



Towards circularity in aquaculture systems: Environmental impact of *Hermetia illucens* meal inclusion in diets for rainbow trout reared in aquaponics

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ABSTRACT

In advancing towards more sustainable aquaculture, the inclusion of insect meals in aquafeeds has significant potential to increase circularity and reduce environmental impacts, especially in aquaponics. Nevertheless, there is a lack of information regarding the environmental performance of these innovative feeding and management solutions. This study assessed the environmental impact associated with the dietary inclusion of *Hermetia illucens* (HI) meal (0%, 6% and 12%) in diets for rainbow trout (*Oncorhynchus mykiss*) reared in a low-tech aquaponic system characterised by nine independent experimental units (6.06 kg/m³ of fish and 14 strawberry plants per unit). A cradle-to-gate attributional life cycle assessment model was used to consider the impacts related to the whole aquaponic system (AQ-FISH) and those only related to the production and use of the aquafeed (AQ-FEED). The impact categories were 100-year global warming (kg CO₂-eq) – with (GWP_{LUC}) and without (GWP) the emissions associated with land-use change (LUC), acidification (AP, g SO₂-eq) and eutrophication (EP, g PO₄-eq) potentials, cumulative energy demand (CED, MJ), land occupation (LO, m²/y) and water scarcity (WS, m³-eq). The functional unit was a 1 kg live weight increase of rainbow trout. Data originated from a previous 76-d fish performance trial. Data regarding HI meal were derived from an interview with the manufacturer. An economic method was used to partition the impacts between HI meal, HI fat and the frass (exhausted substrate). The effect of the HI meal inclusion level on the AQ-FISH and AQ-FEED impact values was tested using one-way analysis of variance.

A 1 kg live weight increase of rainbow trout reared in the aquaponic system (AQ-FISH) produced 15.6 kg CO₂-eq (GWP), 18.3 kg CO₂-eq (GWP_{LUC}), 67 SO₂-eq and 55 g PO₄-eq and used 354 MJ, 3.5 m²/y and 311 m³-eq. The dietary inclusion of HI meal did not affect the AQ-FISH results, except for CED (+2%–5%). When considering the impact resulting from feed production (AQ-FEED), the inclusion of HI meal did not affect GWP_{LUC}, AP, EP and LO, but it negatively affected GWP (+13%–26%) and CED (+34%–68%). Results from AQ-FISH and AQ-FEED scenarios showed low sensitivity to the methodological choices. Overall, our findings suggest that general improvements to reduce the environmental impact associated with rainbow trout production in aquaponics should be concurrently directed towards both the system setting and management, as well as towards the HI meal production process, with a particular emphasis on enhancing energy efficiency. To minimise potential trade-offs, especially from an environmental perspective, future studies should prioritise the investigation of energy use and greenhouse gas emission associated with feed production. This requires a thorough evaluation of all changes in the diet formulation resulting from the inclusion of a new ingredient.

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Abbreviations

AQ-FEED	Life cycle assessment model considering the impact due to the aquafeed used in the aquaponic system
AQ-FISH	Life cycle assessment model considering the impact due to the whole aquaponic system
CED	cumulative energy demand
EP	eutrophication potential
GWP_LUC	global warming potential plus emission due to land use change
GWP	global warming potential
HI-LCA	Life cycle assessment model to assess the impact associated with the production of <i>Hermetia illucens</i> meal
HI	<i>Hermetia illucens</i>
LO	land occupation
OM	organic matter
SE	system expansion
WB	wheat bran

1. Introduction

Food production is projected to increase by 35%–56% by 2050 relative to 2010 (van Dijk et al., 2021). At the same time, the negative effects of food production systems on the natural environment have steadily aroused several concerns in recent years (Steffen et al., 2015; IPCC, 2023). These trends have significant implications for aquaculture, the production of which has increased strongly in the last decades, mainly because of the decreased availability of wild fish stocks (FAO, 2022). In this context, aquaponic systems that integrate aquaculture and hydroponic cultivation in a single water- and nutrient-recirculating system have emerged as a promising sustainable solution to meet the growing demand for seafood products (Joyce et al., 2019; Baganz et al., 2022).

Numerous studies have explored the environmental effects of farmed fish in aquaculture and aquaponic systems, considering various conditions, system settings and fish species (see Bohnes et al., 2019, for a comprehensive review). Recent literature highlights that aquafeed production stands as a primary contributor to the environmental impact of farmed fish. In addition, heavy reliance on fish meal and fish oil to produce aquafeeds exerts increasing pressure on wild fish stocks, leading to the limited availability of these ingredients and stricter regulations on their harvest (FAO, 2020a). Consequently, using the life cycle assessment (LCA) (ISO, 2006) as a standard recognised method to evaluate product impact throughout its life cycle (Vidergar et al., 2021), researchers have explored the feasibility of substituting fish meal and oil with alternative vegetable- or animal-based aquafeed ingredients by adopting attributional (Boissy et al., 2011; Nhu et al., 2016; Smáráson et al., 2017) and consequential (Smetana et al., 2019; Bordignon et al., 2023) LCA approaches.

Among the emerging alternatives to fish meal, one of the most promising options is insect meal. Insect meals derived from species such as *Hermetia illucens*, *Musca domestica* and *Tenebrio molitor* have a favourable nutritional profile, good acceptance and feasible use at the commercial level. Among these species, the meal obtained from *Hermetia illucens* prepupae (HI) shows great potential as it has an essential amino acid profile similar to that of fish meal (Henry et al., 2015) and larvae have a high adaptability to low-cost substrates obtained by food by-products and wastes (Gasco et al., 2020). In addition, HI meal production is expected to leave a small ecological footprint as it requires limited arable land and water and it releases a low amount of greenhouse gases and ammonia (Tran et al., 2022).

Nevertheless, research on the environmental implications of insect meal inclusion in aquafeeds remains poorly investigated. A recent

review assessed the environmental consequences of insect meal inclusion in aquafeeds by retrieving data of fish performance, aquafeed composition and LCA-based impact values from literature (Tran et al., 2022). Although aquafeed has been identified as a significant contributor to the environmental impact associated with aquaculture production, the extent of this contribution varies depending on the specific impacts and the farming system considered (Aubin et al., 2009; Bordignon et al., 2022b; Zoli et al., 2023). Consequently, focusing solely on impacts due to aquafeed may be restrictive, and only few studies have directly assessed the overall environmental impact of fish production systems using insect-based aquafeeds. Notable examples include studies on rainbow trout (Wind et al., 2022) and salmon (*Salmo salar* L.) (Goglio et al., 2022) reared in flow-through aquaculture systems.

