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

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

3

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11

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13 **Abstract (400 words)**

14

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

36

37 **Highlights**

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

42

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

44

45 **1 Introduction**

46 To meet the growing demand for seafood and the need for protein sources, aquaculture is one of the  
47 most dynamic animal production sectors, expanding in volume by 7.7% per year since the 1980s (FAO,  
48 2020) to compensate for the stability of fisheries production in the past decade. Indeed, aquaculture  
49 has been the main source of fish for human consumption since 2015. In 2018, it provided 53% of fish,  
50 a percentage that is expected to increase over the long term as part of the solution to provide sufficient  
51 food and protein to more than nine billion people by 2050.

52 Animal production systems, including aquaculture, are widely criticised for their impacts on the  
53 environment (Bohnes et al., 2019; Ottinger et al., 2016; Steinfeld et al., 2006). For its part, aquaculture  
54 is responsible for many direct impacts related to eutrophication of aquatic ecosystems due to the  
55 emission of nutrients (e.g. nitrogen (N), phosphorus (P), particles) and intensive use of water, land, and  
56 energy; but also for indirect impacts related to the production of fish feed (Wilfart et al., 2013),  
57 especially for carnivorous fish (Aubin et al., 2009; Pelletier et al., 2009). Feed can represent 65-95% of  
58 the environmental impacts of animal products leaving a farm (Wilfart et al., 2018). For rainbow trout,  
59 feed production can contribute 73-87% of climate change impacts, 86% of acidification impacts, and  
60 68-96% of net primary production use (NPPU) (Boissy et al., 2011; Papatryphon et al., 2004; Parker and  
61 Tyedmers, 2012). Aquaculture is also criticised for its heavy dependence on limited resources due to  
62 its massive use of fishmeal and fish oil (Boissy et al., 2011; Edwards, 2015; Papatryphon et al., 2004).

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

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

79 Hence, to reduce the environmental footprint of feed, the formulation algorithm should consider the  
80 cost and environmental impacts of raw ingredients simultaneously. Recently, Garcia-Launay et al.  
81 (2018) developed a multiobjective (MO) formulation that uses the constraints of least-cost formulation  
82 (nutrients and feed-ingredient incorporation rates) and calculates a MO function that includes both  
83 feed cost and environmental impact indicators obtained by life cycle assessment (LCA) (i.e. climate  
84 change, non-renewable energy use, P demand, land occupation). Nevertheless, fish growth is strongly  
85 influenced by the type of raw feed ingredients. For example, replacing all fishmeal and fish oil with raw  
86 plant ingredients decreased rainbow trout growth by 30% (Lazzarotto et al., 2018).

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

92

## 93 **2 Materials and methods**

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

### 99 **2.1 Feed formulation**

100 Two isonitrogenous, isolipidic, and isoenergetic diets were designed to fulfil the nutritional  
101 requirements of rainbow trout (NRC, 2011). The C-diet was formulated to minimise cost while  
102 containing a fixed level of fishmeal and fish oil using Allix® software, whereas the ECO-diet was

103 formulated using the MO formulation algorithm developed by Garcia-Launay et al. (2018) and updated  
 104 by de Quelen et al. (2021). Information on the nutritional composition of feed ingredients was obtained  
 105 from the Feed Ingredients Composition database included in the International Aquaculture Feed  
 106 Formulation Database (IAFFD, 2019). Ingredient prices (as of October 2019) were provided by feed  
 107 company employees (pers. comm.). See the supplementary materials for details.

108 Environmental impacts of feed ingredients were taken from the ECOALIM dataset (v7, accessed 1 Sep  
 109 2019) as included in the AGRIBALYSE database (Wilfart et al., 2016), except for NPPU and water  
 110 dependence, which were calculated for each feed ingredient in the context of this project. The impact  
 111 categories considered were climate change including land-use change (CC, in kg CO<sub>2</sub>-eq) as calculated  
 112 by the International Reference Life Cycle Data (ILCD) system (JRC, 2012), ILCD acidification potential  
 113 (AC, in mol H<sup>+</sup>-eq), eutrophication potential (EU, in kg PO<sub>4</sub><sup>3-</sup>-eq) from the Institute of Environmental  
 114 Sciences (CML), Cumulative Energy Demand 1.8 (CED, in MJ), non-renewable energy demand (NRE, in  
 115 MJ), CML land occupation (LO, in m<sup>2</sup>year), P demand (PD, in kg P) from Wilfart et al. (2016), NPPU (in  
 116 kg C) from Papatryphon et al. (2004) and water dependence (WD, in m<sup>3</sup>) from Boissy et al. (2011). All  
 117 impacts from the ECOALIM dataset were considered to be those at the storage-organisation gate or  
 118 factory gate.

