

1 **Environmental benefits of novel non-human food**
2 **inputs to salmon feeds**

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19 **Abstract**

20 Global population growth and changing diets increase the importance, and challenges, of
21 reducing the environmental impacts of food production. Farmed seafood is a relatively efficient
22 way to produce protein and has already overtaken wild fisheries. The use of protein-rich food
23 crops, such as soy, instead of fishmeal in aquaculture feed diverts these important protein sources
24 away from direct human consumption and creates new environmental challenges. Single cell
25 proteins (SCPs), including bacteria and yeast, have recently emerged as replacements for plant-
26 based proteins in salmon feeds. Attributional life cycle assessment (ALCA) is used to compare
27 salmon feeds based on protein from soy, methanotrophic bacteria, and yeast ingredients. All
28 ingredients are modeled at the industrial production scale and compared based on seven resource
29 use and emissions indicators. Yeast protein concentrate showed drastically lower impacts in all
30 categories compared to soy protein concentrate. Bacteria meal also had lower impacts than soy
31 protein concentrate for five of the seven indicators. When these target meals were incorporated
32 into complete feeds the relative trends remain fairly constant, but benefits of the novel
33 ingredients are dampened by high impacts from the non-target ingredients, Particularly, primary
34 production requirements (PPR) are about equal and constant across all feeds for both analyses
35 since PPR was driven by fishmeal and oil. The bacteria-based feed has the highest climate
36 change impacts due to the use of methane to feed the bacteria who then release carbon dioxide.
37 Overall, the results of this study suggest that incorporating SCP ingredients into salmon feeds
38 can help reduce the environmental impacts of salmon production. Continued improvements in
39 SCP production would further increase the sustainability of salmon farming.

40

41 **Introduction**

42 More efficient food systems are needed to feed a rapidly growing human population in
43 environmentally sustainable ways. How to feed >9 billion people by the year 2050¹ is a major
44 question, but doing so without further degrading or destroying natural ecosystems and their
45 capacity to support food production adds a significant degree of difficulty. Our challenge is
46 exacerbated by a clear trend indicating that as a country develops economically, and per capita
47 income rises, there follows a shift in human behavior towards greater consumption of animal-
48 based products, specifically meat-eating.^{2,3} The FAO estimates that from 2010 to 2050 food
49 production must increase by 70% and meat production in particular must increase ~100% to
50 meet increased demand.^{4,5} But animals must be fed to produce meat, and today most animal
51 feeds are comprised of crop-based cereals, legumes, and seeds that require large tracts of land to
52 grow. These crop-based feeds are also composed mainly of the same food stocks that much of
53 the world's human population, located in the least developed countries, depend upon for their
54 nutrition.^{6,7} Clearly there is need to develop new sources of animal feeds that do not reduce our
55 overall capacity to feed humans, and that minimize further demand on strained resources.

56 Seafood is an important source of protein that can help alleviate some of the major challenges
57 facing food production systems. Wild fisheries have historically provided most of our seafood
58 but recently aquaculture, which includes both freshwater and ocean-based mariculture, has
59 overtaken wild caught fisheries in seafood-based protein production.⁸ An important player in
60 these trends is Atlantic Salmon (*Salmo salar*), whose farmed production has recently overtaken
61 total wild production of the major salmon species, statistics that continue to diverge.⁸ In terms of
62 revenue produced by mariculture, Atlantic Salmon is the highest valued fish species.⁸ A top
63 carnivore, salmon are initially bred and reared in land-based freshwater facilities but the majority
64 of growth occurs in the coastal ocean pens. Salmon grow very rapidly to large sizes thereby

65 requiring large amounts of protein-rich food. Rapid expansion of salmon farming has generated
66 great demand for salmon feeds, which now represent the highest cost in salmon production and
67 comprise the majority of the environmental impacts of salmon farming.^{9,10} As such, developing
68 low cost, environmentally less-impactful feeds is one of the biggest obstacles to aquaculture
69 sustainability.

70 Globally, the aquaculture industry used ~40 million tonnes of feed in 2012, an amount that has
71 grown at an average annual rate of 10.3% per year since 2000 and is expected to reach over 65
72 million tonnes by 2020¹¹. For carnivorous species such as salmon, fish-based feed ingredients
73 (i.e., fishmeal and fish oil) enhance growth rate mainly by providing essential amino acids and
74 lipids¹¹. Through innovations, the conversion rate of 4 kg of fish-based ingredients in feeds to
75 1kg of salmon biomass has decreased to <1:1, due mainly to the emergence and use of
76 alternative, mostly plant-based ingredients. The alternatives have been developed primarily
77 because of the rising cost of fishmeal and oil, as well as a growing concern about overfishing
78 vulnerable wild fish stocks.^{12,13} In response, fish nutritionists and health scientists have produced
79 an impressive array of alternative salmon feed ingredients to replace fish-based inputs while
80 maintaining rapid growth and survivorship rates in salmon.^{12,14}