The inclusion of insect meal in aquafeed used for aquaponic farming has been recently discussed as a promising strategy for the sustainable intensification of aquaculture (Bordignon et al., 2022a; Campanati et al., 2022; Colombo et al., 2022). Aquaponics, which integrates recirculating aquaculture with hydroponics, represents an ideal farming technique for water treatment and nutrient recycling in a closed-loop system, reducing nutrient waste, water consumption and land use. For these reasons, aquaponics is emerging as a potential key player in the framework of a circular bio-economy (Das et al., 2022). In this context, the use of insect meals as a protein source emerges as another potential contributor in closing the loop, transforming the nutrient losses back into the agri-food chain in the form of protein-rich animal feed (Ojha et al., 2020). However, the environmental advantages resulting from the integration between aquaponic systems and the inclusion of insect meals in aquafeeds remain unexplored.

Therefore, the aim of this study was to assess the effect of the partial substitution of fish meal with HI meal in the aquafeed on the environmental impact associated with rainbow trout reared in an aquaponic system. Primary data were obtained from a previous experimental trial performed by our research group (Bordignon et al., 2022a). Then, we performed a specific LCA to quantify the environmental impact of HI meal. This assessment relied on primary data obtained through an interview with the manufacturer, and any lack of data was addressed through scenario analysis.

2. Materials and methods

The LCA model was constructed on the basis of the scheme described by the ISO standards 14040–14044 (ISO, 2006). An attributional cradle-to-gate LCA approach was adopted to compare the environmental impact of a conventional rainbow trout aquafeed with that of an alternative one characterised by the inclusion of HI meal as a protein-rich ingredient fed to rainbow trout reared in an aquaponic system.

The system boundaries (Fig. 1) included the environmental impact associated with the production of aquafeeds and other inputs needed for the aquaponic system functioning, the rainbow trout rearing and the background impact related to the breeding phase of the rainbow trout (from birth to the initial body weight in the aquaponic system) (AQ-FISH, cradle-to-gate model). Because the inclusion of HI meal modified the aquafeed composition but not the functioning of the aquaponic system, a second model (AQ-FEED, partial cradle-to-gate) was used to focus on the impacts derived from the production and use of the aquafeed in the aquaponic system.

The impact categories assessed were the following: global warming potential (horizontal time: 100 years; kg CO₂-eq) with (GWP_LUC) and without (GWP) the emissions associated with the land-use change (LUC), acidification potential (AP, g SO₂-eq), eutrophication potential (EP, g PO₄-eq), cumulative energy demand (CED, MJ), land occupation (LO, m²/y) and water scarcity (WS, m³-eq). The functional unit was a 1 kg live weight increase in rainbow trout. Although the aquaponic system provided not only fish but also a vegetable co-product, the different dietary treatments did not involve any modifications in the management of the vegetable part of the aquaponic system and in the vegetable yield

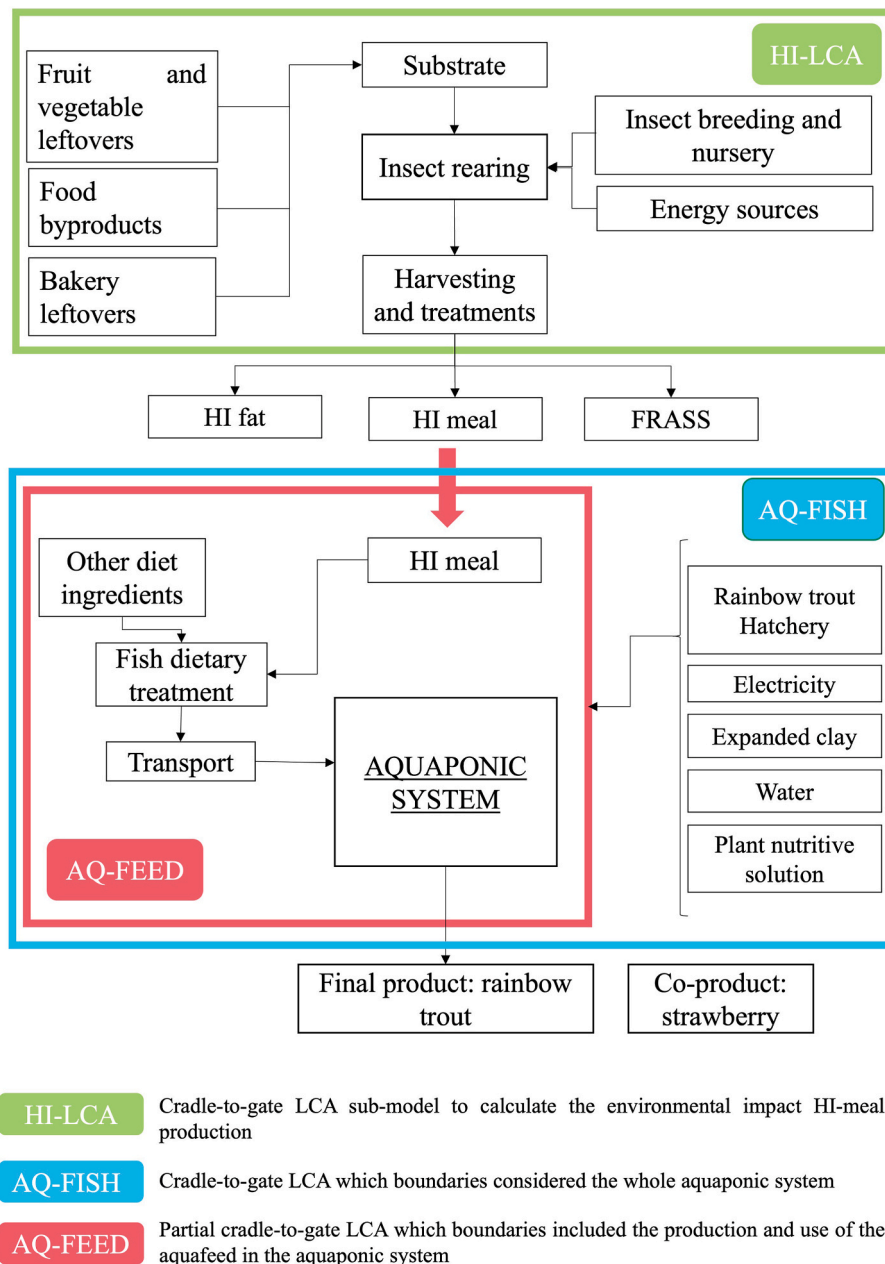


Fig. 1. System boundaries for the HI meal production (HI-LCA) and for the rainbow trout production in an aquaponic system (AQ-FISH and AQ-FEED).

obtained. For this reason and given that fish was the targeted product of the system, no impact was allocated to the vegetable part and the whole impact was attributed to the fish output. Nevertheless, for completeness, we also provide the impact values per kg live weight increase in rainbow trout after the allocation of the whole impact between fish and strawberries based on economic values (4.8 and 4.5 /kg for fish and strawberries, respectively) retrieved for fish from Bordignon et al. (2022b) and for strawberries from BMTI (2022).