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

126

$$f(x) = \sum_{i \in I} \text{coef}_i \frac{\text{Impact}_i^t x - \text{Min}_i}{\text{Ref}_{\text{impact}_i} - \text{Min}_i} \quad \text{Equation 1}$$

$$c^t x \leq \epsilon \quad \epsilon = \{\text{Ref}_{\text{price}}, \dots, \text{Max}_{\text{price}}\} \quad \text{Equation 2}$$

$$\text{Impact}_i^t x \leq 1.05 \times \text{Ref}_{\text{impact}_i} \quad \text{Equation 3}$$

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

$$i = [ \text{CC}, \text{AC}, \text{EU}, \text{NRE}, \text{LO}, \text{PD}, \text{NPPU}, \text{WD} ]$$

127 where  $\text{Impact}_i$  is the vector of impact  $i$  of feed ingredients;  $c$  is the matrix of feed-ingredient prices;  
 128  $\text{Max}_{\text{price}}$  is the price of feed when formulating without constraint  $\epsilon$ ;  $\text{Min}_i$  is the level of impact  $i$  when

129 formulated at the lowest impact  $i$ ;  $x$  is the matrix of incorporation rates of feed ingredients (decision  
130 variables);  $Ref_{impact_i}$  and  $Ref_{price}$  are the impact  $i$  and price of least-cost feed formulation, respectively;  
131  $coef_i$  is the weighting factor of impact  $i$ , with  $coef_{AC}$  and  $coef_{EU}$  equal to 1,  $coef_{LO}$ ,  $coef_{PD}$ ,  $coef_{NRE}$ ,  $coef_{WD}$ ,  
132 and  $coef_{NPPU}$  equal to 2, and  $coef_{CC}$  equal to 3;  $q_{min}$  and  $q_{max}$  are the minimum and maximum  
133 incorporation constraints on feed ingredients, respectively; and  $n_{min}$  and  $n_{max}$  are the lower and upper  
134 limits, respectively, of the nutritional constraints applied to the feed.

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

## 137 2.2 Experiments

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

148

### 149 2.2.1 Digestibility experiment

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

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

$$ADC \text{ of } X (\%) = 100$$

Equation 6

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

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

160

### 161 2.2.3 *In vivo* growth experiment

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

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

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

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

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

173 At the end of the experiment, 3 fish per tank were sedated by immersing them in a 10 mg/L  
 174 benzocaine solution and then euthanised by immersing them in a 60 mg/L benzocaine solution  
 175 (anaesthetic overdose) for 3 minutes. They were then frozen at  $-20^\circ\text{C}$  until body composition  
 176 analysis.

### 177 2.2.4 Biochemical composition of diets, whole body and faeces

178 Proximate analysis of the experimental diets and whole body was determined according to AOAC  
 179 (2006) as follows: dry matter was analysed by drying samples to constant weight at  $105^\circ\text{C}$  for 24 h.  
 180 Crude protein was determined using the Kjeldahl method after acid digestion and estimated by  
 181 multiplying the N content by 6.25. Crude lipid was quantified by petroleum diethyl ether extraction

182 using the Soxhlet method. Gross energy content was determined in an adiabatic bomb calorimeter  
183 (IKA®). Ash content was calculated after combustion in a muffle furnace at 550°C for 16 h. The total  
184 lipid content of feed and whole fish was quantified gravimetrically after extraction by  
185 dichloromethane/methanol (2:1, v/v), containing 0.01% of butylated hydroxytoluene as an antioxidant  
186 (Folch et al., 1957).

### 187 2.2.5 Nutrient and energy use

188 Nutrient and energy intake (g or kJ.kg BW<sup>-1</sup>.day<sup>-1</sup>), gain (g or kJ.kg BW<sup>-1</sup>.day<sup>-1</sup>), and retention (%) were  
189 calculated as follows:

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

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

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

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

193

### 194 2.3 Life cycle assessment

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

202 The potential impacts considered the farm level were the same as those used to assess the feed level.  
203 CC including land-use change is the potential impact of greenhouse gas emissions on heat-radiation  
204 absorption in the atmosphere. It was calculated according to 100-year-horizon global warming  
205 potentials (CC, kg CO<sub>2</sub>-equivalents) of the International Panel on Climate Change (IPCC, 2006) as  
206 calculated by the ILCD (JRC, 2012). Acidification (AC, expressed in mol H<sup>+</sup>-eq/kg) refers to negative  
207 effects on soils, ground and surface water, and ecosystems from acidifying pollutants. It was calculated



208 using average European acidification potential factors from ILCD (JRC, 2012). Eutrophication (EU, kg  
209  $\text{PO}_4^{3-}$  equivalents) refers to potential impacts of high levels of nutrients, especially N and P, in the  
210 environment. It was calculated using factors developed by the CML (Guinee et al., 2002) and adding  
211 the theoretical oxygen demand caused by solid waste from fish farms. P demand (PD, expressed in kg  
212 P) from Wilfart et al. (2016) includes all P and phosphate inputs throughout the life cycle. LO ( $\text{m}^2\text{yr}$ )  
213 refers to human use of land that delays natural restoration of land quality during the period of  
214 occupation (Guinee et al., 2002). CED (MJ) refers to the consumption of all energy sources used, and  
215 non-renewable energy demand (MJ) refers to the consumption of energy from non-renewable sources  
216 used. They were obtained from CED v1.8. NPPU (kg carbon) refers to the amount of net primary  
217 production (biomass produced by photosynthesis) required as a biotic resource input that cannot be  
218 used for other purposes. It was calculated according to Papatryphon et al. (2004). Water dependence  
219 (WD,  $\text{m}^3$ ) equals to the evaporative blue water or the volume of water in ground and surface water  
220 bodies available for abstraction that is not immediately reusable used by the system (Boissy et al.,  
221 2011).

