constraints, cost, and target levels of fishmeal and fish oil incorporation, MO formulation aims for trade-offs among nutritional constraints, cost, and environmental impacts of ingredients. With MO formulation, reducing CC was one of our main goals, according to priorities of the United Nations Paris Agreement (United Nations, 2015). Thus, the weighting factor was 3 for CC; 2 for NRE, LO, PD, WD, and NPPU; and 1 for EU and AC. Consequently, the ECO-diet had lower impacts than the commercial-type C-diet, with the main gains observed for CC (-46%), NPPU, and WD (-44%), and NRE (-42%).

MO formulation changed the composition of the rainbow trout diet drastically, increasing the number of raw ingredients from 16 to 23. We observed sets of replacement between categories of ingredients. First, MO formulation significantly decreased fishmeal and fish oil use by ca. 50%. Fishmeal and fish oil have high NPPU, WD, NRE, and CC impacts, which likely contributed to the decrease in these impacts in the ECO-diet. Likewise, soy products (soy protein concentrate and soya bean meal), which contribute strongly to CC due to their Brazilian origin (i.e. some production after deforestation) contributed 40% of the CC impact of the C-diet. In the ECO-diet, soya bean meal was replaced by rapeseed meal (1.11 vs. 0.347 kg CO_2-eq/kg, respectively). The lower cost of rapeseed meal than soya bean meal likely favoured its incorporation in the ECO-diet (225 vs 400 €/t, respectively). The ECO-diet contained no glutens, which have high impacts per kg for almost all categories (especially CC and NRE) and high cost. Although they were introduced in small percentages in the C-diet (6.0% and 2.5% for corn and wheat gluten, respectively), they were always among the five raw ingredients with the highest impacts per kg regardless of the impact category, as wheat and corn glutens are highly processed co-products from the starch industry. Processed animal co-products (poultry feather and blood meals) appeared in the ECO-diet due to their lower CC and NRE impacts. Faba bean was not included in the ECO-diet because of its high contribution to LO (24.3%), just below that of rapeseed oil (46.6%). Faba bean is used in aquaculture feeds because it provides starch (37.3%), which is essential for extrusion during production, and has a high crude-protein content (26.8%); however, as a legume, it contains relatively little methionine. In the ECO-diet, starch is provided mainly by wheat, which has lower environmental impacts, whereas protein is provided mainly by animal co-products (i.e. feather and blood meals), which have a higher protein content, higher methionine concentration, and lower CC impact. As a protein source, pea protein concentrate appears in the ECO-diet due to its high protein content (77%) and low environmental impacts. Despite its high incorporation rate (20%), it contributed little to impacts of the ECO-diet. In the C-diet, rapeseed oil contributed the most to LO (46.6%), EU (26.1%), and AC (27.3%) and second-most to CC (15.1%) after soy protein concentrate, which likely explains why it was not included in the ECO-diet.

In the context of fishmeal and fish oil replacement, there is now great interest in new feed ingredients, such as insects and yeast as sources of protein (Agboola et al., 2021; Henry et al., 2015; Roques et al., 2018) and microalgae as a source of n-3 long-chain polyunsaturated fatty acids (i.e. EPA and DHA) (Sprague et al., 2017). Although these ingredients were included in the list of those available for MO formulation, only yeast was ultimately included in the ECO-diet but at a low incorporation rate (1.8%). Insect meal (Thévenot et al., 2018), whose CC impact is 3 times as high as that of soya bean meal (3.5 vs. 1.1 kg CO_2 per kg, respectively), was not included in the ECO-diet. DHA-rich microalgae were not included in the ECO-diet either. Even though their ability to replace the DHA provided by fish oil has been clearly demonstrated for rainbow trout (Richard et al., 2021), their high price automatically excludes them from lower-cost formulations.

