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Environmental and biodiversity performance of a novel single cell protein for rainbow trout feed

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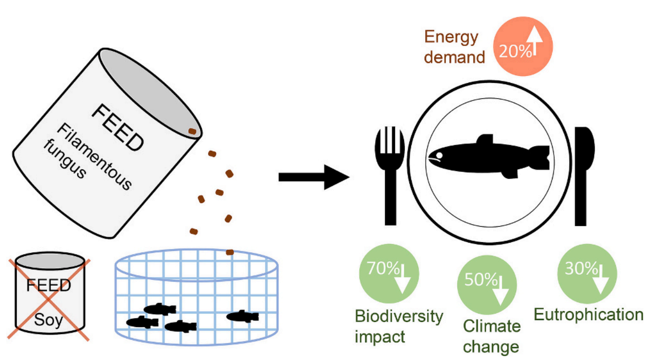
HIGHLIGHTS

- The novel feed ingredient filamentous fungus can be produced from side streams.
- Filamentous fungus protein has lower overall environmental impact than soy protein.
- A diet containing filamentous fungus protein did not affect fish growth.
- The study accounts for biodiversity impacts from exploitation and land use change.
- Omitting fishery impacts can underestimate biodiversity impact from diets and feeds.

GRAPHICAL ABSTRACT



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ABSTRACT

Seafood has an important role to play to achieve a sustainable food system that provides healthy food to a growing world population. Future seafood production will be increasingly reliant on aquaculture where feed innovation is essential to reduce environmental impacts and minimize feed and food competition. This study aimed to investigate whether a novel single cell protein feed ingredient based on *Paecilomyces variotii* grown on a side stream from the forest industry could improve environmental sustainability of farmed rainbow trout (*Oncorhynchus mykiss*) by replacing the soy protein concentrate used today. A Life Cycle Assessment including commonly addressed impacts but also the rarely assessed biodiversity impacts was performed. Furthermore, feeding trials were included for potential effects on fish growth, i.e., an assessment of the environmental impacts for the functional unit 'kg feed required to produce 1 kg live-weight rainbow trout'. Results showed that the best experimental diet containing *P. variotii* performed 16–73 % better than the control diet containing soy protein concentrate in all impact categories except for energy demand (21 % higher impact). The largest environmental benefits from replacing soy protein with *P. variotii* in rainbow trout diets was a 73 % reduction of impact on biodiversity and halved greenhouse gas emissions. The findings have high relevance for the aquaculture industry

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as the production scale and feed composition was comparable to commercial operations and because the effect on fish growth from inclusion of the novel ingredient in a complete diet was evaluated. The results on biodiversity loss from land use change and exploitation through fishing suggest that fishery can dominate impacts and exclusion thereof can greatly underestimate biodiversity impact. Finally, a novel feed ingredient grown on side streams from the forest industry has potential to add to food security through decreasing the dependence on increasingly scarce agricultural land resources.

1. Introduction

The demand for food is increasing with population growth whilst the food system needs to go through extensive transformations to keep global resource use and emissions within the planetary boundaries (Steffen et al., 2015). Currently, the food production stands for around 25 % of anthropogenic greenhouse gas emissions, occupies around 43 % of ice and desert-free land and drives around 90 % of deforestation (FAO, 2022; Poore and Nemecek, 2018). Seafood represents a promising component of healthy and more sustainable diets and could play an important role in future sustainable food security (Crona et al., 2023; Godfray et al., 2010; Tacon and Metian, 2013). Capture fisheries production volume is limited, but aquaculture production volume has exhibited an unprecedented growth rate since the 1980s up until recent years (FAO, 2018). Future growth rate is much dependent on feed innovation to minimize competition with limited wild fish resources and agricultural land (Foley et al., 2005; Froehlich et al., 2018; Troell et al., 2014).

Feed innovation is also key to mitigate a majority of environmental impacts from farmed seafood (Foley et al., 2005; Henriksson et al., 2021; Shepherd and Jackson, 2013; Willett et al., 2019) as repeatedly shown in environmental system analyses of aquaculture (Avadí et al., 2015; Henriksson et al., 2021; Pelletier et al., 2009; Silvenius et al., 2017). Since over a decade, growth of aquaculture has been enabled through increased use of feed from crops, primarily soy, replacing marine ingredients (Aas et al., 2019; Cashion et al., 2016; Shepherd and Jackson, 2013). This development has shifted the environmental burden and resource use towards use of pesticides, land transformation and water use. In addition, the expanding agriculture, especially from soy production, is a main driver of deforestation (Persson et al., 2014), that causes biodiversity loss (Lucas et al., 2021).

Use of crop ingredients such as soy has also introduced fish health issues for the farmed species caused by antinutritional substances (Francis et al., 2001). Combined with profitability and sustainability issues, this has motivated a continuous development and evaluation of novel aquaculture feed ingredients (Pelletier et al., 2018). Novel protein feed ingredients like bacteria, algae, yeast and filamentous fungi, i.e single cell proteins, have the advantage that they can be grown on substrates such as methane and carbohydrate rich waste streams (Martínez-Córdova et al., 2017). They can thus transform energy sources previously unavailable for feed production into protein feed ingredients and subsequently food. Single cell proteins as feed components have been evaluated for a multitude of fish species in regards to effect on fish growth, showing both positive and negative result (Alriksson et al., 2014; Øverland et al., 2013; Wan-Mohtar et al., 2021; Warwas et al., 2023). Though improved sustainability is often mentioned as a motive to develop single cell proteins for aquafeed, very few studies have actually included an evaluation of environmental impacts (Agboola et al., 2021). In addition, previous assessments of aquafeeds have often only included environmental evaluation of the feed itself, disregarding the important parameter of potential influence on fish growth (Couture et al., 2019; Pelletier and Tyedmers, 2007; Silva et al., 2018).