81 Replacements for fish-based ingredients are usually plant based, with soy inputs making up most
82 heavily used substitutes.¹⁵ Soy has high protein and lipid content and other important essential
83 nutrients, which make them great substitutes for nutritious fishmeal and oil ingredients. As a
84 result, soy is now found in almost all aquaculture feeds.¹⁵⁻¹⁹ Impacts on wild fisheries and
85 overall costs associated with predominantly fish-based feeds have declined by integrating
86 soybean and other crops (e.g., wheat, corn, rapeseed) but other environmental costs have
87 apparently increased. Recent studies indicate that intensified crop production, and soy in

88 particular, has increased land conversion and use in farming.²⁰⁻²² Saponins in soybean meal can
89 cause enteritis in salmon which leads to slower growth,²³⁻²⁷ a limitation that can be overcome by
90 condensing the soy product further into soy protein concentrate. Condensing soy, however,
91 requires a greater total input of soy per unit of salmon, which in turn requires additional land use,
92 energy, fossil phosphorous inputs and other limited resources.^{28,29} Finally, and perhaps most
93 importantly for human welfare, using soy and other products as alternatives in salmon feed
94 reduces their availability for direct human consumption, particularly for the least food-secure
95 people living in less developed countries which depend primarily on crop-based foods.^{6,7} Use of
96 these important protein-rich crops to produce high value products such as salmon diverts these
97 important and accessible resources away from those who most need them. Further exacerbating
98 the issue, feeding crops to livestock is inefficient energetically because energy conversion rates
99 between trophic levels is low.³⁰ To address these inefficiencies and inequalities, the livestock
100 feed industry is working to develop feeds that minimize human-food ingredients, with the
101 intended outcomes being increased supply of human-food resources and reduced environmental
102 impacts.^{11,31-33}

103 Emerging single cell proteins (SCP) make up a diverse group of promising feed ingredients.³³
104 SCPs, which include methanotrophic bacteria, *Methylococcus capsulatus (Bath)*, and a common
105 yeast, *Saccharomyces cerevisiae*, are fast growing, protein-rich organisms that are produced at
106 relatively low cost in closed, controlled environments. SCP-derived nutrients are naturally high
107 in protein but can also be manipulated to meet different nutritional requirements, including
108 salmon diets.³⁴ Methanotrophic bacteria oxidize methane into carbon dioxide, which would
109 generate climate change benefits if the methane was to be otherwise released into the
110 environment.³⁵ Salmon fed *M. capsulatus* diets resulted in increased growth compared to salmon

111 fed soy-based diets. Bacteria-inclusive feeds may also produce healthier fish through bioactive
112 components that enhance gut health in Atlantic salmon.^{36,37} For centuries, *S. cerevisiae* yeast
113 have been used for centuries for human consumption in foods like beer and bread and were
114 traditionally grown on simple sugar media. In order to conserve resources yeast producers,
115 particularly those producing yeast for animal feeds, have used byproducts from other industrial
116 processes to feed the yeast cells, such as wheat grains from biofuels production.³³ These low
117 resource methods are being further improved upon by sourcing more non-human food inputs,
118 such as algae and lumber byproducts, to feed cell propagation.³⁸ Whether the replacement of
119 human-food ingredients with SCP ingredients in salmon feeds could decrease the overall
120 environmental impacts of salmon farming has yet to be determined.

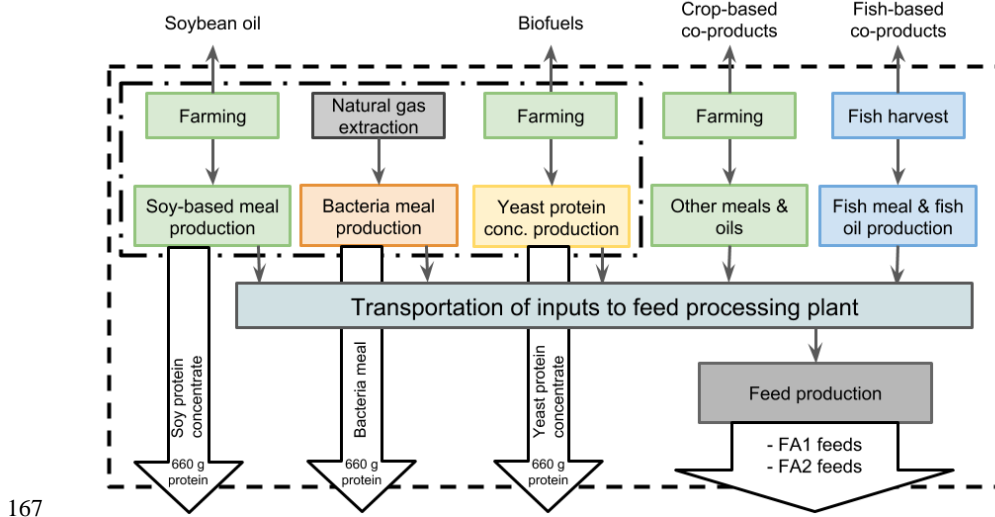
121 High environmental impacts of feeds in aquaculture production are well documented^{9,10,39} and
122 further studies indicate that fish-based ingredients in salmon feeds have higher impacts than soy-
123 based feeds.^{14,39} Still, nutritionists and fish farmers understand the importance of fish ingredients
124 in feeds for carnivorous fish,^{14,39} therefore, salmon feeds today minimize fish inclusion while
125 maintaining nutritious diets. With fish ingredients at a minimum, focus has turned to increasing
126 sustainability of feeds through other highly demanded ingredients, particularly soy.²⁸ Here we
127 test whether the replacement of soy-based ingredients in salmon feed with protein-rich bacteria
128 and yeast can further reduce the environmental impacts of Atlantic salmon production. The use
129 of life cycle assessments to measure the environmental impacts of seafood products is becoming
130 more common, and provides a way to compare disparate production methods (fishing versus
131 aquaculture, different feeds, etc.) side-by-side⁴⁰. We use attributional life cycle assessment
132 (ALCA) to compare the impacts of soy protein concentrate against bacteria meal and yeast
133 protein concentrate directly, then also compare feeds in which soy ingredients are replaced with