Unlike other studies, in which MO formulation was applied to pig and poultry feeds (Meda et al., 2021), the ECO-diet was less expensive than the commercial-type C-diet (-8%). Commercial diets for pigs and poultry are obtained using least-cost formulation. In our study, the C-diet was formulated as closely as possible according to current commercial practices, which consider both least cost and pre-set percentages of fishmeal and fish oil. This approach increased costs. The still high content of fishmeal and fish oil in the C-diet thus explains why the C-diet cost more than the ECO-diet, which was formulated without constraints on these two raw ingredients.

4.2 Effects on animal performances

At the end of the experiment, the mean body weight of fish did not differ between diets, but their growth curves had begun to diverge. Based on these growth curves, fish fed the C-diet would require 5 more days of rearing to reach the size of a human meal portion (250 g), while those fed the ECO-diet would require 20 more days. These results suggest better growth performance for fish fed the C-diet than those fed the ECO-diet, as illustrated by the former's higher protein gain. The decrease in growth performance in the long term is probably mainly related to the decrease in feed intake. The lower protein and fat digestibilities of the ECO-diet could have also contributed to the trend of lower growth but to lower extent compared to feed intake because nutrients ADC could be considered as high in both diets. In any case, this decrease cannot be associated with a decrease in feed efficiency, because the FCR did not differ between the two diets.

Previous studies of Atlantic salmon (Torstensen et al., 2008) and seabream (de Francesco et al., 2007) have shown that a decrease in fishmeal and fish oil in diets leads to a decrease in fish feed intake. This decrease in feed intake was also observed in rainbow trout when fishmeal and fish oil were completely replaced by raw plant ingredients (Panserat et al., 2009). In our study, the decrease in feed intake in trout fed the ECO-diet could have resulted from the decrease in the percentages of fishmeal and fish oil (55% and 45% less, respectively, than in the C diet) and thus more plant ingredients. In addition, a diet with a high percentage of plant ingredients may have a high concentration of antinutritional factors that negatively influence fish feeding behaviour (Krogdahl et al., 2010). Furthermore, Roy et al. (2020) highlighted that rainbow trout prefer a diet with high percentages of n-3 long-chain polyunsaturated fatty acids, especially EPA and DHA. The ECO-diet contained lower percentages of EPA and DHA, which can contribute to a decrease in feed intake.

4.3 Effect on environmental performances

Overall, using MO formulation to decrease environmental impacts of feed made it possible to significantly decrease the environmental footprint of the fish farming system studied per kg of body weight gain. The decrease in impacts was lower at the farm level than that at the feed level, especially for EU and, to a lesser extent, NRE and CC. In contrast, the feed and farm levels had similar decreases for NPPU, WD, LO, AC, and PD. Differences between the feed and farm levels have been observed for other species (pigs and poultry) for which MO formulation was applied (de Quelen et al., 2021; Meda et al., 2021), but they were observed for all environmental impacts, not only for some of them as in the present study. The farm level included emissions due to biological processes of fish but also those to the functioning of the rearing facility. NPPU and PD depended only on the feed, which explains why their decrease was the same for both levels. In the experimental system used, the fish were reared in raceways in which water was taken from a river, continually flowed through the system, and then returned to the river. Because the water could thus be reused, it was not included in water use in life cycle inventory, as recommended by Boissy et al. (2011). Consequently, the decrease in WD at the farm level was the same as that at the feed level. The experiment was performed in 60 L tanks, which contributed less to LO than the areas used to produce the crops that provided feed ingredients. In contrast, the decrease in AC was the same at the farm level, unlike the increases in AC observed for pig and poultry systems. Because ammonia (NH_3) contributes to AC, NH_3 emissions from manure management in terrestrial livestock systems are included in the life cycle inventory, and they influence AC at the farm level strongly. In contrast, due to its very low concentration in water, no NH_3 emissions to the air compartment was accounted for the raceway system in this study, so AC differed little between the feed and farm scales. In aquaculture systems, only transport-related gaseous emissions are included. For EU, the feed and farm levels differed by less than 8%. The contribution analysis showed that rearing activities were responsible for 85-90% of this impact, which is slightly higher than that generally observed in the literature for salmonids (ca. 73-87%) (Boissy et al., 2011; Wilfart et al., 2013). The decreases in CC and NRE were ca. 2 and 4 percentage points lower, respectively, at the farm level than the feed level, due mainly to the transport of raw ingredients, as indicated by the contribution analysis. In this study, MO formulation was applied according to the LCA boundaries of the ECOALIM database (i.e. the factory gate for processed ingredients and the storage-organisation gate for raw plant ingredients), without considering the actual distance between the production site of the ingredients and the experimental facility, where the feeds were manufactured just before the experiment. Some suppliers of raw ingredients were identified before using the latter to formulate feeds at the facility, distances from google map estimations were used for raw ingredients in the LCA at the experimental facility gate. Nevertheless, additional experiments under commercial production conditions (for feed and rearing) will be necessary to validate the environmental gains that can be obtained with MO formulation, both in terms of animal growth and the life cycle inventory of inputs and infrastructure.