The objective of this study is to quantify the environmental pressures and resource use of aquafeeds containing the filamentous fungus *Paecilomyces variotii* grown on residual streams from a pulp mill with an integrated biorefinery. This was done through performing Life Cycle Assessment (LCA) using collected data from production of filamentous fungus, feed production and grow-out trials on rainbow trout (*Oncorhynchus mykiss*). The overall aim was to identify production hotspots as well as to evaluate if inclusion of filamentous fungus could lower the environmental footprint of salmonid feeds when replacing soy protein concentrate.

2. Material and methods

Life Cycle Assessment is an ISO standardized tool commonly used to quantify resource use and a broad suite of environmental impacts of products through their supply chain to safe-guard that impacts are not shifted between environmental impacts or production phases.

2.1. Goal and scope

The goal of this attributional LCA was to quantify the environmental impacts and hotspots from the filamentous fungus (*P. variotii*), using spent sulfite liquor from a side stream from the forest industry as substrate, and evaluate the outcome when replacing soy protein concentrate in feed for rainbow trout. The system boundary was cradle to feed plant gate for the functional unit ‘kg feed required to produce 1 kg rainbow trout’. The effect of different inclusion levels and treatment potentially enhancing nutrient uptake of the filamentous fungus ingredient was also evaluated by comparing four versions of the experimental feed to a control feed.

Environmental impacts associated with micro ingredients (e.g. vitamins and amino acids) used in the feeds were excluded. This as the Life Cycle Inventory data was insufficient and the levels included were the same across diets, thus not affecting comparability. Including the micro ingredients may however add to absolute values since they can be a hot spot for e.g., greenhouse gas emissions (Ziegler et al., 2021). Co-products arise in many stages of the life cycle, for example in the pulp production where pulp, lignosulfonate and spent sulfite liquor are different outputs of the same production. Allocation based on physical relationships was not possible as that information was lacking. The main allocation strategy was therefore economic allocation. The monetary value of spent sulfite liquor in comparison to pulp and lignosulfonate was assumed to be equal to the value of bioethanol that is presently produced from the spent liquor sulfite (González-García et al., 2011).

2.2. Life Cycle Inventory

2.2.1. Production of filamentous fungus ingredient

A pulp mill biorefinery utilizing wood from Swedish forestry to produce pulp and lignosulfonate, among other products, provided the growth substrate for the filamentous fungus. The substrate, spent sulfite liquor, is a side stream generated during cellulose production using the sulfite pulping method. Spent sulfite liquor is rich in monomeric sugars that mainly originate from hemicellulose degradation and lignosulfonates that are derived from lignin. Inventory data on spent sulfite liquor production was obtained from an LCA of the pulp mill biorefinery by González-García et al. (2011). It was confirmed with the biorefinery that the LCA was still representative for the production in 2019. The amount of spent sulfite liquor produced at the pulp mill in relation to other products was however not available and instead calculated using information directly from the pulp mill (Johanna Eriksson, Domsjö fabriker, pers. comm.).

The filamentous fungus was cultivated at demo plant scale at RISE Processum in Sweden with spent sulfite liquor as the substrate and using phosphoric acid, ammonia and potassium hydroxide as additional nutrients (Table 1). The cultivation was carried out in an aerated bioreactor with controlled temperature, pH, and oxygen level and was initiated by adding an inoculum of *P. variotii*. Sodium hydroxide was used to achieve optimal pH and high-pressure air, electricity and steam was used for mixing, aeration and heating. The electricity used for cultivation and processing was the Swedish consumption mix. The biomass was harvested from the cultivation broth by filtration and subsequently washed, dried and grinded into a powder feed ingredient. Drying was facilitated by surplus steam and heat generated in the biorefinery during production of specialty cellulose and lignosulfonate. The resource use and emissions associated are therefore already accounted for in the life cycle of spent sulfite liquor. Water used for cooling was recycled and therefore not included as a consumed input.

2.2.2. Feeds

The control diet consisted of 40 % marine ingredients, 58 % plant-based ingredients and 2 % micro ingredients such as vitamins and amino acids (Table 2). Fish meal was the main ingredient (26 %) followed by soy protein concentrate (20 %). It was confirmed with a commercial producer that the composition in the control feed matched a commercial feed for freshwater salmonids. The following experimental diets were evaluated:

- Treated 15 % FF feed: extruded filamentous fungus replaced three quarters of the soy protein concentrate (15 % of the feed in total)
- 15 % FF feed: untreated filamentous fungus replaced three quarters of the soy protein concentrate (15 % of the feed in total)
- Treated 20 % FF feed: extruded filamentous fungus replaced all soy protein concentrate (20 % of the feed)
- 20 % FF feed: untreated filamentous fungus replaced all soy protein concentrate (20 % of the feed)

The inclusion rates of filamentous fungus were determined based on the chemical composition of the ingredient and on results from pre-trials (unpublished data). Two dietary treatments included pre-extruded fungus, to evaluate a potential increase in nutrient uptake. Diets were formulated to fulfill nutrient requirement of rainbow trout and were iso-nitrogenous (crude protein content of 45.6 %) and iso-energetic (21.9 MJ/kg feed).