2.1. LCA of rainbow trout production in aquaponics

The model used to assess the environmental impact of the inclusion of HI meal in the aquafeed for rainbow trout reared in aquaponics was derived from Bordignon et al. (2022b) based on the experimental trial settings reported in Bordignon et al. (2022a). All data used for the LCA analysis of rainbow trout production in aquaponics (i.e. aquafeed composition and intake, fish initial and final body weights, mortality

and aquaponic system setup) were obtained from a previous experimental trial of our research group (Bordignon et al., 2022a) and reported in Table 1. Briefly, the aquaponic system was installed in a plastic greenhouse with 50% shading. The experimental setup comprised nine independent aquaponic units, each consisting of a fish tank (volume, 500 L; height, 0.80 m), a sedimenter (volume, 100 L; height, 0.60 m), two tanks for hydroponic cultivation (volume, 275 L each; height, 0.35 m) and a storage tank (volume, 50 L; height, 0.45 m). The tanks used in the trial were constructed from high-density polyethylene. The aquaponic units were designed as 'low-tech', featuring a simple hydroponic section that also acted as a bio-filter. The system had no energy consumption for water temperature regulation and minimal environmental control (lacking continuous water evaluation probes, remote management systems and water sanitation devices such as UV and ozone chambers). Water flow within the system was ensured through overflow, moving from the main fish tank to the plant tanks and then to the storage tank. A flow rate of 120 L/h allowed complete water turnover every 5 h.

Table 1
Inventory of the experimental trial per dietary treatment (H0, H6 and H12 diets) (modified from Bordignon et al., 2022a).

Variable	Unit	Diet		
		H0	H6	H12
Initial fish biomass per tank	kg/m ³	6.07 ± 0.7	6.03 ± 0.6	6.07 ± 0.5
Tanks	N	3	3	3
Trial duration	D	76	76	76
Fish body weight at 0 days	g/fish	158	160	154
Fish body weight at 76 days	g/fish	310	313	285
Feed conversion ratio		1.50	1.54	1.55
Aquafeed, ingredients per treatment				
Fish meal (CP 73% DM)	g/kg	200	150	100
Hermetia illucens meal	g/kg	0	62	124
Gelatinised starch, D500	g/kg	150	138	126
Corn gluten meal	g/kg	119	119	119
Soybean (SB) meal	g/kg	215	215	215
SB protein concentrate	g/kg	70	70	70
Porcine haemoglobin	g/kg	30	30	30
Wheat flour	g/kg	55	55	55
Fish oil	g/kg	70	70	70
Soybean oil	g/kg	70	70	70
Hydrolysed krill	g/kg	5	5	5
Mineral premix	g/kg	2.5	2.5	2.5
Vitamin premix	g/kg	2.5	2.5	2.5
DL-methionine	g/kg	8	8	8
L-lysine	g/kg	3	3	3
Aquaponic system set up				
Clay	kg	4.05	4.05	4.05
Greenhouse	m ²	0.46	0.46	0.46
Nutrient solution	kg	0.11	0.11	0.11
Electricity consumption	kwh	82	82	82
Water (evaporated)	L	97	97	97
Hatchery, eggs	N	31	31	31

H0: control diet including 0% of *Hermetia illucens* (HI) meal; H6: diet including 6% of HI meal; H12: diet including 12% of HI meal.

Water oxygenation was facilitated by a porous stone connected to an aerator (Scubla D100; Scubla Srl, Remanzacco, Italy) positioned in the main fish tanks. In all aquaponic units, the hydroponic sub-unit was destined for the cultivation of strawberries (*Fragaria x ananassa* Duch). Three aquaponic units were assigned to each of the three dietary treatments.

A total of 173 rainbow trout (initial body weight: 156 ± 40 g) were allocated in the nine main tanks (initial biomass, 6.06 ± 0.6 kg/m³; balanced among tanks) during a 76-d feeding period. Three experimental diets were formulated to be iso-nitrogenous and iso-energetic. These included a control diet without HI meal (H0) that contained 200 g/kg of fish meal and no HI meal. Then, two alternative diets were prepared: diet H6, containing 150 g/kg of fish meal and 62 g/kg of HI meal; diet H12, containing 100 g/kg of fish meal and 124 g/kg of HI meal. Because of the lower crude protein content of HI meal (60.5% dry matter; DM) compared with that of fish meal (74.3% DM), HI meal was included at higher rates than the substituted fish meal. In addition, the level of included gelatinised starch was slightly adjusted. The main information on the inventory regarding the aquaponic system, fish rearing and ingredients of the aquafeeds is reported in Table 1 and described in detail by Bordignon et al. (2022a).

Nitrogen (N) and phosphorous (P) input–output balance was computed according to Cho and Kaushik (1989), with excretion calculated as the difference between intake (from aquafeed) and retention in fish body weight. The net nutrient release in the water was computed by subtracting the plant nutrient uptake from the fish excretion. The strawberry fruit yield (21 ± 4 g/100 g fish) was derived from Bordignon et al. (2022a) and nutrient content (N: 0.14%; P: 0.28%) from CREA (2019). The strawberry vegetative portion was assumed equal to fruit yield, and its nutrient content was derived from Nestby et al. (2005). Electricity consumption was calculated as fish biomass growth multiplied by kWh/kg fish factor, obtained from Bordignon et al. (2022b). Fish background data for the period from hatching to the initial body

weight of the trial were derived from the Ecoinvent v3.7 database (Wernet et al., 2016). Furthermore, we assumed that aquafeed and fish transport distance from the producers to the aquaponic system was equal to 200 km (average distances covering the area of the Po valley where the main producers are located). The impact factors of the different inputs were derived from the Ecoinvent v3.7 (Wernet et al., 2016), Agri-footprint v5.0 (Blonk Agri-footprint, 2020), and Agribalyse (Colomb et al., 2015) databases (see Supplementary Table S1).

Regarding life cycle impact assessment, the single emissions and contributions were standardized to the common unit of the correspondent impact category (e.g. conversion of the single greenhouse gases – GHG – into the common unit associated with GWP category, kg CO₂-eq). We derived the characterisation factors for GWP from Myhre et al. (2013) (CO₂:1, biogenic CH₄: 28; fossil CH₄: 30 and N₂O: 265); those for AP, EP and LO from CML-IA (CML, 2016); then, we used the method CED v1.11 (Frischknecht et al., 2003) for CED and AWARE method (Ansoorge and Beránková, 2017) for WS. Both the databases with impact factors and the characterisation methods were implemented in the Simapro v9.3 software.

2.2. LCA of HI meal production

A sub-model was used to estimate the impact associated with the HI meal used in the trial (1 kg as a functional unit), also in compliance with the LCA recommendations to use primary data, whenever possible, for impact evaluations (ISO, 2006; FAO, 2020b). An interview was conducted with the HI meal manufacturer to obtain the input data. To be compliant with AQ-FISH and AQ-FEED models, the questionnaire was set to cover all stages of insect rearing (reproduction and larvae rearing) and processing to obtain the meal using a cradle-to-gate approach and to allow the computation of the same impact categories (GWP, GWP_LUC, AP, EP, CED, LO and WS).