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

226

## 227 2.5 Statistical analysis

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

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

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

240

241 where  $BW_{ij}$  is the mean body weight in tank  $i$  at time  $j$ ,  $Duration_{ij}$  is the duration of the experiment (in  
242 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  
243 mean growth rate,  $a_i$  is the effect of tank  $i$  on  $A$ , and  $e_{ij}$  are random errors.

244

### 245 **3 Results**

#### 246 3.1 Feed formulas

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

258 MO formulation changed the contribution of ingredients to the protein, lipid, and starch contents of  
259 the feed greatly (Figure 1). While soy protein concentrate (38%), fishmeal (25%), faba bean (11%), and  
260 corn gluten (8%) contributed the most to the protein content of the C-diet, pea protein concentrate  
261 (36%) became the major protein contributor to the ECO-diet, followed by hydrolysed feather meal  
262 (27%), fishmeal (11%), and rapeseed meal (6%). Lipids in the C-diet were provided by rapeseed oil  
263 (61%) and fish oil (30%), both of which were halved in the ECO-diet (-32 and -17 percentage points,  
264 respectively) to the benefit of soy lecithin (24% of lipids) and poultry oil (16% of lipids). Most of the  
265 starch was provided by faba bean (76%) in the C-diet, followed by wheat (13%) and gluten (10%),  
266 whereas nearly all of the starch (95%) came from wheat in the ECO-diet.

267 MO formulation decreased environmental impacts by 21-57% depending on the impact considered:  
268 compared to the C-diet, the ECO-diet decreased CC by 46%, NRE by 57%, AC by 30%, EU by 35%, LO by  
269 24%, NPPU by 44%, WD by 44%, and PD by 21%. This reduction in the environmental footprint was  
270 associated with a decrease in feed cost of 8%.

271 Table 1. Composition of the experimental diets (pellets)

	<b>C-diet</b>	<b>ECO-diet</b>
<b>Ingredient, %</b>		
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 <sup>1</sup>	1.16	1.37
Vitamin premix <sup>2</sup>	1.16	1.00
<b>Number of ingredients</b>	<b>16</b>	<b>23</b>
<b>Chemical composition, g/kg</b>		
Dry matter <sup>3</sup>	966.40	973.40
Crude protein <sup>3,4</sup>	473.70	476.70

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

272 <sup>1</sup>Provided per 100 g of premix: calcium hydrogen phosphate 49 478 mg, calcium carbonate 21 500 mg, sodium  
273 chloride 4000 mg, potassium chloride 9000 mg, magnesium oxide 12 400 mg, iron sulphate 2000 mg, zinc  
274 sulphate 900 mg, manganese sulphate 300 mg, copper sulphate 300 mg, cobalt chloride 2 mg, potassium iodide  
275 15 mg, selenite sodium 5 mg, fluoride sodium 100 mg.

276 <sup>2</sup>Provided per 100 g of premix: vitamin A 500 000 IU, vitamin D3 250000 IU, vitamin E 5 00 mg, vitamin C 1429  
277 mg, vitamin B1 10 mg, vitamin B2 50 mg, vitamin B3 100 mg, vitamin B5 200 mg, vitamin B6 30 mg, vitamin B7  
278 3000 mg, vitamin B8 100 mg, vitamin B9 10 mg, vitamin B12 100 mg, vitamin K3 200 mg, folic acid 10 mg, biotin  
279 100 mg, choline chloride 16 700 mg, cellulose 76 921mg.

280 <sup>3</sup>Analysed values

281 <sup>4</sup>Expressed per kg of dry matter

282 <sup>5</sup>CC = climate change, NRE = non-renewable energy demand, AC = acidification, EU = eutrophication, LO = land  
283 occupation, NPPU = net primary production demand, WD = water dependence, PD = P demand.

284

## 285 3.2 Animal performance

### 286 3.2.1 Feed digestibility

287 For the ECO-diet, MO formulation significantly decreased the ADC of lipid, energy, and ash (by 1.6%,  
288 2%, and 13.6%, respectively) but increased the ADC of starch by 5.5% (Table 2).