Background data for all other feed ingredients was collected from the agri-footprint database version 4.0 (Blonk consultants, 2017) motivated from agri-footprint providing the most comprehensive set of feed data.

Database processes were selected to best match origin, production and processing methods of ingredients in European fish feed, based on information at hand (Table S1 in Supplementary materials). For soy protein concentrate, Brazilian production was assumed as Brazil is the largest soy producer and the EU is the largest user of South American soy (Fraanje et al., 2020).

Input data on feed production through extrusion (batch size 30 kg) was gathered from the Center for Feed Technology (FôrTek) at the Norwegian University of Life Sciences who produced the feeds. Energy use for the pre-extrusion was assumed to be equal to that of the final feed production. Based on this data, the average electricity consumption during the production of feed pellets through extrusion was 168 kJ/kg feed. An additional 254 kJ of electricity was needed for drying of the pellets.

The total transport distance for 15 % and 20 % filamentous fungus protein feed was 1400 km and 1000 km respectively, the latter having lower total transport distance as it fully avoids transportation of soy from Brazil. For the control feed containing soy the transport distance was 2900 km.

Table 1
Inputs and outputs per ton dried filamentous fungus.

Inputs	
Spent sulfite liquor (m^3)	0.041
High pressure air (m^3)	0.245
Phosphoric acid (kg)	0.041
Ammonia (kg)	0.108
Sodium hydroxide (kg)	0.414
Potassium hydroxide (kg)	0.016
Electricity (kWh)	1.645
Steam (kJ)[a]	0.002
Cooling water (m^3)	0.645
Heat (MJ)[a]	4.000
Outputs	
Dried filamentous fungus (ton)	1.000
Waste water total (ton)[b]	0.100

[a] Excluded from the LCA as heat and steam come from the pulp mills own production already accounted for in upstream LCA of spent liquor.
[b] Excluded from analysis as it was recycled.

Table 2
Inclusion rates (%) of feed ingredients in the four experimental filamentous fungus diets and the control diet. FF=filamentous fungus.

Ingredients	Treated 15 % FF feed	15 % FF feed	Treated 20 % FF feed	20 % FF feed	Control feed
Fish meal, herring	26.0	26.0	26.0	26.0	26.0
Soy protein concentrate	4.0	4.0	–	–	20.0
Filamentous fungus protein	–	15.0	–	20.0	–
Treated filamentous fungus protein	15.0	–	20.0	–	–
Fish oil, herring	13.3	13.3	13.3	13.3	13.3
Wheat meal	12.0	12.0	12.0	12.0	12.0
Wheat gluten	16.0	16.0	16.5	16.5	11.0
Pea meal	6.9	6.9	5.8	5.8	9.9
Rapeseed oil	3.0	3.0	2.6	2.6	4.0
Potato starch	2.0	2.0	2.0	2.0	2.0
Micro ingredients[a]	1.8	1.8	1.8	1.8	1.8

[a] Excluded in the LCA due to lack of data.

2.2.3. Feeding trial with rainbow trout

Feeding trials were conducted on 300 rainbow trouts (mean weight 151.0 g at start), randomly allocated in triplicate groups (20 fish per tank) to 700 L tanks in a flow through system. Fish were fed in excess for 11 weeks, and data on fish growth was provided by the Swedish University of Agricultural Sciences. Fish were individually weighed at start of the trial as well as at the end (mean weight 330.2 g), and the amount of feed given and feed waste were measured in order to calculate feed use.

There were no statistically significant differences in feed conversion ratios between diets. The average feed conversion ratios varied from 1.11 to 1.23 kg feed per kg fish (Table S2 in Supplementary materials). Fish fed with the two treated filamenous fungus diets had similar feed use as the fish fed with the control diet and slightly lower feed conversion ratios compared to the untreated filamentous fungus diets.

2.2.4. Transportation and remaining background data

Transportation mode and distances for the feed ingredients to a feed production plant was approximated using a representative location for a commercial feed production plant (Stavanger, Norway). Using google maps and seadistances.com, the transport distances for all ingredients according to their country of origin of were gathered and truck transportation chosen for all transportation within Europe. For the transportation of Brazilian soy, barge ship was used as transport mode.

The Ecoinvent database version 3.5 (Wernet et al., 2016) was used for all remaining background data e.g. on energy and chemicals.

2.3. Life Cycle Impact Assessment

The impacts and indicators and respective LCIA methods included in the LCA were the commonly applied impact categories in aquaculture LCAs (Bohnes and Laurent, 2019): climate change (IPCC 2013 GWP 100a; Stocker et al., 2013), freshwater eutrophication (ReCiPe v. 1.05; Huijbregts et al., 2017), acidification (ILCD 2011 midpoint+; Posch et al., 2008; Seppälä et al., 2006), land use (CML-IA non-baseline version 3.04) and cumulative energy demand (CED version 1.11; Frischknecht and Jungbluth, 2003). Furthermore, this LCA included the impact category biodiversity loss, given that both marine and terrestrial feed production give rise to substantial biodiversity impacts. The assessment was limited to the two most important drivers of biodiversity loss globally: land use and exploitation through fishing. Two recently developed methods for assessing biodiversity loss were applied. For terrestrial feed ingredients, land use related impact was quantified using characterization factors presented in Kuipers et al. (2021). This method captures species loss for the taxonomic groups amphibians, birds, mammals and reptiles caused by occupation, transformation and fragmentation of land for anthropogenic land use. It was selected over other available methods as it includes effects of fragmentation and as it is recent. For marine feed ingredients, fishery related impacts were assessed using the method presented in Hélias et al. (2023). This method takes into account the depleted fraction and growth rate of a fish stock to capture ecosystem quality impact from capture fisheries. It is presently the most recent of Life Cycle Impact Assessment methods regarding exploitation from fishing and it is compatible with the method used to assess biodiversity impact from land use, making it suitable for this LCA. The six selected impact categories were chosen due to their importance when evaluating a feed with ingredients deriving from agriculture, forestry and fisheries. Ecotoxicity impact was excluded as no impact assessment method that could capture the effects of all pesticides used was found. The LCA software SimaPro 9.0.0.48 was used for modelling for all impacts except for those related to biodiversity. Biodiversity impact from land use on global scale was calculated using average approach characterization factors for ecoregions (see Table S1 in Supplementary material for information on ecoregions selected). For soy, a weighted mean between the two ecoregions Cerrado and Alto Paraná Atlantic Forest was used as they are the two most important ecoregions for soybean production in terms of area (46 and 33 % respectively; Lucas et al., 2021). Land transformation area associated with the crop ingredients was gathered from the Blonk LUC impact tool and from Ecoinvent for Swedish wood for sulfite liquor production.