5. Conclusions and perspectives

MO formulation seems to be a promising tool to reduce environmental impacts of trout farming without necessarily decreasing their growth performance. Nevertheless, some points deserve further investigation. For example, because growth performance could decrease over the long term, the rearing period should be extended to validate the performance of these diets in portion-size trout or to evaluate them when producing large trout intended for smoked fillets, which requires longer rearing periods. These feeds should also be tested with other production stages such as fry, which are sensitive to the composition of their feed, but also on broodstock, to assess consequences of these feeds on egg production and quality, and on environmental impacts of these production systems. Similarly, effects of these feeds at the farm level must be studied to assess potential environmental gains for commercial production.

Acknowledgements

The authors thank Phileo by Lesaffre for providing the yeast used in the project and the technical staff of the NuMeA experimental fish farm facility for their help with experiment preparation and data collection.

Funding source

This project was supported by a grant from the PHASE Division of the French National Research Institute for Agriculture, Food and Environment (INRAE).

Credit role

Study design: AW, SSC, FGL, FT
Data curation: SSC, ES, AW, FT, FGL
Funding acquisition: AW
Animal experiments: FT, PA
LCA model and assessment: AW
Feed formulation: FGL, FT, AW
Statistical analysis: ES, FGL, SSC, AW
Project administration and supervision: AW
Writing - original draft: AW, SSC
Writing - review & editing: AW, SSC, FGL, FT, ES, PA

References

Agboola, J.O., Øverland, M., Skrede, A., Hansen, J.Ø., 2021. Yeast as major protein-rich ingredient in aquafeeds: a review of the implications for aquaculture production. Rev. Aquacult. 13, 949-970. https://doi.org/10.1111/raq.12507.

AOAC, 2006. Official Methods of Analysis of AOAC International. Association of Official Analytical Chemists, Washington, USA.

Aubin, J., Papatryphon, E., van der Werf, H.M.G., Chatzifotis, S., 2009. Assessment of the environmental impact of carnivorous finfish production systems using life cycle assessment. J. Cleaner Prod. 17, 354-361. https://doi.org/10.1016/j.jclepro.2008.08.008.

Bohnes, F.A., Hauschild, M.Z., Schlundt, J., Laurent, A., 2019. Life cycle assessments of aquaculture systems: a critical review of reported findings with recommendations for policy and system development. Rev. Aquacult. 11, 1061-1079. https://doi.org/10.1111/raq.12280.

Boissy, J., Aubin, J., Drissi, A., van der Werf, H.M.G., Bell, G.J., Kaushik, S.J., 2011. Environmental impacts of plant-based salmonid diets at feed and farm scales. Aquaculture. 321, 61-70. https://doi.org/10.1016/j.aquaculture.2011.08.033.