The primary data about the production inventory (obtained from the manufacturer) regarded several production cycles occurred in 2021–2022 and included average information regarding the amount of substrate (fresh and dry weight) used to feed insects, the list of ingredient types included in the substrate and the amount of fresh insect larvae, HI meal, HI fat and frass obtained at the end of the production cycle, along with their respective chemical composition. The detailed inventory of all data used for LCA and associated with the production of 1 kg HI meal per production cycle based on both manufacturer information and literature is reported in Table 2.

Because of a non-disclosure agreement, a complete inventory from the manufacturer could not be compiled. To address this, we supplemented the unavailable primary data with information from scientific literature. Regarding the substrate composition, the inclusion percentage of each ingredient type (vegetable plus fruit leftovers, food by-products – wheat bran (WB) and brewery grains – and bakery leftovers) was estimated based on the DM content of each ingredient type. An optimization model (Solver Add-in in Microsoft Excel) was used to obtain the inclusion percentage of each ingredient type so that the estimated DM content of the substrate was equal to that reported by the manufacturer (30%). The inclusion percentage of WB and brewery grains in the food by-products ingredient type was based on experts' suggestions (25% and 75% of the food by-product ingredient type, respectively) (see Supplementary Table S2). The DM content of vegetable plus fruit leftovers was obtained from the manufacturer, and the DM contents of WB, brewery grains and bakery leftovers were obtained from FEDNA tables (de Blas et al., 2021). To complete the inventory, data on insect reproduction and larvae rearing stages were derived from Bava et al. (2019) and data on the amount of energy (thermal energy and electricity) needed for the insect rearing and post-harvesting processes were obtained from Smetana et al. (2019) and Spykman et al. (2021). Machinery and equipment were not considered because of a lack of data.

Furthermore, HI larvae rearing is a multi-functional process because HI fat and exhausted rearing substrate (frass) were co-produced with HI

Table 2
Inventory associated with the production of 1 kg of *Hermetia illucens* meal.

Variable	Unit	Value	Reference
<i>Breeding and nursery</i>			
Eggs	n	3400	Bava et al. (2019)
Substrate	kg	0.04	Bava et al. (2019)
Aquafeed, transport	tkm	0.2	Bava et al. (2019)
Electricity (insect rearing, feed management)	kwh	0.41	Smetana et al. (2019); Spykman et al., 2021
Thermal energy (insect rearing, feed management)	MJ	0.43	Smetana et al. (2019); Spykman et al., 2021
<i>Insect grow-out phase</i>			
Insect larvae	kg	5	Insect meal manufacturer
Substrate	kg	25	Insect meal manufacturer
Substrate, transport	tkm	2.5	Insect meal manufacturer
Electricity (insect rearing, feed management)	kwh	5.52	Smetana et al. (2019); Spykman et al., 2021
Thermal energy (insect rearing, feed management)	MJ	1.08	Smetana et al. (2019); Spykman et al., 2021
Electricity (meal extraction)	kwh	1.32	Smetana et al. (2019); Spykman et al., 2021
Thermal energy (meal extraction)	MJ	7.70	Smetana et al. (2019); Spykman et al., 2021
<i>Insect outputs</i>			
Protein meal	kg	1.0	Insect meal manufacturer
Oil (fat)	kg	0.4	Insect meal manufacturer
Frass	kg	5.0	Insect meal manufacturer
<i>Frass composition</i>			
Organic matter	%	51	Insect meal manufacturer
Nitrogen	%	2	Insect meal manufacturer
P ₂ O ₅	%	2	Insect meal manufacturer
K ₂ O	%	3	Insect meal manufacturer

meal. Therefore, the whole impact associated with HI-LCA was partitioned among these three outputs (HI meal, HI fat and frass) by applying an economic allocation method, in accordance with several previous studies (Salomone et al., 2017; Ites et al., 2020; Maiolo et al., 2020). Economic values were derived from Maiolo et al. (2020), i.e. frass with null economic value and HI meal and HI fat equal prices.

Regarding the impact computation of the inventory data, the impact factors associated with the different inputs were derived from the Ecoinvent v3.7 (Wernet et al., 2016) and Agri-footprint v5.0 (Blonk Agri-footprint, 2020) databases. In particular, vegetable and fruit leftovers, as well as bakery leftovers, were assumed to have a negligible environmental impact as their environmental burden was attributed to the activities that generated them. Conversely, WB and brewery grains are products useable in animal feeding (and WB also as human food). Consequently, they are associated with an impact, economically allocated with respect to their production supply chain, according to the PEF standard for feed (FEFAC, 2018). Methane emissions related to organic waste and insect rearing were not included, in accordance with Salomone et al. (2017). Life cycle impact assessment was performed using the same procedure described in Section 2.1 for trout production in aquaponics.

2.3. Sensitivity and uncertainty analysis

The LCA model and the associated inventory data for evaluating the impact of HI meal production were subjected to uncertainty arising from the lack of few primary data and to variability related to available primary data. To address this, we performed an uncertainty analysis on the HI-LCA model using the Monte Carlo analysis (1000 iteration) by Simapro software v.9.3. The uncertainty range for the energy consumption values (thermal and electrical) was derived from Smetana et al. (2019) (range: $\pm 25\%$), and the uncertainty for insect substrate composition was $\pm 10\%$ (as reported by the manufacturer). The insect breeding and nursery phase lacked uncertainty data, so we applied the same uncertainty range as that for energy values ($\pm 25\%$).

The impact results could also be influenced by the choices operated in the computation of HI-LCA, such as the method to resolve the multi-

functionality of HI meal production, the estimated inclusion rate of WB in the food by-product ingredient used for the insect substrate and the potential effect of the uncertainty analysis output obtained for HI-LCA on the results for AQ-FISH and AQ-FEED. Consequently, the following four sensitivity scenarios were designed to increase the robustness of the impact results with respect to the baseline scenario (economic allocation, WB included at 25% in the food by-product ingredient type used in the insect substrate):

- 1) SENS_SE: Resolution of HI-LCA multi-functionality using the system expansion method, a method used in some previous studies dealing with LCA evaluation of insect meal production (Bava et al., 2019; Smetana et al., 2019; Spykman et al., 2021). In this approach, nutrients (nitrogen, phosphorous and potassium) in the frass co-product were assumed to substitute the nutrients in the mineral fertiliser with a 0.5:1 ratio (i.e. 1 kg nitrogen in the frass substitutes and 0.5 kg in the mineral fertiliser). Conversely, the use of HI fat is still underexplored, despite emerging applications as an ingredient in animal feed formulation (Schäfer et al., 2023) and in cosmetic production (Verheyen et al., 2023). For this reason, the partition of the impact between HI meal and HI fat (excluding the avoided impact due to frass) was based on the same allocation factors obtained for economic allocation.
- 2) SENS_OM: Resolution of HI-LCA multi-functionality using an allocation based on the organic matter content of different outputs. Unlike economic allocation and system expansion, which rely on external factors, this method uses an internal reference based on organic matter content.
- 3) SENS_WB: Inclusion level of wheat bran increased from 25% to 50% of the food by-product ingredient type used in the insect substrate.
- 4) UNCERT_HI: On the basis of the results of the uncertainty analysis applied on HI-LCA, impact values per 1 kg HI meal were modified as impact value $\pm 2SD$, where SD was the standard deviation obtained from the Monte Carlo analysis for each impact category.