2.4. Treatment of uncertainty

Numerous types of uncertainty can influence the reliability of LCA results. The approaches to deal with uncertainty were selected based on the nature of the most important sources of uncertainty in the study and depending on data availability and limitations of the modelling software.

Primary data and data that most influenced the results were feed conversion ratios and inventory data related to filamentous fungus and soy protein concentrate. Capturing the variability and quality of that data was therefore prioritized. However, the primary data available for filamentous fungus production as well as a part of the secondary data were lacking distributions and consequently did not allow for probabilistic simulation such as Monte Carlo. For feed conversion ratios for the different diets, data on spread around the means was available, and impacts were calculated for the lowest and highest feed conversion ratios achieved for each diet.

For soy protein concentrate, climate impact is highly variable depending on whether the production is associated with land transformation and whether the greenhouse gas emissions from that land transformation like deforestation are taken into account (Persson et al., 2014). Furthermore, allocation strategy always influences LCA results, and in this case, side-streams formed the base of the new ingredient to be evaluated. Choosing economic allocation generally favors co-products, and there are guidelines available on preferred allocation strategies for different production systems. Handling of climate impacts related to land use together with choice of allocation method for feed ingredients were therefore identified as modelling choices with large influence on results. Their impact on the results was thus evaluated with a sensitivity analysis including four important modelling choices:

- Consequences of the allocation strategy for dividing burden between co-products for all crop and marine feed ingredients. In the sensitivity analysis, allocation based on mass was applied instead of allocation after economic value.
- The effect from exclusion of the greenhouse gas emissions from land transformation.
- The impact on results from allocating all environmental burden from pulp production to pulp and none to spent sulfite liquor. In the studied system, spent sulfite liquor is used for bioethanol production and therefore carries the burden from upstream activities in the main results. However, spent sulfite liquor is generally a less used waste stream in pulp production (Humpert et al., 2019) and should, according to the Renewable Energy Directive (Swedish Energy Agency, 2012), not be burdened with any environmental pressures from upstream processes as it is not a main product of pulp production.
- Potential effect on results from a future upscaled production that might have to rely on additional energy for cultivation and drying of filamentous fungus. In this scenario, heat and steam required did not come from surplus energy from the pulp mill (main results) but had to be produced and added as an additional input.

3. Results and discussion

3.1. Life Cycle Impact Assessment

The production activity that contributed most to the impact from filamentous fungus on climate change, freshwater eutrophication and acidification was the production of sodium hydroxide used for adjusting pH in the cultivation process (Fig. 1). Sodium hydroxide production is heavily dependent on electricity which in turn contributes to environmental impacts through production, especially if produced from fossil sources (Wernet et al., 2016). The land area required to produce filamentous fungus protein as well as the biodiversity impact from land use was almost exclusively associated to the spent sulfite liquor which relies on forestry. The most energy consuming parts of the life cycle was electricity used for cultivation and the energy used to produce spent sulfite liquor (Fig. 1).

Protein level was lower in the filamentous fungus (57 % of dry weight) compared to the soy protein concentrate used in the feeding trials (64 % of dry weight). However, per kg protein, filamentous fungus was still associated with lower environmental impacts than soy protein concentrate in terms of climate change, freshwater eutrophication, acidification and biodiversity impact from land use, and required smaller land area. The energy demand was on the other hand higher for filamentous fungus protein, although almost half of the energy from the production in Sweden was from renewable sources (Table 3).

The filamentous fungus diets were associated with lower impacts per functional unit than the control diet on climate change, freshwater eutrophication, acidification, land use and biodiversity impacts from land use and fisheries (Table 4). The extra energy required for the treatment of filamentous fungus protein used on two of the experimental diets had negligible effect on the impacts measured (less than 1 %). The slightly higher average fish growth for treated filamentous fungus protein thus resulted in lower impacts. The 20 % treated filamentous fungus diet resulted in the lowest impacts of all experimental diets except in regards to energy demand (Table 4). This diet compared to the control diet performed 50 % lower for climate change, 30 % for eutrophication,