Choubert, G., De la Noue, J., Luquet, P., 1982. Digestibility in fish: Improved device for the automatic collection feces. Aquaculture. 29, 185-189. https://doi.org/10.1016/0044-8486(82)90048-5.

de Francesco, M., Parisi, G., Pérez-Sanchez, J., Gomez-Réquini, P., Médale, F., Kaushik, S.J., Mecatti, M., Poli, B.M., 2007. Effect of high-level fishmeal replacement by plant proteins in gilthead sea bream (Sparus aurata) on growth and body/fillet quality traits. Aquacult. Nutr. 13, 361-372. https://doi.org/10.1111/j.1365-2095.2007.00485.x.

de Quelen, F., Brossard, L., Wilfart, A., Dourmad, J.-Y., Garcia-Launay, F., 2021. Eco-Friendly Feed Formulation and On-Farm Feed Production as Ways to Reduce the Environmental Impacts of

Pig Production Without Consequences on Animal Performance. Frontiers in Veterinary Science. 8. https://doi.org/10.3389/fvets.2021.689012.

Edwards, P., 2015. Aquaculture environment interactions: Past, present and likely future trends. Aquaculture. 447, 2-14. https://doi.org/10.1016/j.aquaculture.2015.02.001.

FAO, 2020. The state of world fisheries and aquaculture (SOFIA). Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 244.

Folch, J., Lees, M., Sloane-Stanley, G., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-509.

Garcia-Launay, F., Dusart, L., Espagnol, S., Laisse-Redoux, S., Gaudre, D., Meda, B., Wilfart, A., 2018. Multiobjective formulation is an effective method to reduce environmental impacts of livestock feeds. Brit. J. Nutr. 120, 1298-1309. https://doi.org/10.1017/s0007114518002672.

Guinee, J.B., Gorree, M., Heijungs, R., Huppes, G., Kleijn, R., de Koning, A., van Oers, L., Wegener Sleeswijk, A., Suh, S., Udo de Haes, H.A., 2002. Handbook on Life Cycle Assessment. An Operational Guide to the ISO Standards. Springer, Netherlands.

Henry, M., Gasco, L., Piccolo, G., Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: Past and future. Anim. Feed Sci. Technol. 203, 1-22. https://doi.org/10.1016/j.anifeedsci.2015.03.001.

IAFFD, 2019. Feed Ingredient Composition Database (FICD) v5.2. https://www.iaffd.com (septembre 2019).

IE Numea, 2021. Fish nutrition and aquaculture, INRAE. https://doi.org/10.15454/GPYD-AM38.

IPCC, 2006. IPCC guidelines for national greenhouse gas inventories, volume 4: agriculture, forestry and other land use. . Kanagawa: Institute for Global Environmental Strategies.

JRC, 2012. Characterisation factors of the ILCD recommanded life cycle impact assessment methods. database and supporting information. in: European Commission, J.R.C., Institute for Environment and Sustainability (Ed.). European Commission, Joint Research Centre, Institute for Environment and Sustainability, pp. 31

Krogdahl, Å., Penn, M., Thorsen, J., Refstie, S., Bakke, A.M., 2010. Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. Aquacult. Res. 41, 333-344. https://doi.org/10.1111/j.1365-2109.2009.02426.x.

Lazzarotto, V., Medale, F., Larroquet, L., Corraze, G., 2018. Long-term dietary replacement of fishmeal and fish oil in diets for rainbow trout (Oncorhynchus mykiss): Effects on growth, whole body fatty acids and intestinal and hepatic gene expression. Plos One. 13. https://doi.org/10.1371/journal.pone.0190730.

Mackenzie, S.G., Leinonen, I., Ferguson, N., Kyriazakis, I., 2016. Towards a methodology to formulate sustainable diets for livestock: accounting for environmental impact in diet formulation. Brit. J. Nutr. 115, 1860-1874. https://doi.org/10.1017/s0007114516000763.

Meda, B., Garcia-Launay, F., Dusart, L., Ponchant, P., Espagnol, S., Wilfart, A., 2021. Reducing environmental impacts of feed using multiobjective formulation: What benefits at the farm gate for pig and broiler production? Animal. 15. https://doi.org/10.1016/j.animal.2020.100024.