134 either bacteria- or yeast-based ingredients. Environmental performance was assessed based on
135 seven resource use and emissions metrics. Results are intended to inform feed producers, salmon
136 industry, researchers, consumers and consumer awareness campaigns of the tradeoffs between
137 current and emerging feeds and feed inputs. There are many different ways to assess
138 environmental impacts from food production; while the use of LCA is growing in popularity, it is
139 important to remember that LCA does not measure all environmental impacts equally well and
140 should be complemented with other types of assessments.

141 **Methods**

142 ISO-compliant ALCA was used to assess the environmental impacts of replacing soy ingredients
143 with novel single cell protein (SCP) meals, focusing on resource use and emissions to the natural
144 environment.^{41,42} We first compared these ingredients directly by assessing the impacts of soy
145 protein concentrate compared to bacteria meal and yeast protein concentrate. Bacteria meal is
146 produced through fermentation and drying of methanotrophic bacteria. The bacteria are fed fossil
147 methane as a growth medium and also require additional chemical inputs for the initial
148 fermentation step. Cells are then harvested from the fermenter, condensed and heat dried into a
149 meal. For yeast protein concentrate, yeast cells are fed a wheat byproduct from biofuels
150 production. The wheat byproduct requires enzyme treatment to make a viable growth medium
151 for the yeast cells. These cells are similarly harvested, condensed and dried into a concentrated
152 meal. These meals were compared on an equal protein basis: 660g of protein, which is the
153 protein content of 1kg of soy protein concentrate. Two feed analyses were also conducted. One
154 assessed how total feed impacts change when soy protein concentrate is replaced by the SCP
155 meals on an equal protein basis, with all other ingredients held constant (Feeds Analysis 1, FA1).

156 FA1 allows for comparison of the different protein meals in a whole feed context without
 157 conflating the meal impact differences with impact changes due to varying the non-target
 158 ingredients. In the second analysis, soy protein concentrate is replaced by the SCP meals on an
 159 equal mass basis and non-target ingredients were adjusted to meet the nutrient requirements of
 160 salmon, they were formulated to have equal protein and lipid levels (Feeds Analysis 2, FA2).
 161 FA2 is believed to be a more realistic scenario in commercial feed formulations. All products
 162 were assessed from cradle-to-factory-gate at the industrial scale. System boundaries for the three
 163 analyses are defined in Figure 1. In each analysis, each of the three treatments were assessed
 164 based on the following seven midpoint impact categories: climate change impacts, acidification,
 165 aquatic eutrophication (freshwater and marine separately), land occupation, water consumption,
 166 and primary production requirement (Table S1).



167

168 **Figure 1:** System boundary. *These analyses assess the processes of novel single cell protein*
 169 *meals and salmon feeds production from cradle to factory gate. Boundaries for the meals*

170 *analysis are represented by the dot-dashed line, and each of the FA1 (380g of protein*
171 *equivalent) and FA2 feeds (1 kg of feed) by the black dashed line.*

172 ReCiPe^{43,44} methods (v.1.11) were used to calculate environmental impact indicators for all
173 impact categories except land occupation and primary production requirements (PPR) (Table
174 S1). Climate change impacts quantify all emitted greenhouse gases converted to kilograms of
175 CO₂-equivalent.⁴⁴ We excluded biogenic carbon from this analysis since any uptake of carbon in
176 crop material will quickly be digested and respired through consumption of the food items by
177 salmon and human consumers in a relatively short timespan so no true sequestration is
178 achieved.⁴⁵ Acidification impacts measure the emissions of acidifying compounds from the
179 process (SO_x, NO_x, NH₃). Aquatic eutrophication (freshwater and marine) impacts were
180 considered separately since each system is limited by different nutrients (kilograms of
181 phosphorous and nitrogen, respectively). Land occupation measures the total area of land
182 occupation (m²) per portion of a year (a) from agriculture, urban and transformation activities.⁴³
183 Freshwater consumption was measured in meters cubed of water removed from the local
184 watershed.⁴⁴ For simplicity of analysis and interpretation the land occupation and water
185 consumption indicators used here are accounting metrics and lack characterization factor
186 calculations. PPR is quickly becoming an important impact indicator in food and aquaculture
187 LCAs with developments still emerging⁴⁰ (Table S1). The calculations used here employed the
188 methods of Cashion et al. (2016), which uses Pauly and Christiansen's (1995) equation for
189 primary production requirement with updated trophic level and trophic efficiency data^{8,30,46,47}.
190 PPR was calculated for each feed ingredient and weighted sums were used to assess the total
191 PPR for each feed. Impacts from fish-based ingredients were calculated using species specific
192 data for transfer efficiencies⁴⁷ and trophic level⁸ while standard values were used for carbon