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

<b>Component</b>	<b>C-diet</b>		<b>ECO-diet</b>		<i>P</i>
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	
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

290

### 291 3.2.2 Growth performance

292 Only certain indicators of growth performance and nutrient use by the rainbow trout after 12 weeks  
 293 of feeding differed by feed (Table 3). At the end of the growth experiment, body weight and SGR did  
 294 not differ significantly between diets, but analysis of the growth curves revealed a significant  
 295 interaction between diet and time ( $p = 0.0005$ ) (Figure 2). FCR were similar, but DFI was significantly  
 296 higher (7%) for fish fed the C-diet. Feed formulation did not influence the body composition of fish  
 297 (Table 4). Fish fed the ECO-diet showed lower protein gain due to lower protein intake, as the two diets  
 298 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

<b>Characteristic<sup>1</sup></b>	<b>C-diet</b>		<b>ECO-diet</b>		<b>P</b>
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	
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 <sup>-1</sup> day <sup>-1</sup>	16.2	0.03	15.0	0.02	0.009
FCR	1.2	0.02	1.2	0.05	0.93
<b>Nutrient intake</b>					
Protein, g kg <sup>-1</sup> day <sup>-1</sup>	7.4	0.08	6.9	0.06	0.01
Lipid, g kg <sup>-1</sup> day <sup>-1</sup>	3.6	0.04	3.5	0.03	0.09
Energy, kJ kg <sup>-1</sup> day <sup>-1</sup>	4.0	0.04	3.6	0.03	0.003
<b>Nutrient gain</b>					
Protein, g kg <sup>-1</sup> day <sup>-1</sup>	2.4	0.07	2.1	0.06	0.04
Lipid, g kg <sup>-1</sup> day <sup>-1</sup>	2.7	0.06	2.6	0.06	0.60
Energy, kJ kg <sup>-1</sup> day <sup>-1</sup>	1.6	0.03	1.6	0.03	0.17
<b>Nutrient retention</b>					
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 <sup>1</sup>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

<b>Body composition<sup>1</sup></b>	<b>C-diet</b>		<b>ECO-diet</b>		<b>P</b>
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	
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 <sup>-1</sup>	10.2	0.15	10.2	0.18	0.86

303 <sup>1</sup>BW= body weight

304 3.3 LCA impacts per kg of fish body weight gain

305 The environmental impacts calculated from the growth experiment and expressed per kg of body  
306 weight gain (Table 5) or as a percentage of the highest impact by category (Figure 3) varied. Except for  
307 EU (mean of both diets: 0.049 kg PO<sub>4</sub><sup>3-</sup>-eq), compared to the C-diet, the ECO-diet decreased CC, NPPU,  
308 and WD by 44%; NRE by 38%; AC by 27%; LO by 23%; and PD by 24% per kg of body weight gain.

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

Impact category <sup>1</sup>	C-diet		ECO-diet		P
	Mean	SD	Mean	SD	
CC (kg CO <sub>2</sub> -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 <sup>+</sup> -eq)	0.021	0.0004	0.015	0.0008	0.0003
EU (kg PO <sub>4</sub> <sup>3-</sup> -eq)	0.051	0.0014	0.047	0.0034	0.16
LO (m <sup>2</sup> year)	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 <sup>3</sup> )	11.86	0.256	6.64	0.383	0.05
PD (g P)	0.0084	0.0002	0.0064	0.0004	0.001

311 <sup>1</sup>CC = climate change, NRE = non-renewable energy demand, AC = acidification, EU = eutrophication, LO = land  
312 occupation, NPPU = net primary production demand, WD = water dependence, PD = P demand.

313

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

319

320 4 Discussion

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

324

#### 325 4.1 Consequences of MO formulation on the composition and environmental impacts of diets

326 Unlike current commercial aquaculture feed-formulation practices, which aim for trade-offs among  
327 nutritional constraints, cost, and target levels of fishmeal and fish oil incorporation, MO formulation  
328 aims for trade-offs among nutritional constraints, cost, and environmental impacts of ingredients. With  
329 MO formulation, reducing CC was one of our main goals, according to priorities of the United Nations  
330 Paris Agreement (United Nations, 2015). Thus, the weighting factor was 3 for CC; 2 for NRE, LO, PD,  
331 WD, and NPPU; and 1 for EU and AC. Consequently, the ECO-diet had lower impacts than the  
332 commercial-type C-diet, with the main gains observed for CC (-46%), NPPU, and WD (-44%), and NRE  
333 (-42%).