The SENS_SE, SENS_OM and SENS_WB sensitivity scenarios were performed on HI-LCA, AQ-FISH and AQ-FEED models, whereas UNCERT_HI was performed on AQ-FISH and AQ-FEED only.

Allocation factors for the three outputs (HI meal, HI fat and frass) obtained from insect production in the baseline and sensitivity scenarios related to the different multi-functionality resolution methods are detailed in Table 3.

2.4. Data quality evaluation

Data quality was evaluated using the pedigree matrix proposed by Weidema et al. (2013), which explores qualitative aspects related to reliability, completeness and temporal, geographical and technological correlations, using scores from 1 (indicating the best quality) to 5 (the least favourable). The pedigree matrix and the comprehensive data quality evaluation are reported in Supplementary Table S3. Briefly, a

Table 3

Allocation factors (%) to resolve the multifunctionality of *Hermetia illucens* (HI) production.

Partitioning method	HI meal	HI fat	Frass
Organic matter	32	14	54
Economic	71	29	0
System expansion			
Global warming potential (GWP)	57	23	20
GWP plus emissions due to land use change	57	23	20
Acidification potential	37	15	48
Eutrophication potential	58	24	18
Cumulative energy demand	64	26	10
Land occupation	65	26	9
Water scarcity	17	7	76

cradle-to-gate approach was used to assess all main impact categories of interest (see Tran et al., 2022). Both primary and secondary data were systematically collected and retrieved to ensure consistency with this approach and to encompass all primary production processes. Data related to the experimental trial in the aquaponic system and HI meal production were directly collected on-field or documented through interviews. Energy consumption for insect production, which was covered by a non-disclosure agreement, was obtained from literature sources.

The insect substrate formula was accurately estimated using the data provided by the HI meal manufacturer. Whenever feasible, inventory sheets from the Ecoinvent, Agri-footprint and Agribalyse databases were selected to align with the origin and technological setup of the inputs. Furthermore, uncertainty analysis was conducted to address uncertainties associated with secondary data sourced from literature and databases.

2.5. Statistical analysis

A hotspot analysis (EC, 2010) was performed to assess the contribution of the different impact sources within each impact category. Impact values associated with the AQ-FISH and AQ-FEED models were analysed using a GLM model (PROC GLM; SAS, 2013) to test the effect of the inclusion level of HI meal in rainbow trout feed (three levels) on the environmental impact associated to rainbow trout reared in aquaponics. The Bonferroni's test was used to compare least square means. Differences between least square means with $P < 0.05$ were assumed to be statistically significant.

3. Results

3.1. Environmental impact of HI meal production (HI-LCA)

The impact category values obtained for 1 kg of HI meal are reported in Table 4. The production of 1 kg of HI meal to feed rainbow trout in the aquaponic system generated an average emission of 2.48 kg CO₂-eq (GWP and GWP_LUC), nearly 6 g SO₂-eq (AP) and 3.3 g PO₄-eq (EP) and was associated with the utilisation of 84 MJ of energy (CED), 0.45 m² of land (LO) and 0.2 m³-eq of water (WS). The uncertainty associated with the impact category values was generally low (coefficient of variation, CV, lower than 13%), except for CED (CV: 33%) and especially WS (CV: 3830%).

3.2. Environmental impact of rearing rainbow trout in aquaponics

The descriptive statistics of the environmental impact associated with a 1 kg live weight increase of rainbow trout reared in the aquaponic system (AQ-FISH) are reported in Table 5. On average, a 1 kg increase in fish live weight was linked to 15.6 kg CO₂-eq (GWP), 18.3 kg CO₂-eq (GWP_LUC), 67 g SO₂-eq (AP), 55 g PO₄-eq (EP), 354 MJ (CED), 3.5 m² (LO) and 311 m³-eq (WS). Notably, almost 85% of these impacts were

Table 4

Impact category values for 1 kg of *Hermetia illucens* meal and results of uncertainty analysis.

Impact category ¹	Unit	Value	Uncertainty analysis			
			CV (%)	2.50%	97.50%	SEM
GWP	kg CO ₂ -eq	2.48	9.9	1.99	2.95	0.01
GWP_LUC	kg CO ₂ -eq	2.48	9.9	1.99	2.95	0.01
AP	g SO ₂ -eq	5.98	12.9	4.6	7.6	0.02
EP	g PO ₄ -eq	3.28	13.8	2.5	4.3	0.01
CED	MJ	84.2	33.2	41.5	148.0	0.9
LO	m ² /y	0.45	9.3	0.38	0.54	0.00
WS	m ³ -eq	0.206	3830.0	-17.4	14.2	1.1

GWP: global warming potential; GWP_LUC: GWP plus emissions due to land use change; AP: acidification potential; EP: eutrophication potential; CED: cumulative energy demand; LO: land occupation; WS: water scarcity.

Table 5

Descriptive statistics of the impact (values expressed per 1 kg live weight increase of rainbow trout) associated with the rainbow trout reared in aquaponics (whole system: AQ-FISH model; considering only the impact due to feed production: AQ-FEED).

Impact category	Unit	AQ-FISH		AQ-FEED	
		Mean	SD	Mean	SD
GWP	kg CO ₂ -eq	15.64	0.23	1.67	0.19
GWP_LUC	kg CO ₂ -eq	18.27	1.78	3.75	0.28
AP	g SO ₂ -eq	66.71	7.07	9.49	0.54
EP	g PO ₄ -eq	54.81	3.96	6.16	0.39
CED	MJ	354	29	29	7
LO	m ² /y	3.45	0.14	2.44	0.14
WS	m ³ -eq	311	7	1.20	0.07

GWP: global warming potential; GWP_LUC: GWP plus emissions due to land use change; AP: acidification potential; EP: eutrophication potential; CED: cumulative energy demand; LO: land occupation; WS: water scarcity.

attributed to fish and 15% to strawberry when allocating outputs within the aquaponic system (Supplementary Table S4). The variability attributed to different tank units was minimal (CVs ranging from 1% to 10%).

When considering only the impact due to aquafeed production as a focus on the aspect – i.e. the diet composition – that changed between the three theses (AQ-FEED), a 1 kg live weight increase of rainbow trout caused the emission of 1.7 kg CO₂-eq (GWP), 3.8 kg CO₂-eq (GWP_LUC), 9.5 g SO₂-eq (AP) and 6.2 g PO₄-eq (EP) and the exploitation of 29 MJ (CED) of energy, 2.4 m² (LO) of land and 1.2 m³-eq (WS) of water.