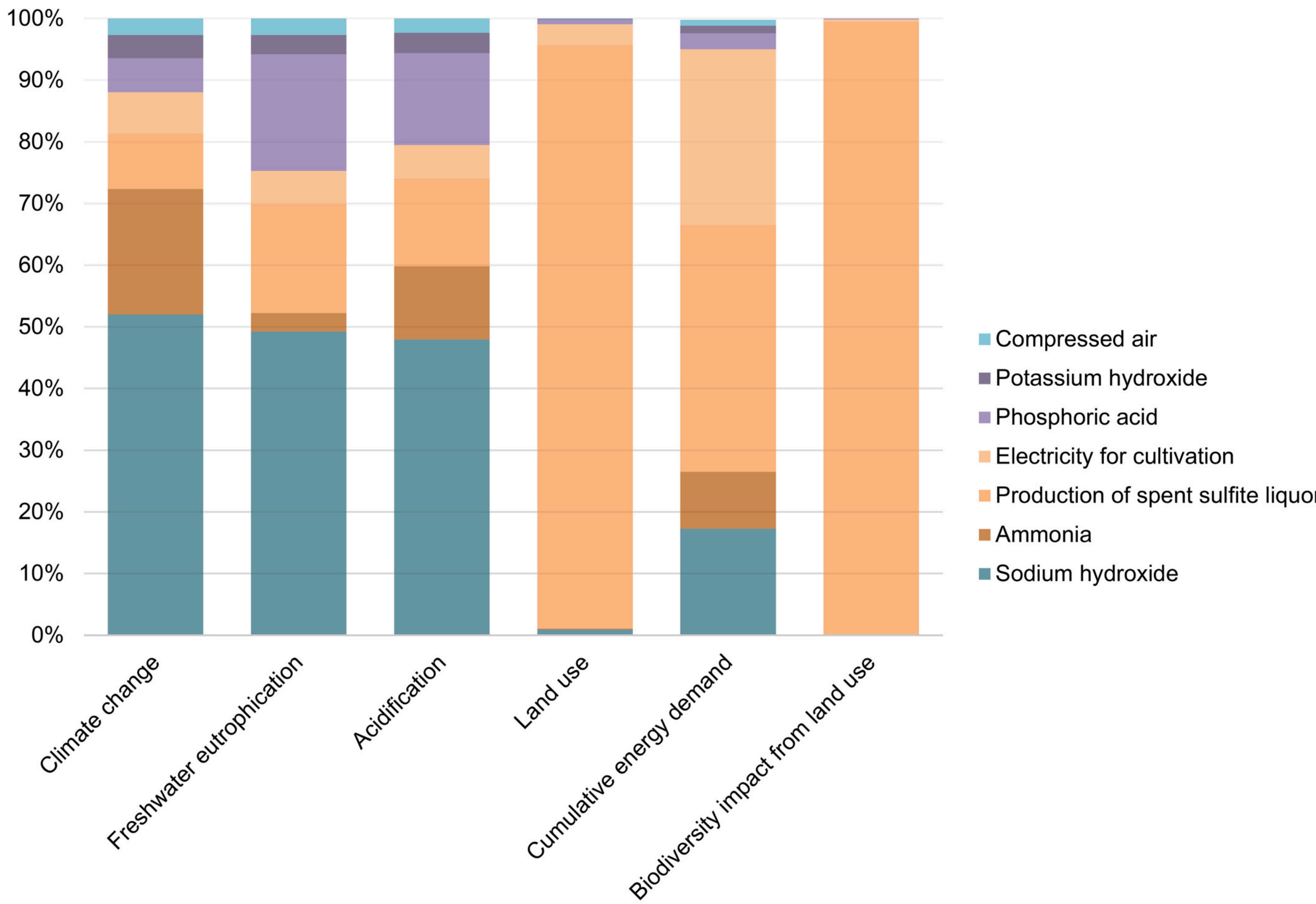

Fig. 1. Relative contribution to environmental impacts and indicators from different production inputs per kg filamentous fungus.

Table 3
Impact assessment results per kg protein of filamentous fungus and soy protein concentrate from Brazil.

Impact/indicator	Unit	Filamentous fungus protein	Brazilian soy protein concentrate
Climate change	kg CO_2 eq	1.89E+00	1.05E+01
Freshwater eutrophication	kg P eq	1.18E-03	1.56E−03
Acidification	molc H+ eq	1.24E−02	1.56E−02
Land use	m^2a	5.16E+00	6.50E+00
Cumulative energy demand, non-renewable	MJ	4.79E+01	3.49E+01
Cumulative energy demand, renewable	MJ	3.86E+01	3.82E+00
Biodiversity impact from land use	PDF_{glo}	4.84E−15	2.26E−14

16 % for acidification, 26 % for land use and 73 % for biodiversity impact from land use and fisheries (Fig. 2). The control diet scored better in one impact category, cumulative energy demand (Table 4), as the energy requirement for producing the filamentous fungus feed was 21 % higher compared to soy concentrate (Table 3). The higher energy demand may increase environmental impacts but the magnitude and pressure depend highly on the energy source, e.g., coal or hydropower. The filamentous fungus was produced with close to 50 % renewable energy as a result of the Swedish electricity mix having a large share of renewables, thus keeping impacts of the extra energy use required low.

Soy protein concentrate alone was the dominating driver of impacts on climate change, freshwater eutrophication and biodiversity impacts from land use and fisheries in the control feed per functional unit (Fig. 2). The filamentous fungus ingredient was the driver of impacts on freshwater eutrophication and cumulative energy demand but generally had lower impacts than soy protein concentrate resulting in the overall lower impact of that diet.