334 MO formulation changed the composition of the rainbow trout diet drastically, increasing the number  
335 of raw ingredients from 16 to 23. We observed sets of replacement between categories of ingredients.  
336 First, MO formulation significantly decreased fishmeal and fish oil use by ca. 50%. Fishmeal and fish oil  
337 have high NPPU, WD, NRE, and CC impacts, which likely contributed to the decrease in these impacts  
338 in the ECO-diet. Likewise, soy products (soy protein concentrate and soya bean meal), which contribute  
339 strongly to CC due to their Brazilian origin (i.e. some production after deforestation) contributed 40%  
340 of the CC impact of the C-diet. In the ECO-diet, soya bean meal was replaced by rapeseed meal (1.11  
341 vs. 0.347 kg CO<sub>2</sub>-eq/kg, respectively). The lower cost of rapeseed meal than soya bean meal likely  
342 favoured its incorporation in the ECO-diet (225 vs 400 €/t, respectively). The ECO-diet contained no  
343 glutes, which have high impacts per kg for almost all categories (especially CC and NRE) and high cost.  
344 Although they were introduced in small percentages in the C-diet (6.0% and 2.5% for corn and wheat  
345 gluten, respectively), they were always among the five raw ingredients with the highest impacts per  
346 kg regardless of the impact category, as wheat and corn glutes are highly processed co-products from  
347 the starch industry. Processed animal co-products (poultry feather and blood meals) appeared in the  
348 ECO-diet due to their lower CC and NRE impacts. Faba bean was not included in the ECO-diet because  
349 of its high contribution to LO (24.3%), just below that of rapeseed oil (46.6%). Faba bean is used in  
350 aquaculture feeds because it provides starch (37.3%), which is essential for extrusion during  
351 production, and has a high crude-protein content (26.8%); however, as a legume, it contains relatively  
352 little methionine. In the ECO-diet, starch is provided mainly by wheat, which has lower environmental  
353 impacts, whereas protein is provided mainly by animal co-products (i.e. feather and blood meals),  
354 which have a higher protein content, higher methionine concentration, and lower CC impact. As a  
355 protein source, pea protein concentrate appears in the ECO-diet due to its high protein content (77%)  
356 and low environmental impacts. Despite its high incorporation rate (20%), it contributed little to  
357 impacts of the ECO-diet. In the C-diet, rapeseed oil contributed the most to LO (46.6%), EU (26.1%),



358 and AC (27.3%) and second-most to CC (15.1%) after soy protein concentrate, which likely explains  
359 why it was not included in the ECO-diet.

360 In the context of fishmeal and fish oil replacement, there is now great interest in new feed ingredients,  
361 such as insects and yeast as sources of protein (Agboola et al., 2021; Henry et al., 2015; Roques et al.,  
362 2018) and microalgae as a source of n-3 long-chain polyunsaturated fatty acids (i.e. EPA and DHA)  
363 (Sprague et al., 2017). Although these ingredients were included in the list of those available for MO  
364 formulation, only yeast was ultimately included in the ECO-diet but at a low incorporation rate (1.8%).  
365 Insect meal (Thévenot et al., 2018), whose CC impact is 3 times as high as that of soya bean meal (3.5  
366 vs. 1.1 kg CO<sub>2</sub> per kg, respectively), was not included in the ECO-diet. DHA-rich microalgae were not  
367 included in the ECO-diet either. Even though their ability to replace the DHA provided by fish oil has  
368 been clearly demonstrated for rainbow trout (Richard et al., 2021), their high price automatically  
369 excludes them from lower-cost formulations.

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

377

#### 378 4.2 Effects on animal performances

379 At the end of the experiment, the mean body weight of fish did not differ between diets, but their  
380 growth curves had begun to diverge. Based on these growth curves, fish fed the C-diet would require  
381 5 more days of rearing to reach the size of a human meal portion (250 g), while those fed the ECO-diet  
382 would require 20 more days. These results suggest better growth performance for fish fed the C-diet  
383 than those fed the ECO-diet, as illustrated by the former's higher protein gain. The decrease in growth  
384 performance in the long term is probably mainly related to the decrease in feed intake. The lower  
385 protein and fat digestibilities of the ECO-diet could have also contributed to the trend of lower growth  
386 but to lower extent compared to feed intake because nutrients ADC could be considered as high in  
387 both diets. In any case, this decrease cannot be associated with a decrease in feed efficiency, because  
388 the FCR did not differ between the two diets.

389 Previous studies of Atlantic salmon (Torstensen et al., 2008) and seabream (de Francesco et al., 2007)  
390 have shown that a decrease in fishmeal and fish oil in diets leads to a decrease in fish feed intake. This  
391 decrease in feed intake was also observed in rainbow trout when fishmeal and fish oil were completely  
392 replaced by raw plant ingredients (Panserat et al., 2009). In our study, the decrease in feed intake in  
393 trout fed the ECO-diet could have resulted from the decrease in the percentages of fishmeal and fish  
394 oil (55% and 45% less, respectively, than in the C diet) and thus more plant ingredients. In addition, a  
395 diet with a high percentage of plant ingredients may have a high concentration of antinutritional  
396 factors that negatively influence fish feeding behaviour (Krogdahl et al., 2010). Furthermore, Roy et al.  
397 (2020) highlighted that rainbow trout prefer a diet with high percentages of n-3 long-chain  
398 polyunsaturated fatty acids, especially EPA and DHA. The ECO-diet contained lower percentages of EPA  
399 and DHA, which can contribute to a decrease in feed intake.