193 content³⁰ and inclusion in fishmeal and fish oil for all input fish species. PPR values for plant-
194 based ingredients were sourced from the literature⁹ and weighted based on their inclusion in each
195 feed.

196 These three analyses compare how impacts will change with replacement of soy ingredients with
197 novel SCP meals. The meal analysis compares soy protein concentrate, bacteria meal and yeast
198 protein concentrate at equal protein levels (660g protein). Since the meals have different protein
199 concentrations, the masses of meals differ for this analysis: 1kg soy protein concentrate, 0.94kg
200 bacteria meal, 1.07 kg yeast protein concentrate. The FA1 analysis is based on a standard
201 industrial salmon feed use in Norway which uses 25% soy ingredients. These ingredients are
202 directly replaced with one each of bacteria meal and yeast protein concentrate at masses that
203 maintain equal protein levels for the entire feed (Table 1). The FA2 analysis similarly replaces
204 the soy ingredients with the novel feeds, while also maintaining consistent lipid levels by varying
205 the other ingredients in the feed. Total feed masses as well as inclusion of fish ingredients,
206 fishmeal and fish oil, were held constant in the FA2 feeds (Table 1). Full product inventories are
207 documented in the supporting information file: “customProcessesLCI.xlsx” for review and
208 reuse.⁴⁸

209 Since the feed commodities market fluctuates widely, and therefore commercial feed
210 compositions as well, the standard feed used in this study was formulated based on the relative
211 amounts of feed ingredients imported by the Norwegian fish feed industry in 2016⁴⁹ and
212 balanced to meet the nutritional needs of salmon. This feed includes fishmeal and soy protein
213 concentrate as the main protein ingredients. Mineral and vitamin mixes were included at a
214 consistent rate in all feeds but comprised only 2% of the total feeds so were excluded from this
215 analysis. Globally, Norway is the largest producer of farmed salmon, so we assumed production

216 of all three feeds occurs in Oslo, Norway and transportation⁵⁰ of feed ingredients to Norway
 217 were based on the sourcing and imports data from the Norwegian government.⁴⁹

	Standard	FA1		FA2	
		Bacteria	Yeast	Bacteria	Yeast
Meals					
Soy protein concentrate	220.0	0.0	0.0	0.0	0.0
Bacteria meal	0.0	227.1	0.0	250.0	0.0
Yeast protein concentrate	0.0	0.0	257.3	0.0	250.0
Soybean meal	30.0	0.0	0.0	0.0	0.0
Non-target ingredients					
Wheat starch	90.0	90.0	90.0	122.6	92.8
Wheat gluten	90.0	90.0	90.0	55.6	86.2
Corn gluten	20.0	20.0	20.0	19.5	23.1
Sunflower meal	40.0	40.0	40.0	51.7	49.6
Fava/field beans	40.0	40.0	40.0	48.5	47.8
Rapeseed oil	180.0	180.0	180.0	162.2	160.5
Fishmeal	160.0	160.0	160.0	160.0	160.0
Fish oil	110.0	110.0	110.0	110.0	110.0
Mineral & vitamin mix	20.0	20.0	20.0	20.0	20.0
Nutrients					
Protein	381.3	381.3	381.3	381.3	381.3
Lipid	314.5	331.4	317.8	314.5	314.5
Starch	83.2	82.6	82.6	109.6	90.0

218 **Table 1:** Formulations for each feed analyzed. *Values are in grams, FA1 feeds may not sum to*
 219 *1kg. Mineral and vitamin mix was excluded from analysis.*

220 Data for our analyses were gathered from a variety of sources. Feed formulations were designed
 221 with the goal of achieving nutritional balance between the feeds. Through close collaborations
 222 with industry we understand that feed formulations change often and quickly, so we use
 223 Norwegian imports data combined with nutritional requirements to guide the formulation of the
 224 standard feed and design the novel feeds to match this nutritionally (Table 1). Data for the novel
 225 meals were more difficult to acquire. Bacteria meal data were obtained directly from a company
 226 producing bacteria meal at an industrial scale. Yeast protein concentrate production data were

227 sourced from Tallentire et al. 2018. Data for input ingredients to these feeds were mainly sourced
228 from the Gabi and Ecoinvent databases.^{51,52} Additional data were gathered from the scientific
229 literature (Table S2).