The biodiversity impact from land use of the filamentous fungus from Sweden was remarkably low in comparison to that of soy from Brazil considering that the difference in land use area was much smaller. The combined impact on biodiversity from land use and fisheries show that the impact from the soy protein concentrate surpassed the impact of the marine ingredients fish meal and fish oil from the herring fishery. For the experimental diet lacking soy, marine ingredients however accounted for 80 % of the total biodiversity impact.

3.2. *Uncertainty and sensitivity analysis*

Impacts were calculated for the lowest and highest feed conversion ratios achieved for each diet in addition to the average feed conversion ratios. When comparing the lowest (most efficient) feed conversion ratio achieved for the control feed with the highest (least efficient) feed conversion ratio achieved for the 20 % treated filamentous fungus diet, the results remained unchanged in regards to which feed performed best in the different impact categories.

The sensitivity analysis showed that changing four crucial modelling choices did not alter the relative results regarding which diets scored higher or lower in the different impact categories and indicator. The magnitude of differences in environmental footprint between rainbow

Table 4
Impact assessment results for kg feed required to produce 1 kg rainbow trout for five different diets (range based on min and max feed conversion ratio in brackets). FF=filamentous fungus.

Impact/indicator	Unit	15 % treated FF diet	15 % FF diet	20 % treated FF diet	20 % FF diet	Control diet
Climate change	kg CO2 eq	1.56 (1.43–1.95) E+00	1.73 (1.63–2.3) E+00	1.32 (1.22–1.58) E+00	1.42 (1.36–1.92) E+00	2.62 (2.48–3.17) E+00
Freshwater eutrophication	kg P eq	2.94 (2.70–3.13) E−04	3.26 (3.07–3.39) E−04	2.78 (2.58–2.88) E−04	2.99 (2.87–3.17) E−04	3.95 (3.74–4.09) E−04
Acidification	molc H+ eq	1.15 (1.06–1.22) E−02	1.28 (1.20–1.33) E−02	1.11 (1.03–1.15) E−02	1.20 (1.15–1.27) E−02	1.33 (1.26–1.37) E−02
Land use	m2a	1.52 (1.40–1.62) E+00	1.68 (1.59–1.75) E+00	1.44 (1.34–1.49) E+00	1.56 (1.49–1.65) E+00	1.96 (1.85–2.03) E+00
Cumulative energy demand, non-renewable	MJ	1.98 (1.82–2.11) E+01	2.19 (2.06–2.27) E+01	2.05 (1.90–2.12) E+01	2.19 (2.10–2.32) E+01	1.93 (1.83–2.00) E+01
Cumulative energy demand, renewable	MJ	4.37 (4.02–4.65) E+00	4.79 (4.51–4.98) E+00	5.54 (5.15–5.74) E+00	5.92 (5.67–6.26) E+00	1.12 (1.06–1.16) E+00
Biodiversity impact from land and fishery	PDF_{glo}·year	5.46 (5.02–5.80) E−15	6.05 (5.71–6.30) E−15	3.60 (3.34–3.73) E−15	3.89 (3.73–4.11) E−15	1.34 (1.27–1.39) E−14