400

#### 401 4.3 Effect on environmental performances

402 Overall, using MO formulation to decrease environmental impacts of feed made it possible to  
403 significantly decrease the environmental footprint of the fish farming system studied per kg of body  
404 weight gain. The decrease in impacts was lower at the farm level than that at the feed level, especially  
405 for EU and, to a lesser extent, NRE and CC. In contrast, the feed and farm levels had similar decreases  
406 for NPPU, WD, LO, AC, and PD. Differences between the feed and farm levels have been observed for  
407 other species (pigs and poultry) for which MO formulation was applied (de Quelen et al., 2021; Meda  
408 et al., 2021), but they were observed for all environmental impacts, not only for some of them as in  
409 the present study. The farm level included emissions due to biological processes of fish but also those  
410 to the functioning of the rearing facility. NPPU and PD depended only on the feed, which explains why  
411 their decrease was the same for both levels. In the experimental system used, the fish were reared in  
412 raceways in which water was taken from a river, continually flowed through the system, and then  
413 returned to the river. Because the water could thus be reused, it was not included in water use in life  
414 cycle inventory, as recommended by Boissy et al. (2011). Consequently, the decrease in WD at the  
415 farm level was the same as that at the feed level. The experiment was performed in 60 L tanks, which  
416 contributed less to LO than the areas used to produce the crops that provided feed ingredients. In  
417 contrast, the decrease in AC was the same at the farm level, unlike the increases in AC observed for  
418 pig and poultry systems. Because ammonia (NH<sub>3</sub>) contributes to AC, NH<sub>3</sub> emissions from manure  
419 management in terrestrial livestock systems are included in the life cycle inventory, and they influence  
420 AC at the farm level strongly. In contrast, due to its very low concentration in water, no NH<sub>3</sub> emissions  
421 to the air compartment was accounted for the raceway system in this study, so AC differed little

422 between the feed and farm scales. In aquaculture systems, only transport-related gaseous emissions  
423 are included. For EU, the feed and farm levels differed by less than 8%. The contribution analysis  
424 showed that rearing activities were responsible for 85-90% of this impact, which is slightly higher than  
425 that generally observed in the literature for salmonids (ca. 73-87%) (Boissy et al., 2011; Wilfart et al.,  
426 2013). The decreases in CC and NRE were ca. 2 and 4 percentage points lower, respectively, at the farm  
427 level than the feed level, due mainly to the transport of raw ingredients, as indicated by the  
428 contribution analysis. In this study, MO formulation was applied according to the LCA boundaries of  
429 the ECOALIM database (i.e. the factory gate for processed ingredients and the storage-organisation  
430 gate for raw plant ingredients), without considering the actual distance between the production site  
431 of the ingredients and the experimental facility, where the feeds were manufactured just before the  
432 experiment. Some suppliers of raw ingredients were identified before using the latter to formulate  
433 feeds at the facility, distances from google map estimations were used for raw ingredients in the LCA  
434 at the experimental facility gate. Nevertheless, additional experiments under commercial production  
435 conditions (for feed and rearing) will be necessary to validate the environmental gains that can be  
436 obtained with MO formulation, both in terms of animal growth and the life cycle inventory of inputs  
437 and infrastructure.

438

## 439 5. Conclusions and perspectives

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

450

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460 Study design: AW, SSC, FGL, FT  
461 Data curation: SSC, ES, AW, FT, FGL  
462 Funding acquisition: AW  
463 Animal experiments: FT, PA  
464 LCA model and assessment: AW  
465 Feed formulation: FGL, FT, AW  
466 Statistical analysis: ES, FGL, SSC, AW  
467 Project administration and supervision: AW  
468 Writing - original draft: AW, SSC  
469 Writing - review & editing: AW, SSC, FGL, FT, ES, PA  
470