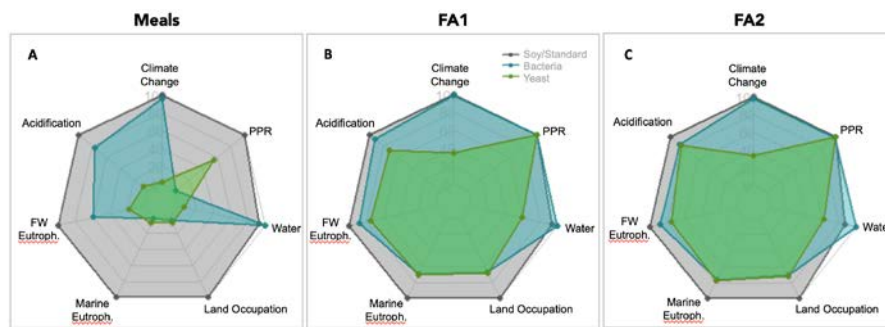
230 Economic-based allocation was used for the many inputs that are co-produced in this analysis.
231 Price allocation was employed for all input ingredients since production and use of input
232 resources is driven by the more valuable product. Also, no common nutritional allocation
233 (calories, protein content, etc.) exists for the co-produced pairs. A sensitivity analysis of
234 allocation choice compared results from economic versus mass allocations. To test sensitivity of
235 results to our assumption that production occurs in Norway, we also model production of the
236 FA1 in high salmon producing (farming) and geographically disparate locations: Chile and
237 British Columbia, Canada. Data for these analyses used imports data from each country to
238 determine the sources of feed ingredients and calculate transportation distances from the source
239 locations.^{50,53,54}

240 Life cycle assessments come with large uncertainty in data and methods, but unfortunately
241 uncertainty for this analysis was hindered by lack of actual uncertainty measures and use of
242 uniform distributions would not add to the results.

243 **Results & Discussion**

244 Based on the data used here, the novel meals are able to decrease environmental impacts of
245 protein meals and feeds compared to human food, soy-based ingredients and feeds. These
246 benefits are realized at varying degrees due to trade-offs between environmental performance
247 and nutritional quality of the SCP meals (Figure 2). For all of the impact indicators tested, yeast
248 protein concentrate had much lower impacts than soy protein concentrate. Bacteria meal was also

249 able to decrease impacts for most indicators compared to soy protein concentrate with two
 250 exceptions: climate change impacts and freshwater consumption, for which bacteria meal and
 251 soy protein concentrate had similar impacts. Low impacts of the yeast protein concentrate give
 252 the yeast-based feed lower overall impacts in the FA1 feed compared to the standard feed. The
 253 bacteria-based feed showed similar impacts to soy protein concentrate for five of the seven
 254 indicators and lower impacts in the remaining two, with impacts matching the yeast-based feed.
 255 Despite yeast protein concentrate having lower environmental impacts at the protein level, higher
 256 protein and lipid levels in the bacteria meal result in equal impacts in five of the seven indicators
 257 from their respective FA2. Low climate change and water consumption impacts of the yeast feed
 258 relative to the other two feeds make it the overall lowest impact feed for the FA2 analysis
 259 (Figures 2C and 3). Sensitivity analyses of the geographic location and allocation methods show
 260 that these assumptions only modestly affect the results and did not change the overall findings.