The contribution of each source of impact (i.e. system setup, feed and transport) to each impact category obtained in the AQ-FISH model is reported in Fig. 2. Electricity consumption emerged as the most significant contributor to GWP, GWP_LUC, AP and CED (73%–86% of the whole impact). The other primary contributions were associated with aquafeed production (approximately 10% for GWP and CED, 14% for AP and 20% for GWP_LUC) and the setup of the aquaponic system (i.e. clay, starting nutrient solution and greenhouse), accounting for 5%–68% of the GWP, GWP_LUC, AP and CED categories. Conversely, EP was mainly influenced by nutrient release in water (55%) and electricity consumption (32%), and aquafeed production and the aquaponic system setup were limited to 5%–6% of the total impact. Regarding LO, aquafeed production showed the largest contribution (70%) and the remaining contribution was substantially represented by electricity consumption (26%). Conversely, the hotspot pattern associated with WS was quite different from all other impact categories because almost all impacts were related to the breeding and hatchery phases of the rainbow trout (98%).

3.3. Environmental impact of HI meal inclusion in aquafeed for rainbow trout reared in aquaponics

The results of the ANOVA for the impact values associated with the production of 1 kg of the different aquafeeds used to feed rainbow trout in the aquaponic system are shown in Table 6. The inclusion of HI meal in the H6 and H12 diets resulted in a significant increase in the values of all impact categories compared to the H0 diet, except for WS. Specifically, the H0 diet showed significantly and remarkably lower GWP, GWP_LUC and CED values than the H6 diet (–10%, –4% and –25%, respectively), which, in turn, showed significantly lower values than the H12 diet (–10%, –5% and –20%, respectively). In terms of the AP, EP and LO categories, the differences were statistically significant, aligning with an equal ranking observed for GWP, GWP_LUC and CED, although the relative differences were of a lower magnitude (up to 1%).

The results of the ANOVA for the impact values related to the AQ-FISH and AQ-FEED models are reported in Table 7. Regarding AQ-FISH, dietary treatment did not affect any impact category, except for CED. Concerning CED, the H12 diet was associated with the highest

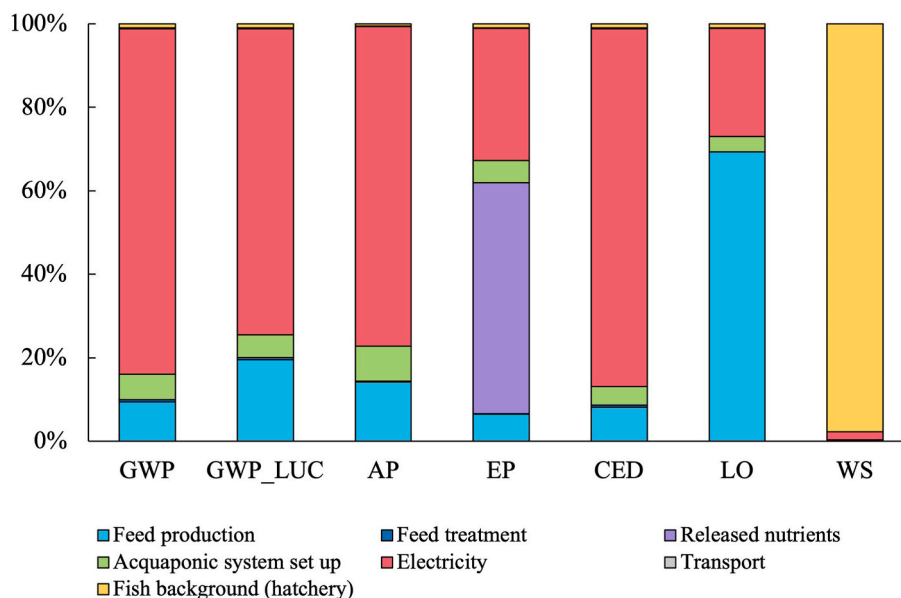


Fig. 2. Hotspot analysis for the aquaponic system. GWP: global warming potential; GWP_LUC: GWP plus emissions due to land use change; AP: acidification potential; EP: eutrophication potential; CED: cumulative energy demand; LO: land occupation; WS: water scarcity.

Table 6

Results of ANOVA (F-value, P-value, root mean square error – RMSE and least square means) for the impact category values for 1 kg of the different aquafeeds utilized as dietary treatment in the aquaponic system (variability distribution obtained with 1000-iterations Monte Carlo analysis).

Impact category	F	P	RMSE	Diet		
				H0	H6	H12
GWP, kg CO ₂ -eq	42.7	<0.001	0.05	0.93	1.03	1.15
GWP_LUC, kg CO ₂ -eq	44.8	<0.001	0.05	2.29	2.395	2.51
AP, g SO ₂ -eq	35.8	<0.001	0.12	6.18	6.22	6.24
EP, g PO ₄ -eq	810.5	<0.001	0.05	4.01	4.05	4.11
CED, MJ	2048.4	<0.001	2.29	13.5	18.0	22.5
LO, m ²	11.8	<0.001	0.03	1.58	1.59	1.60
WS, m ³ -eq	0.3	0.71	3.17	0.78	0.78	0.76

GWP: global warming potential; GWP_LUC: GWP plus emissions due to land use change; AP: acidification potential; EP: eutrophication potential; CED: cumulative energy demand; LO: land occupation; WS: water scarcity. H0: conventional diet, H6: diet including 6% insect meal, H12: diet including 12% insect meal.

value, significantly higher than that of the H6 diet (+3%), which, in turn, was significantly higher than H0 diet CED (+2%) (Fig. 3).

Conversely, for AQ-FEED, the dietary treatment had a notable impact, the degree of which varied according to the impact category. In detail, GWP_LUC, AP, EP, LO and WS were not significantly affected by the level of HI meal inclusion in rainbow trout aquafeed. Conversely, GWP was statically affected, with the use of H12 diet producing a higher GWP per 1 kg live weight increase in rainbow trout compared to the H0 diet (+26%), and the use of the H6 diet showed an intermediate value (Fig. 4a). In addition, CED was significantly influenced by HI meal dietary inclusion: the use of the H0 diet was associated with the lowest CED (Fig. 4b), the use of the H6 diet exhibited a 35% higher CED than H0 and the use of the H12 diet resulted in a 25% increase compared to the H6 diet.

3.4. Sensitivity analysis

The results of the sensitivity scenarios, namely, SENS_SE, SENS_OM and SENS_WB, performed on the impact category values associated with HI meal production are reported in Fig. 5. Both the selection of the method used to address the multi-functionality of the HI meal

Table 7

Results of ANOVA (F-value and P-value and root mean square error - RMSE) for the impact category values for 1 kg live weight increase of rainbow trout in the aquaponic system (whole system: AQ-FISH model; only impact due to aquafeed production: AQ-FEED model).