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596 Figure captions

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

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

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

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

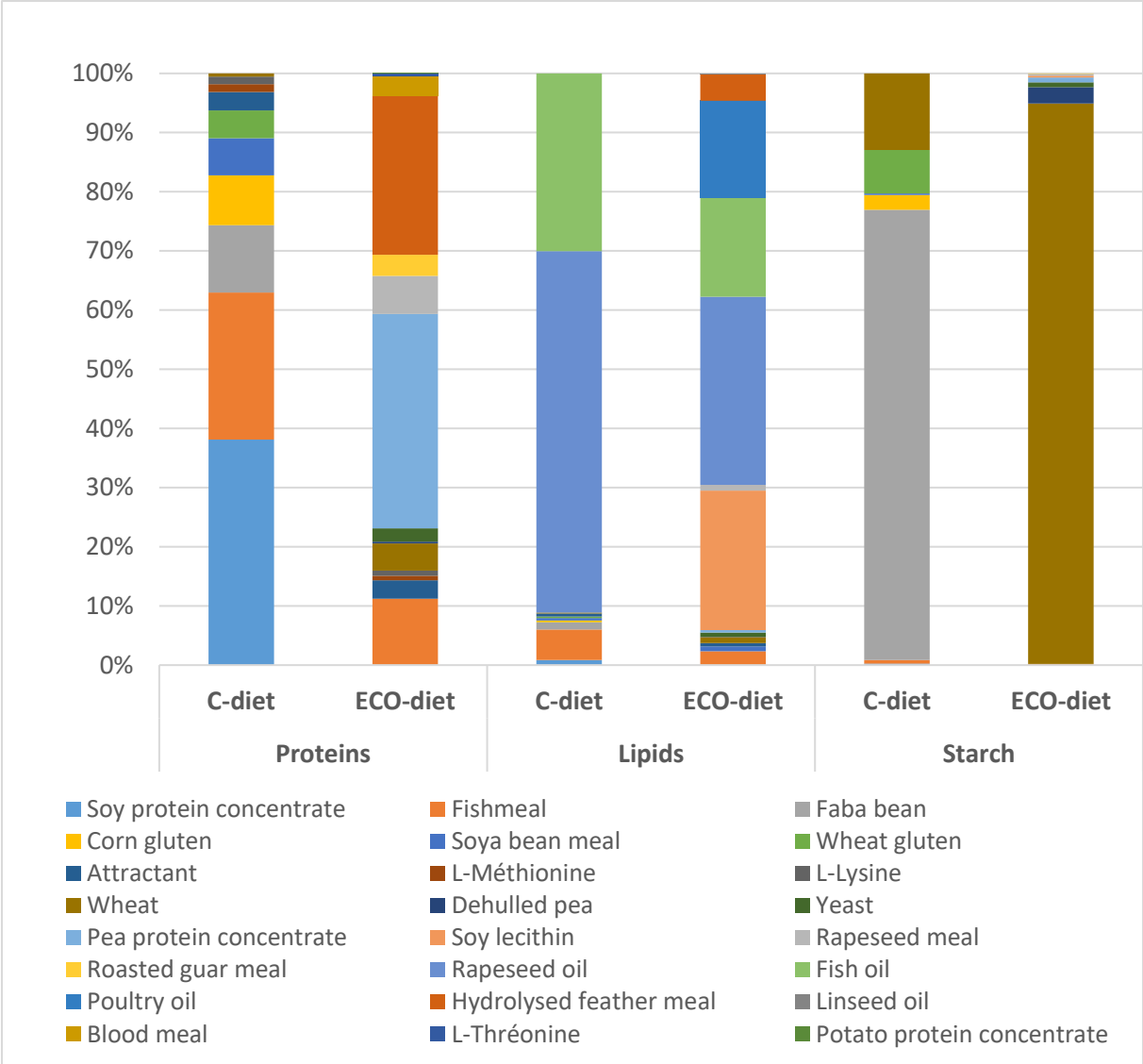
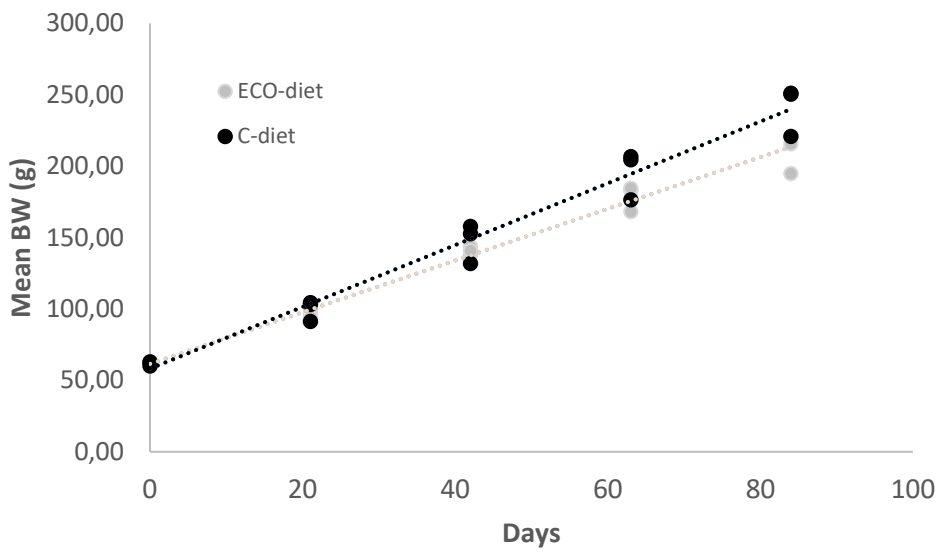