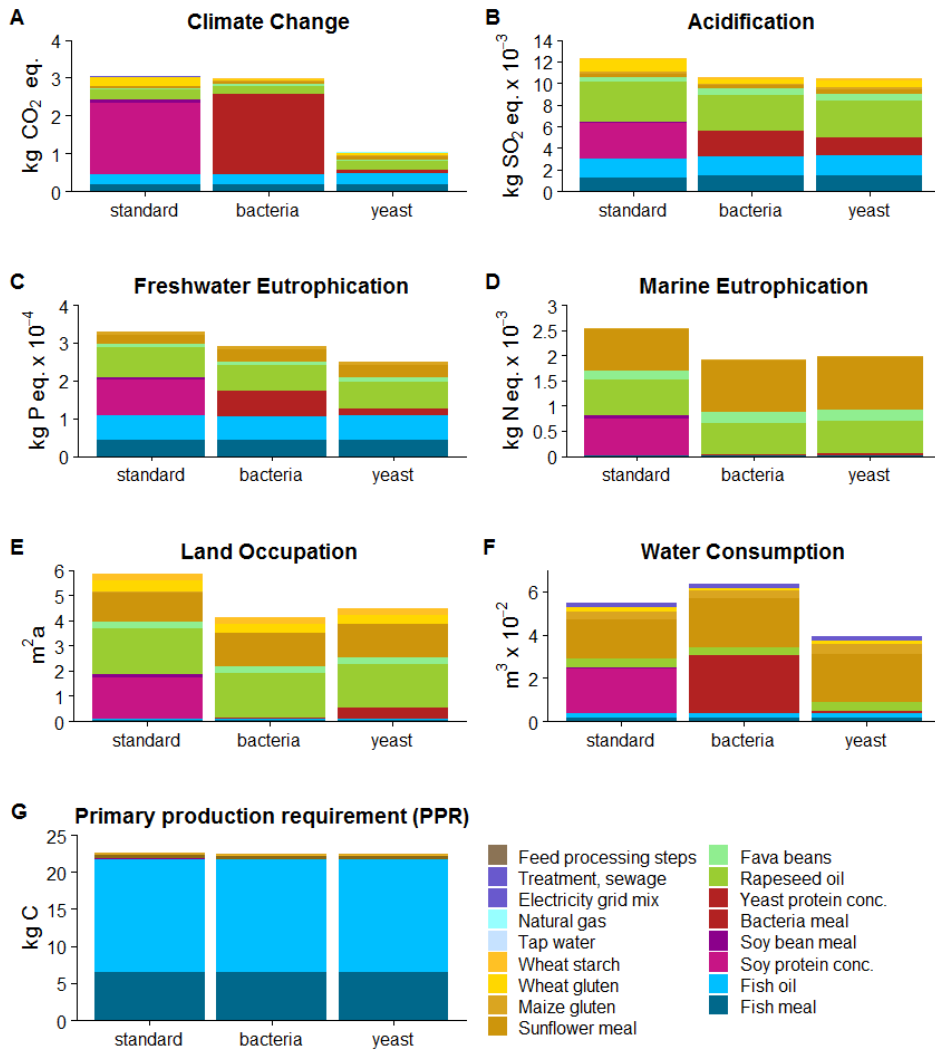
261
 262 **Figure 2:** Radar charts comparing the three (soy-based (gray), bacteria-based (blue), yeast-based
 263 (green)) meals (A) and feeds (B, C) based on seven impact indicators. Axes for each of the
 264 impact indicators: (from the top counter clockwise) climate change, acidification, freshwater
 265 eutrophication, marine eutrophication, land occupation, water consumption, primary production
 266 requirement (PPR). Results are scaled to the highest value for each indicator.

267 **Meals**

268 For all seven environmental indicators assessed, soy protein concentrate had or was among the
269 highest impacts of the three meals. Yeast protein concentrate showed the lowest impacts for all
270 indicators except primary production requirements (PPR), and had the lowest impacts overall.
271 Both SCP meals performed better than soy protein concentrate in five of the seven impact
272 indicators tested. Of these five, yeast and bacteria both had considerably lower impacts than soy
273 protein concentrate in marine eutrophication and land, with bacteria meal showing intermediate
274 freshwater eutrophication impacts; between the yeast and soy protein concentrates. These impact
275 indicators are associated with farming, which is necessary for soy production. While yeast
276 protein concentrate also uses crop-based inputs, allocation with valuable biofuels, makes these
277 impacts low for the yeast product. PPR impacts for yeast protein concentrate (0.582 kg C) are
278 therefore intermediate between bacteria meal, which does not depend on primary production at
279 all (0.00 kg C), and soy protein concentrate (1.06 kg C). While soy protein concentrate impacts
280 are also allocated with soybean oil co-production, soy protein concentrate receives a higher
281 percent of the impacts than yeast protein concentrate. In a similar trend as we saw with the
282 freshwater eutrophication impacts, bacteria meal and yeast protein concentrate both had lower
283 acidification impacts than soy protein concentrate, yeast protein concentrate causing significantly
284 lower impacts, and bacteria meal only marginally lower. Many of the differences in relative
285 impacts are likely due to the low allocation of yeast impacts in the biofuels production (both
286 economically and mass-based). Climate change impacts and water consumption were remarkably
287 similar for bacteria meal and soy protein concentrate production. Bacteria meal produces 8.26 kg
288 CO₂ eq. per 660 grams of protein and soy protein concentrate produces 8.55 kg CO₂ eq. High
289 climate change impacts in bacteria meal production are expected, given the use of methane to

290 feed the bacteria and carbon dioxide release from the cells during the fermentation phase.
291 Comparable climate change impacts in soy protein concentrate production are caused, in large
292 part, by land use changes for soy farming (64%). Water consumption was also similar for
293 bacteria meal ($1.03 \times 10^{-1} \text{ m}^3$) and soy protein concentrate ($9.56 \times 10^{-2} \text{ m}^3$), with bacteria meal
294 requiring slightly more water to produce 660g of protein. Yeast protein concentrate, in contrast,
295 had considerably lower climate change and water consumption impacts (0.21 kg CO₂ eq. and
296 $5.90 \times 10^{-3} \text{ m}^3$). Fermentation of the methanotrophic bacteria requires aqueous chemical inputs,
297 which increase the water requirements for this process (particularly calcium chloride (41%) and
298 ammonia (35%)), despite attempts by the producer to recycle water internally. Higher
299 acidification in bacteria meals is likely the result of greenhouse gas emissions from the
300 fermentation process as well, although, they are below the acidifying emissions of soy protein
301 concentrate production.