Impact category	Unit	AQ-FISH			AQ-FEED		
		F	P-value	RMSE	F	P-value	RMSE
GWP	kg CO ₂ -eq	2.54	0.16	0.14	10.05	0.01	0.10
GWP_LUC	kg CO ₂ -eq	0.45	0.66	0.27	2.93	0.13	0.23
AP	g SO ₂ -eq	0.72	0.52	7.3	0.43	0.67	0.59
EP	g PO ₄ -eq	0.12	0.89	4.5	1.29	0.34	0.38
CED	MJ	26.77	<0.001	2.6	46.81	<0.001	1.87
LO	m ² /y	0.50	0.63	0.15	0.48	0.64	0.15
WS	m ³ -eq	0.61	0.57	30.5	0.42	0.67	0.07

RMSE: Root mean square error; GWP: global warming potential; GWP_LUC: GWP plus emissions due to land use change; AP: acidification potential; EP: eutrophication potential; CED: cumulative energy demand; LO: land occupation; WS: water scarcity.

production and the inclusion level of WB in the insect substrate influenced the impact category values. Specifically, the use of the organic matter allocation method nearly halved all impact category values. Conversely, the system expansion method resulted in a variegated decrease across impact categories, ranging from -20% (GWP and GWP_LUC) to -146% (WS). Then, HI meal production contributed negatively to WS, indicating a reduction in the pressure on water resources. Conversely, SENS_WB was associated with a general impact increase, ranging from +9% (CED) to +140% (LO).

By expanding the perspective and considering these three sensitivity scenarios on the entire rainbow trout production system (AQ-FISH) (Fig. 6) and on the impact due to feed production in the aquaponic system (AQ-FEED) (Fig. 7), the results showed similar trends within each impact category (a decrease due to the use of organic matter allocation or system expansion and an increase due to the higher WB inclusion level in the insect substrate). However, the results diverged from those

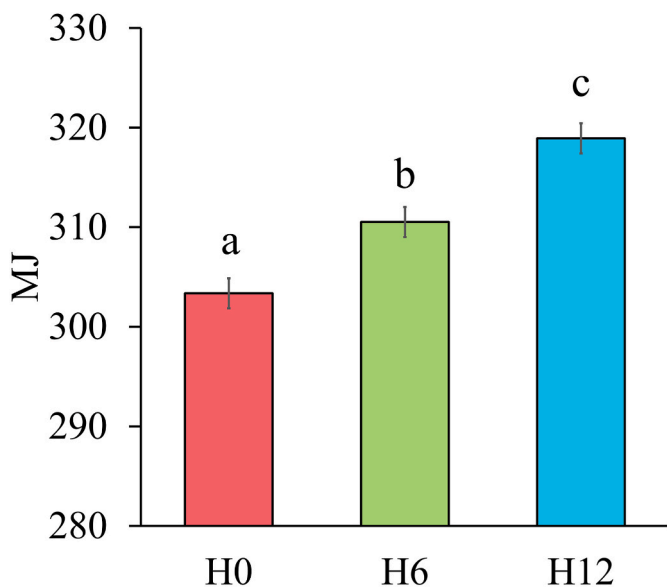


Fig. 3. Least square means of cumulative energy demand (MJ) per 1 kg live weight increase of rainbow trout according to the AQ-FISH model.

observed for HI meal production alone as the absolute change in the AQ-FISH impact values ranged only from -3% to +3% compared to the baseline scenario (Fig. 6). Regarding AQ-FEED, the absolute changes with respect to the baseline scenario were of low magnitude for all categories, except for CED (up to 10%), and CED observed a decrease of approximately 15% and 20% in SENS_OM compared to the baseline scenario (Fig. 7).

When the SENS_SE, SENS_OM and SENS_WB sensitivity scenarios were analysed using the same ANOVA test for the baseline scenarios, the results on AQ-FISH and AQ-FEED did not show any differences compared to the baseline (all detailed results are in Supplementary Tables S5-S7).

Concerning the fourth sensitivity scenario (UNCERT_HI; Fig. 8), the AQ-FISH impact values per unit of rainbow trout increase were slightly influenced by the increase or decrease in the impact related to 1 kg of HI meal (from -3.3% to +3.3%). Conversely, AQ-FEED impact values resulted affected by the increase or decrease in the impact related to 1 kg of HI meal, but depending on the single impact category considered. In particular, the CED category showed variations of nearly 20% and 23% (H6 and H12 diet, respectively) and WS of nearly 161% and 202% (H6 and H12 diet, respectively). When this sensitivity scenario was used for testing the effect of the diet on the impact categories associated with AQ-FISH and AQ-FEED, in most cases, no significant difference was observed with respect to the baseline scenario. The sole differences

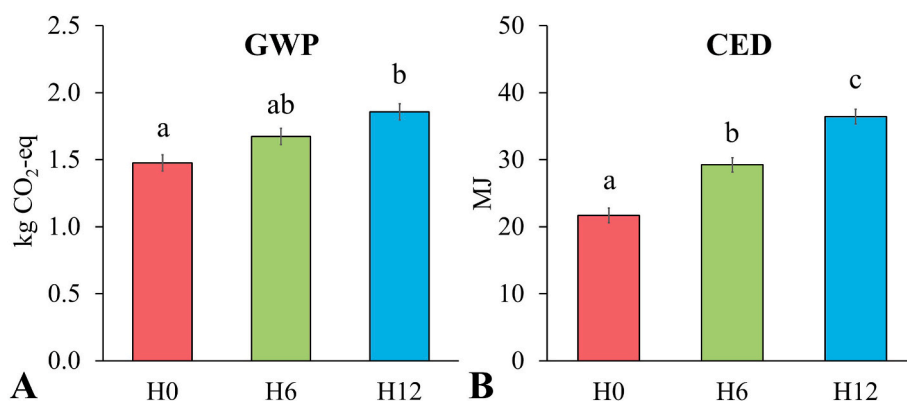


Fig. 4. Least square means of the global warming potential (GWP) and cumulative energy demand (CED) per 1 kg live weight increase of rainbow trout, impact due to aquafeed production (AQ-FEED).