Nguyen, T.T.H., Bouvarel, I., Ponchant, P., van der Werf, H.M.G., 2012. Using environmental constraints to formulate low-impact poultry feeds. J. Cleaner Prod. 28, 215-224. https://doi.org/10.1016/j.jclepro.2011.06.029.

NRC, 2011. Nutrient requirements of fish and shrimp. National Research Council, Washington (DC), USA.

Ottinger, M., Clauss, K., Kuenzer, C., 2016. Aquaculture: Relevance, distribution, impacts and spatial assessments - A review. Ocean Coastal Manage. 119, 244-266. http://doi.org/10.1016/j.ocecoaman.2015.10.015.

Panserat, S., Hortopan, G.A., Plagnes-Juan, E., Kolditz, C., Lansard, M., Skiba-Cassy, S., Esquerré, D., Geurden, I., Médale, F., Kaushik, S., Corraze, G., 2009. Differential gene expression after total replacement of dietary fishmeal and fish oil by plant products in rainbow trout (Oncorhynchus mykiss) liver. Aquaculture. 294, 123-131. https://doi.org/10.1016/j.aquaculture.2009.05.013.

Papatryphon, E., Petit, J., Kaushik, S.J., van der Werf, H.M.G., 2004. Environmental impact assessment of salmonid feeds using Life Cycle Assessment (LCA). Ambio. 33, 316-323.

Parker, R.W.R., Tyedmers, P.H., 2012. Life Cycle Environmental Impacts of Three Products Derived from Wild-Caught Antarctic Krill (Euphausia superba). Environ. Sci. Technol. 46, 4958-4965. https://doi.org/10.1021/es2040703.

Pelletier, N., Tyedmers, P., Sonesson, U., Scholz, A., Ziegler, F., Flysjo, A., Kruse, S., Cancino, B., Silverman, H., 2009. Not All Salmon Are Created Equal: Life Cycle Assessment (LCA) of Global Salmon Farming Systems. Environ. Sci. Technol. 43, 8730-8736. https://doi.org/10.1021/es9010114.

Pomar, C., Dubeau, F., Letourneau-Montminy, M.P., Boucher, C., Julien, P.O., 2007. Reducing phosphorus concentration in pig diets by adding an environmental objective to the traditional feed formulation algorithm. Livest. Sci. 111, 16-27. https://doi.org/10.1016/j.livsci.2006.11.011.

Richard, N., Costas, B., Machado, M., Fernández-Boo, S., Girons, A., Dias, J., Corraze, G., Terrier, F., Marchand, Y., Skiba-Cassy, S., 2021. Inclusion of a protein-rich yeast fraction in rainbow trout plant-based diet: Consequences on growth performances, flesh fatty acid profile and health-related parameters. Aquaculture. 544, 737132. https://doi.org/10.1016/j.aquaculture.2021.737132.

Roques, S., Deborde, C., Richard, N., Sergent, L., Kurz, F., Skiba-Cassy, S., Fauconneau, B., Moing, A., 2018. Characterizing alternative feeds for rainbow trout (O. mykiss) by 1H NMR metabolomics. Metabolomics. 14, 155. https://doi.org/10.1007/s11306-018-1454-5.

Roy, J., Mercier, Y., Tonnet, L., Burel, C., Lanuque, A., Surget, A., Larroquet, L., Corraze, G., Terrier, F., Panserat, S., Skiba, S., 2020. Rainbow trout prefer diets rich in omega-3 long chain polyunsaturated fatty acids DHA and EPA. Physiol. Behav. 213, 112692. https://doi.org/10.1016/j.physbeh.2019.112692.