302 Requirements for cell growth are already being addressed by the feeds industry, although
303 innovations are still in development. Yeast protein concentrate producers are learning to extract
304 lignocellulose from non-human food sources such as lumber by-products to be used as a growing
305 medium for yeast cells and also testing yeast growth on sugars from fast-growing macroalgae.
306 Similarly, labs that produce methanotrophic bacteria are investigating ways to efficiently
307 sequester methane from existing sources, to create a net reduction of greenhouse gases during
308 this fermentation phase. These innovations could help further decrease the environmental
309 impacts of these SCP inputs. Since these meals would likely not be used in isolation, the impacts
310 may change when these meals are incorporated into compound salmon feeds.



311
 312 **Figure 3:** Results of environmental indicators for the FA2 feeds. Single cell protein feeds were
 313 formulated to match the standard feed for protein and lipid content, fish inclusion, and total feed
 314 mass. (A) Climate change impacts, (B) acidification potential, (C) freshwater eutrophication, (D)

315 *marine eutrophication, (E) land occupation, (F) freshwater consumption, (G) primary*
316 *production requirement.*

317 **Feeds Analysis 1 (FA1)**

318 Benefits of the novel SCP salmon feeds are significantly muted in the FA1 feed results since the
319 impacts of the target meals are low compared to impacts from the other feed ingredients. Since
320 Feed Analysis 1 holds all other ingredients constant and allows the meals to vary to meet
321 consistent protein levels, it is predictable that the soy-based standard feed would maintain the
322 highest impacts, as we saw in the meals analysis. Differences in target meal inclusion in the FA1
323 feeds were insufficient to alter which treatments had the lowest and highest impacts. In fact,
324 many of the same trends described for the meals analysis are maintained, with the notable
325 exception that PPR impacts are so dampened by the high (and equal) fishmeal and fish oil
326 inclusion in the FA1 feeds, that differences in PPR impacts between treatments are lost
327 (standard: 22.62 kg C, bacteria: 22.54 kg C, yeast: 22.55 kg C). Relatively low impacts of yeast
328 protein concentrate production lead to overall lower impacts in the yeast feeds, despite lower
329 protein content in this meal. Particularly for climate change impacts bacteria (3.25 kg CO₂ eq.)
330 and standard (3.23 kg CO₂ eq.) feeds remain about equal, and the yeast feed (1.05 kg CO₂ eq.)
331 results in much lower impacts. The other five impact indicators track similarly to the meals
332 results, again, with muted impacts relative benefits of the SCP feeds (Figure 2B). Yeast again,
333 has the lowest impacts for acidification, freshwater eutrophication, and water consumption.
334 In contrast to the meals results, bacteria had only two indicators that are considerably lower than
335 the standard feed, the remaining five are about equal to the standard feed. The bacteria feed did
336 show slightly lower acidification (1.28×10^{-2} kg SO₂ eq.) and freshwater eutrophication ($2.91 \times$
337 10^{-4} kg P eq.) compared to the standard feed (1.34×10^{-2} kg SO₂ eq., 3.31×10^{-4} kg P eq.), but

338 the relative benefits of the SCP ingredient for these metrics were diminished when included in
339 the FA2 feed. Marine eutrophication and land occupation impacts for bacteria (1.78×10^{-3} kg N
340 eq., $1.90 \text{ m}^2\text{a}$) and yeast (1.97×10^{-3} kg N eq., $1.85 \text{ m}^2\text{a}$) FA1 feeds were about equal to each
341 other and lower than the standard feed (2.55×10^{-3} kg N eq., $2.68 \text{ m}^2\text{a}$), although less
342 significantly than in the meals analysis.

343 When feeds are compared on an equal protein basis, the yeast feed results in the lowest
344 environmental impacts overall, with lower impacts than the standard feed for all indicators and
345 lower impacts than the bacteria feed for four indicators and similar results for three. The
346 bacteria-based feed also improves environmental performance compared to the standard feed for
347 marine eutrophication and land occupation, and shows similar results for the remaining five
348 indicators. Since this feeds analysis uses an equal base of non-target ingredients the results
349 closely mirror the results of the meals analysis, but show that when incorporated into a whole
350 feed, the impacts of non-target feed ingredients reduce the differences in impact. The FA1 results
351 highlight that impacts from the non-target ingredients are large compared to those of the target
352 meals, leading to relatively similar impacts across treatment feeds.

353 **Feeds Analysis 2 (FA2)**

354 To learn more about how the non-target feeds might impact the environmental impacts of salmon
355 feeds with the SCP meals, FA2 substitutes the meals on an equal mass basis and then varies the
356 other ingredients to produce feeds with equivalent protein and lipid concentrations. In FA2, the
357 impacts of the novel SCP feeds became even more similar to each other, with about equal
358 impacts for four of the seven indicators, and maintained similar or lower impacts compared to
359 the standard feed for all indicators except water consumption, for which the bacteria feed
360 exceeded the standard feed (Figure 2C). Impacts from the FA2 SCP feeds converged for