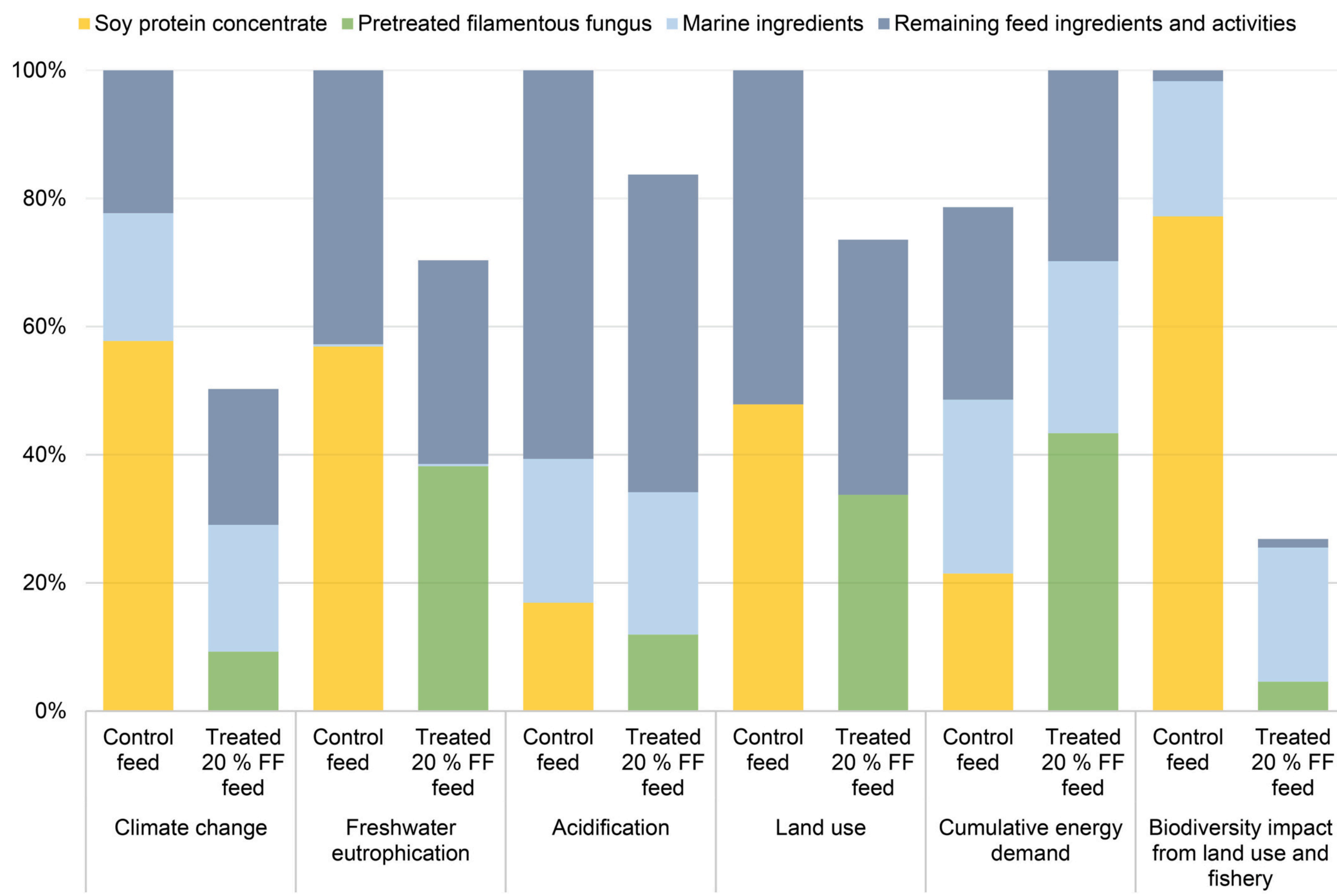

Fig. 2. Relative impact and ingredient contribution per kg feed required to produce 1 kg rainbow trout for control diet and best performing experimental diet.

trout fed the two diets however changed (Table 5). The modelling choice with the most pronounced effect on the outcome was whether to include or exclude greenhouse gas emissions from land use change. Exclusion of those emissions lowered the climate change impact for the control diet with 49 %, decreasing the difference between the control diet and 20 % treated filamentous fungus to 4 % instead of 50 %.

3.3. Influence by land use change modelling and biodiversity impact methods

One important benefit of replacing soy protein with filamentous fungi in rainbow trout diets was the halved greenhouse gas emissions. The outcome is however highly dependent on which type of soy is used. Without including emissions associated to deforestation in South America for soybean farming, the greenhouse gas emissions are only slightly decreased (4 %) when replacing soy with the filamentous fungus *P. variotii*. We argue that it is more correct to include emissions from land use change for soybeans. Firstly, the production areas where land use change is an issue (Brazil, Argentina and Paraguay) produce more than half of all soybeans globally, with the EU being the main export market (Fraanje et al., 2020). Secondly, the certification schemes in place to prevent transformation of natural land into farmland only guarantee that no land transformation has taken place after 2008, even though a large part of the change has taken place as recently as 2000-

Table 5
Sensitivity analysis showing changes from main results in %. GHG = greenhouse gas, LUC = land use change, FF = filamentous fungus.

Scenario	Products	Climate change	Freshwater eutrophication	Acidification	Land use	Cumulative energy demand	Biodiversity impact from land use and fishery
Mass allocation	Control diet	−9 %	−16 %	−9 %	−20 %	−4 %	−8 %
	20 % treated FF diet	−2 %	−9 %	−12 %	−14 %	0 %	+4 %
GHG emissions from LUC excluded	Control diet	−49 %	–	–	–	–	–
	20 % treated FF diet	−2 %	–	–	–	–	–
0 % allocation to spent liquor	Control diet	–	–	–	–	–	–
	20 % treated FF diet	−2 %	−10 %	−2 %	−43 %	−17 %	−16 %
Heat & steam included	Control diet	–	–	–	–	–	–
	20 % treated FF diet	+7 %	+4 %	+3 %	0 %	+5 %	+1 %

2015 (Fraanje et al., 2020). Related to this, there is an ongoing discussion within LCA research how to account for greenhouse gas emissions from land use change (Persson et al., 2014), and consequently, carbon footprints reported for soy are highly variable (Poore and Nemecek, 2018). We address this uncertainty through the sensitivity analysis. Thirdly, the production of spent sulfite liquor is dependent on wood from forestry in Sweden, and land use change emissions from that forestry were included in this LCA. Land use change caused by forestry in Sweden is not well documented but it is estimated that since 2003 as much as 19 % of clear-cuts occurred in natural previously uncut forests (Ahlström et al., 2022). This highlights the issue of baselines and methodological aspects of assessing land use change in LCAs.

The concerns about land use change are not limited to effects on greenhouse gas emissions. Land use change is also the largest driver of biodiversity loss in terrestrial ecosystems (IPBES, 2019). The second largest driver of biodiversity loss globally, and the largest driver in marine ecosystems, is overexploitation (Jaureguiberry et al., 2022), mainly from fishing. The inclusion of land use and fisheries related biodiversity impact in this study is an attempt to better account for this important ecosystem impact from aquafeeds that has so far been left out in LCAs of aquaculture (Bohnes and Laurent, 2019).