Sprague, M., Betancor, M.B., Tocher, D.R., 2017. Microbial and genetically engineered oils as replacements for fish oil in aquaculture feeds. Biotechnol. Lett. 39, 1599-1609. https://doi.org/10.1007/s10529-017-2402-6.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., Haan, C.d., 2006. Livestock's long shadow: environmental issues and options. Food and Agriculture Organization of the United Nations (FAO), Rome Italy, pp. 390.

Thévenot, A., Rivera, J.L., Wilfart, A., Maillard, F., Hassouna, M., Senga-Kiesse, T., Le Féon, S., Aubin, J., 2018. Mealworm meal for animal feed: Environmental assessment and sensitivity analysis to guide future prospects. J. Cleaner Prod. 170, 1260-1267. https://doi.org/10.1016/j.jclepro.2017.09.054.

Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbø, R., Hemre, G.I., Fontanillas, R., Nordgarden, U., Hevrøy, E.M., Olsvik, P., Berntssen, M.H.G., 2008. Novel production of Atlantic salmon (Salmo salar) protein based on combined replacement of fishmeal and fish oil with plant meal and vegetable oil blends. Aquaculture. 285, 193-200. https://doi.org/10.1016/j.aquaculture.2008.08.025.

United Nations, 2015. Paris Agreement, Paris.

Wilfart, A., Prudhomme, J., Blancheton, J.-P., Aubin, J., 2013. LCA and emergy accounting of aquaculture systems: Towards ecological intensification. J. Environ. Manag. 121, 96-109. https://doi.org/10.1016/j.jenvman.2013.01.031.

Wilfart, A., Espagnol, S., Dauguet, S., Tailleur, A., Gac, A., Garcia-Launay, F., 2016. ECOALIM: A Dataset of Environmental Impacts of Feed Ingredients Used in French Animal Production. PLOS ONE. 11, e0167343. https://doi.org/10.1371/journal.pone.0167343.

Wilfart, A., Dusart, L., Meda, B., Gac, A., Espagnol, S., Morin, L., Dronne, Y., Garcia-Launay, F., 2018. Reducing feed environmental impacts for livestock. Inra Productions Animales. 31, 289-306. https://doi.org/10.20870/productions-animales.2018.31.2.2285.

Figure captions

Figure 1. Contributions of feed ingredients to (a) feed protein, (b) lipid, and (c) starch contents for the C-diet (left) and ECO-diet (right).

Figure 2. Mean body weight of fish fed the C-diet and ECO-diet during the 84-day experiment.

Figure 3. Relative environmental impacts at the experimental facility gate and total feed intake, final body weight (BW), and initial BW of the C-diet and ECO-diet. Results are represented as a percentage of the largest impact in each category (more impact further from the centre). CC = climate change; NRE = non-renewable and fossil energy demand; AC = acidification; EU = eutrophication; LO = land occupation; NPPU = net primary production use; WD = water dependence; PD = phosphorus demand. * P< 0.05, **P<0.01, ***P<0.001

Figure 4. Contribution of production stages and inputs to environmental impacts of the C-diet and ECO-diet.