361 acidification and marine eutrophication impacts. Bi-directional shifts in acidification impacts led
362 to about equal impacts for the SCP feeds, with the FA2 bacteria feed (1.06×10^{-3} kg SO₂ eq.)
363 causing lower impacts than its FA1 counterpart and the FA2 yeast feed (1.04×10^{-3} kg SO₂ eq.)
364 causing higher impacts than in FA1. Both feeds had lower acidification impacts than the standard
365 feed. Both novel SCP-based feeds saw increases in marine eutrophication impacts compared to
366 the FA1 feeds. Increases were greater for the bacteria-based FA2 feed (1.93×10^{-3} kg N eq.),
367 which led to equal impacts (yeast: 1.98×10^{-3} kg N eq.) between the novel feeds. Water
368 consumption was also marginally higher for both SCP feed than in the previous analysis,
369 increasing the disparity between the standard and bacteria feed. It should be noted, that in the
370 FA2 analysis, bacteria meal (which has relatively high water consumption impacts) inclusion
371 was higher than in FA1, whereas yeast protein concentrate inclusion was decreased compared to
372 FA1 (Table1). Climate change impacts, PPR, freshwater eutrophication and land occupation did
373 not change compared to FA1 for any of the treatments.
374 Compared to the FA1 feeds, the FA2 feeds held the mass of target meals constant which resulted
375 in higher bacteria meal and lower yeast protein concentrate inclusion than the FA1 feed
376 compositions. Overall, the FA2 yeast feed remains the lowest impact feed due to significantly
377 lower impacts for the climate change and water consumption indicators. Based on these data,
378 these novel SCP meals are both strong alternatives to soy protein concentrate in salmon feeds
379 and improvements in these technologies could help make them even more beneficial.

380 **Sensitivity analyses**

381 Our analysis required a number of assumptions. We assumed that each of the feeds were
382 produced in Norway, which is the largest producer of farmed salmon,⁷ and therefore accounted

383 for transportation from source locations to Oslo, Norway. Fish oil and fishmeal species
384 compositions were based on weighted combinations of species caught in each source country.
385 Importantly, yeast was produced domestically in Norway, whereas both soy protein concentrate
386 and bacteria meal were produced in the Americas and therefore required much further shipping.
387 Bacteria meal was produced in the United States in this study since this is the location of a plant
388 set to open this year. Ingredient sourcing was based on recent Norwegian imports data for feed
389 production. To test the sensitivity of our results to the feed production location, we also modeled
390 the same FA1 feeds produced in Chile and British Columbia, Canada. Results from this
391 sensitivity analysis show that this model is not sensitive to the location of production (Figure
392 S1). Allocation was based on price for all co-produced ingredients. A second sensitivity analysis
393 tested the impacts of our allocation method by comparing results from an economically allocated
394 model to mass allocated results. While results varied slightly between the methods, relative
395 results between the treatments were consistent, suggesting our model is robust with regard to
396 allocation methods (Figure S2).

397 **Future work**

398 Future studies should incorporate ongoing developments in bacteria and yeast meals production
399 to assess whether these changes can further reduce the environmental footprint of SCP feeds.
400 Bacteria cells could potentially be grown using diverted methane rather than newly extracted
401 natural gas, but realized efficiencies and proof of concept have not yet been tested. Similarly,
402 work continues developing industry byproducts for yeast production rather than human-food
403 wheat inputs,⁵⁵ which could further decrease land use and primary production requirements as
404 well as outputs from farming. This analysis stops at the feed factory gate due to lack of

405 information on the effects of these novel feeds on salmon growth and waste production.
406 Following a series of robust feeding trials, a more inclusive study from cradle to grave would
407 provide a more complete picture of the impacts of these feeds.

408 The presented life cycle assessment suggests that replacing soy protein concentrate with bacteria
409 meal or yeast protein concentrate in salmon feeds has the potential to decrease the environmental
410 impacts of salmon farming in addition to easing stress on human-food resources. Tallentire et al.
411 2018 suggest that the climate change impacts of bacteria meal could be even lower than was
412 estimated here. These SCP meals are still being developed with a focus on improving efficiency
413 and reducing impacts of these novel ingredients, particularly through feeding cells byproducts
414 from other industries. Additional single celled proteins such as microalgae could prove
415 environmentally beneficial or supplement these benefits as well.^{56,57} Many of these SCPs are still
416 in the developmental stages but feed companies are rapidly developing industrial scale
417 production lines for these feed ingredients. Developments such as these are essential for moving
418 aquaculture towards the food security solution our planet needs.

419 **Supporting information:**

420 The supporting information files include a word document and two data files. The word
421 document includes a description of the impact indicators used in these analyses as well as a table
422 with the indicator definitions and descriptions and results of the sensitivity analyses: Table S1:
423 Impact categories with definitions, units and data sources; Figure S1: production location
424 sensitivity analysis results; Figure S2: Allocation sensitivity analysis results. Data file
425 “customProcessesLCI.xlsx” includes a complete description of the product inventories use to

426 generate the study results, to facilitate independent review and reuse.⁴⁸ Product impacts and data
427 for figures 2 and 3 are included in the data file, "IciaResults.xlsx"

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