Figure 1





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

Figure 2

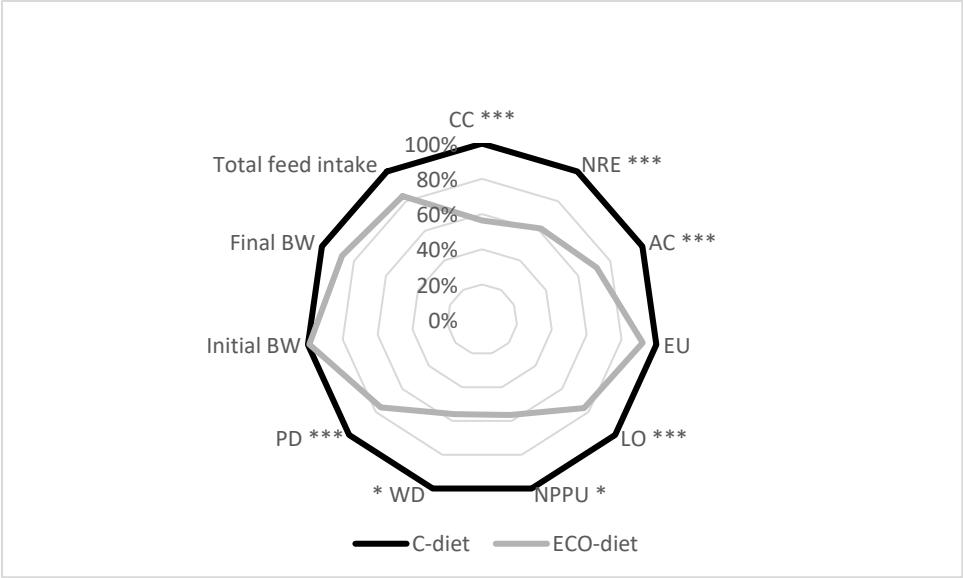
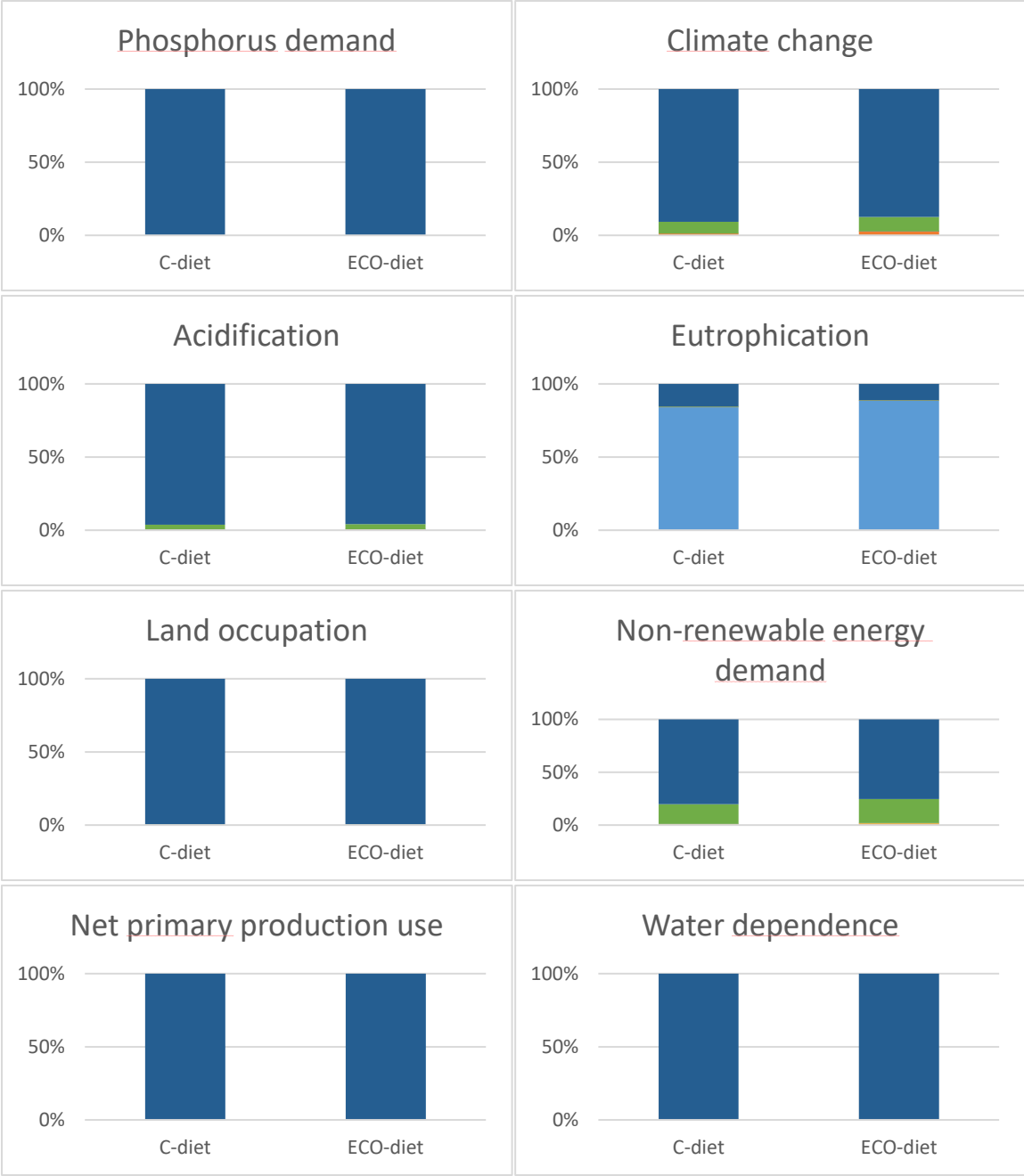


Figure 3



- Rearing activities
  - Liquid oxygen
  - Diesel
  - Feed
- Trout tank
  - Electricity
  - Raw ingredient transportation

Figure 4