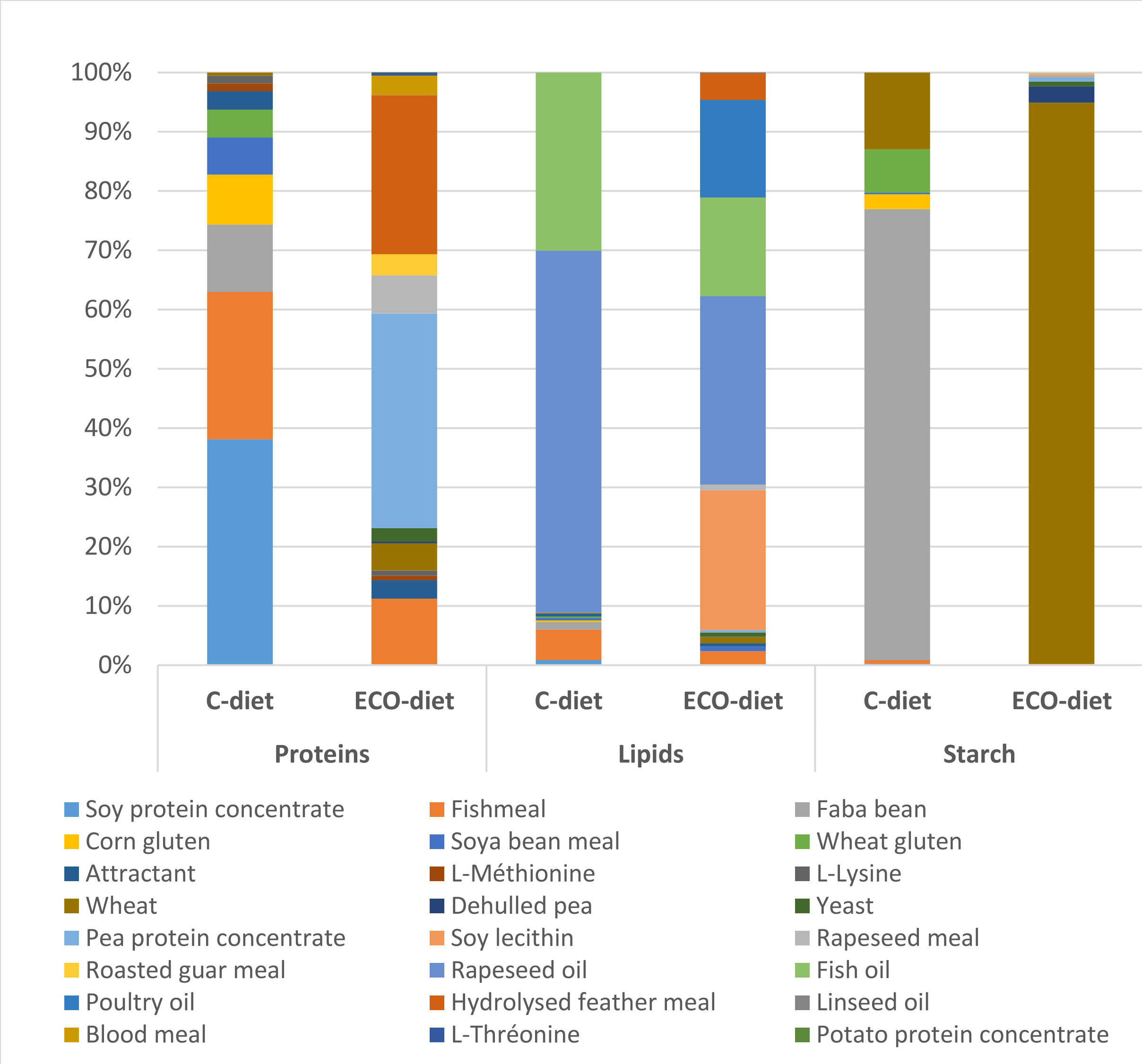
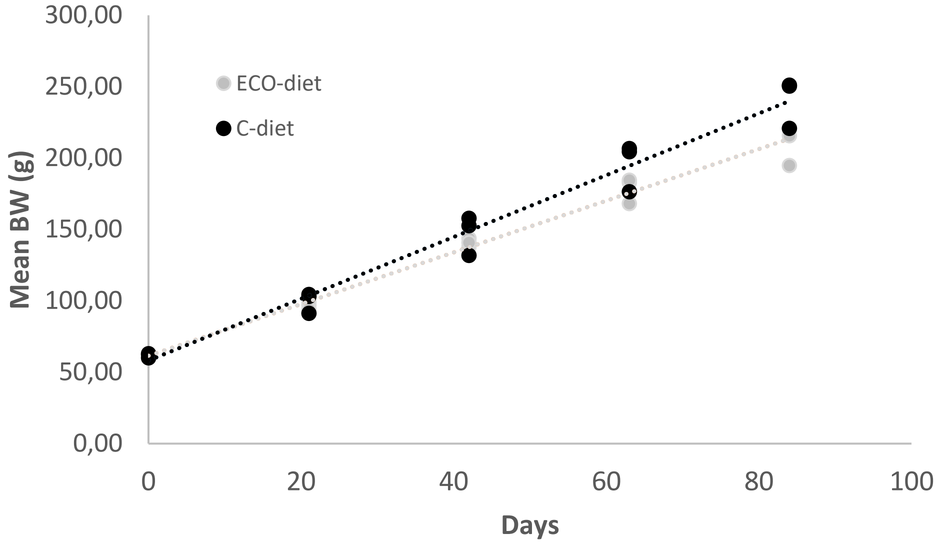