Concerns have been raised regarding the negative biodiversity impacts from land use both for the South American soy production industry as well as Swedish forestry. The results from this study showed a 73 % reduction in ecosystem impacts from land use and fisheries per functional unit when replacing Brazilian soy protein concentrate with filamentous fungus grown on side streams from Swedish forestry. It is notable that the difference in land use area was only 26 %. As ecosystem impact from fisheries was equal between diets, the large difference in ecosystem impact is thus explained by more severe impact from land occupation, transformation and fragmentation in Brazil in comparison to Sweden. Development of methods to capture biodiversity or ecosystem impacts is presently accelerating and impact assessment methods are still in their infancy. It should be noted that the impact on several taxa, e.g. plants, insects and fungi is not captured by the method used (Kuipers et al., 2021); hence future evaluations using new methods currently under development might yield different results. The newly developed method used to assess ecosystem impacts from fisheries, Hélias et al. (2023), has limitations in terms of effects captured and geographical and temporal resolution. The characterization factors are based on data from the year 2015 and are on a geographic area level (FAO major fishing area) that for some species encompasses more than one distinct fish stock of the same species. Using the proposed method, the resolution can however be improved through tailoring characterization factors using more detailed underlying fish stock data if available. It should further be noted that additional impacts need to be taken into account in order to capture all major impacts of fisheries on biodiversity e.g. seabed damage and altered species composition. Despite the current limitations, the results on biodiversity impacts from fishing presented in this study highlights the importance of including additional drivers of biodiversity loss besides land use. For the experimental diet, the impact on biodiversity from fisheries largely exceeded land use related impacts. The results thus show that leaving out marine biodiversity impacts such as fisheries impacts when evaluating diets or feeds can lead to large underestimations of the impact on biodiversity and misidentification of the most efficient interventions to reduce impacts.

3.4. Perspectives and relevance for up-scaled operations

Feed has repeatedly been shown to be the key driver of environmental impacts from farmed fish in LCA studies, making reduction of impacts from feed an effective action to reduce impacts from farmed fish overall (Avadí et al., 2015; Henriksson et al., 2012; Pelletier et al., 2009; Silvenius et al., 2017). As an example of magnitude, feed represented 75-83 % of the climate change impact of Atlantic salmon and rainbow trout farmed in Norway, including life cycle impacts all the way to the wholesaler (Winther et al., 2020).

Salmonids, being a carnivore with high protein feed requirements may not be the first choice when identifying future sustainable aquaculture species. However, salmonid production volume is growing (FAO, 2018) and it is a popular seafood commodity in wealthier countries such as Sweden and other European countries (EUMOFA, 2019; Ziegler and Bergman, 2017). As such, it is a product that generates a particularly high monetary value and may hereby function as an important driver of sustainable feed development that the whole feed sector could benefit from. Due to the current commercial interest in salmonids, improvement potentials to current production systems may also be particularly important.

When interpreting the results from this study, it is important to take into consideration that the feed conversion ratios observed in this study are lower than what can be expected from a commercial grow-out. This is primarily due to that the fish did not reach slaughter size and growth rate decreases with size and age of the fish (Tlusty et al., 2011). In addition, economic feed conversion ratio was not considered, i.e., fish mortality and feed loss were excluded from the calculation of the feed conversion ratios used.

The results of this study are more conservative in environmental benefits found compared to another LCA of single cell protein as a feed ingredient for salmonids (Couture et al., 2019). Six times lower climate change impact per kg protein was presented in Couture et al. (2019). The difference in results is likely explained by both methodological differences between the studies as well as the differences in growth substrates and nutrients used to cultivate the single cell proteins. In comparison with LCA results for other commonly used conventional ingredients, the novel protein source from this study however still displays overall a lower environmental footprint (Silva et al., 2018). To produce filamentous fungi in sufficient volumes for commercial feed manufacturers to be interested, additional substrates than spent sulfite liquor might be needed, for example other abundant residues such as saw dust. Other substrates could require additional process steps which might influence the environmental footprint.

Future areas of research for this feed concept are investigations of fish and gut health, potential to increase protein level and quality of the fungus and potential effect on quality and taste of the final fish product. Other relevant aspects that would merit from further investigations are ecotoxicity effects. It was not included here as no LCA methods were found that could capture the potential effects of all pesticides used in soybean farming. Replacing soy with filamentous fungus would decrease ecotoxicity from pesticides but could potentially increase ecotoxicity linked to other activities such as electricity and chemical production (González-García et al., 2011; Wernet et al., 2016).

4. Conclusion

Replacing soy protein concentrate in salmonid feed with the single cell protein *P. variotii* was shown to decrease environmental pressures for all aspects considered (climate change, eutrophication, acidification, land use and biodiversity impacts) except for energy demand. To improve the environmental performance of *P. variotii*, focus should be on finding alternatives to resource demanding chemicals used in cultivation and on reducing electricity consumption in production. The feed and production system studied here shows high relevance to current commercial aquaculture production due to the feed composition and scale of the production. Furthermore, data from feeding trials were included to examine potential effect on fish growth, an aspect often overlooked in environmental evaluations of novel feed ingredients.

This study accounts for biodiversity impacts from the two largest drivers of biodiversity loss globally by applying two recently developed methods to capture both land use change related biodiversity loss as well as exploitation related biodiversity loss from fishing. Methods to account for marine biodiversity impact have so far largely been missing and impacts have consequently been omitted from LCAs (Crenna et al., 2020). Our results showed that the biodiversity impact from the fishery greatly overshadowed land use related impact for the experimental diets. This suggests that limiting biodiversity loss accounting to the impacts from land use change can lead to false conclusions on a systems biodiversity hotspots and their magnitude.

Finally we conclude that the novel single cell protein feed ingredient *P. variotii* has potential to decrease many environmental pressures of farmed rainbow trout when replacing soy protein concentrate and add to food security through further decoupling from increasingly scarce agricultural land resources.

CRediT authorship contribution statement

Kristina Bergman: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Anna Woodhouse:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Markus Langeland:** Investigation, Writing – review & editing. **Aleksandar Vidakovic:** Investigation. **Björn Alriksson:** Investigation, Resources, Writing – review & editing. **Sara Hornborg:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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