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
C-diet
ECO-diet
C-diet
ECO-diet
C-diet
ECO-diet
Proteins
Lipids
Starch
Soy protein concentrate
Fishmeal
Faba bean
Corn gluten
Soya bean meal
Wheat gluten
Attractant
L-Méthionine
L-Lysine
Wheat
Dehulled pea
Yeast
Pea protein concentrate
Soy lecithin
Rapeseed meal
Roasted guar meal
Rapeseed oil
Fish oil
Poultry oil
Hydrolysed feather meal
Linseed oil
Blood meal
L-Thréonine
Potato protein concentrate

Figure 1



C-diet	**ECO-diet**
$y = 2.1634x + 58.173$	$y = 1.9095x + 61.531$
R^2 = 0.97	R^2 = 0.98

Figure 2

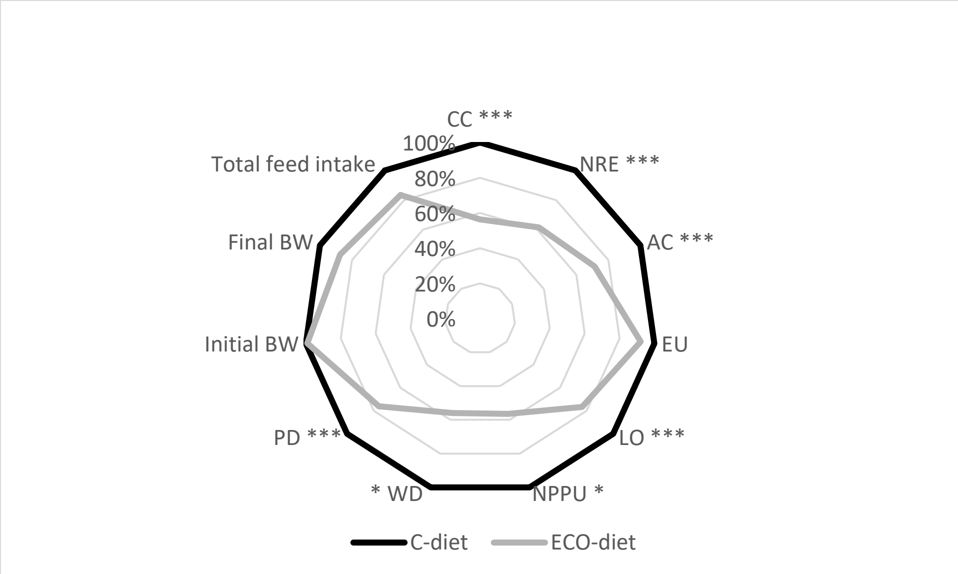

CC ***
NRE ***
AC ***
EU
LO ***
NPPU *
* WD
PD ***
Initial BW
Final BW
Total feed intake
100%
80%
60%
40%
20%
0%
C-diet
ECO-diet

Figure 3


Phosphorus demand
Climate change
Acidification
Eutrophication
Land occupation
Non-renewable energy demand
Net primary production use
Water dependence
100%
50%
0%
C-diet
ECO-diet
Rearing activities
Liquid oxygen
Diesel
Feed
Trout tank
Electricity
Raw ingredient transportation

Figure 4