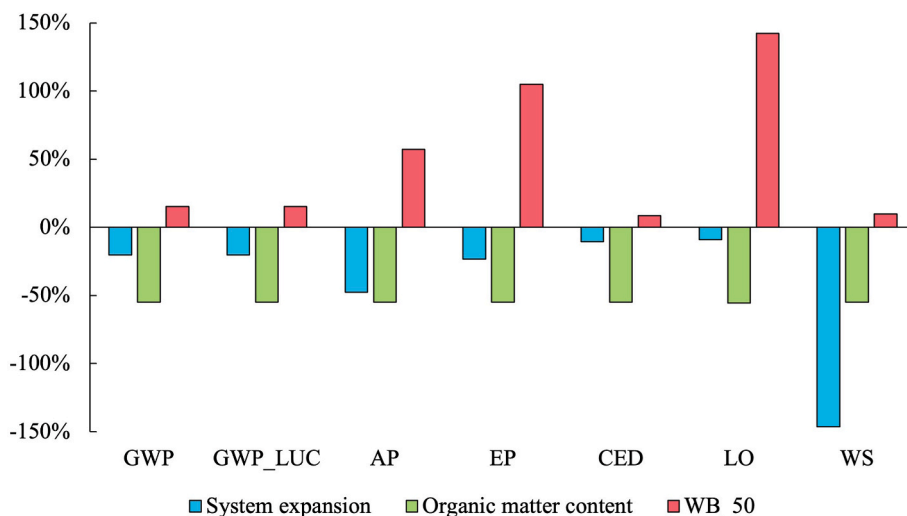


Fig. 5. Sensitivity difference in the impact category values associated with 1 kg *H. illucens* (HI) meal due to the method to allocate the impact between HI meal and the co-products (HI fat and frass) (allocation on organic matter content or system expansion; baseline scenario: economic allocation) and due to different inclusion level of wheat bran in the food by-products composing the insect substrate (inclusion at 50% (WB_50); baseline scenario: inclusion at 25%).

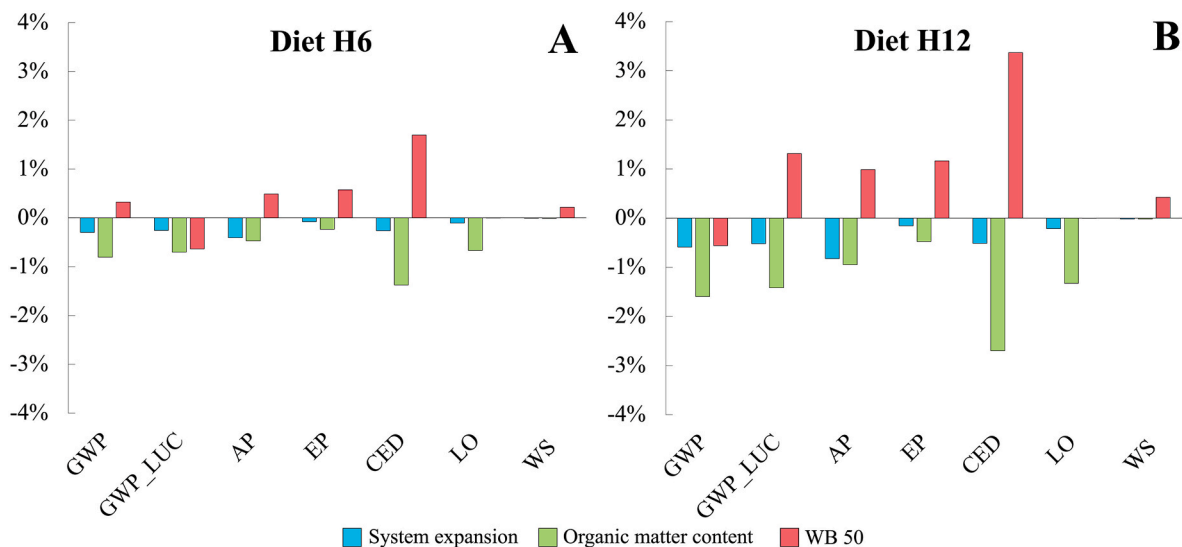


Fig. 6. Sensitivity difference (%) in the impact category values associated with 1 kg live weight increase of rainbow trout in the aquaponic system (whole system) due to the method to allocate the impact between *H. illucens* (HI) meal and the co-products (HI fat and frass) (allocation on organic matter content or system expansion; baseline scenario: economic allocation) and due to different inclusion level of wheat bran in the food by-products composing the insect substrate (inclusion at 50% (WB_50); baseline scenario: inclusion at 25%). A: experimental units with rainbow trout fed with diet including 6% HI meal (Diet H6); B: experimental units with rainbow trout fed with diet including 12% HI meal (Diet H12). GWP: global warming potential; GWP_LUC: GWP plus emissions due to land use change; AP: acidification potential; EP: eutrophication potential; CED: cumulative energy demand; LO: land occupation; WS: water scarcity.

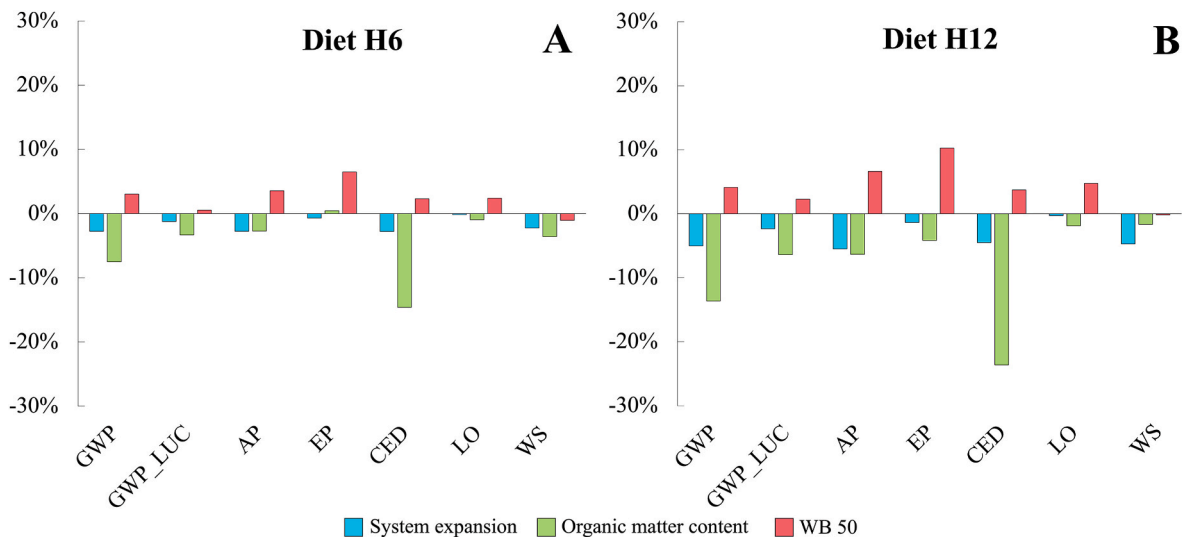


Fig. 7. Sensitivity difference (%) in the impact category values associated with 1 kg live weight increase of rainbow trout in the aquaponic system (only impact due to feed production, AQ-FEED) due to the method to allocate the impact between *H. illucens* (HI) meal and the co-products (HI fat and frass) (allocation on organic matter content or system expansion; reference method: economic allocation) and due to different inclusion level of wheat bran in the food by-products composing the insect substrate (inclusion at 50% (WB50); reference: inclusion at 25%). A: experimental units with rainbow trout fed with diet including 6% HI meal (Diet H6); B: experimental units with rainbow trout fed with diet including 12% HI meal (Diet H12). GWP: global warming potential; GWP_LUC: GWP plus emissions due to land use change; AP: acidification potential; EP: eutrophication potential; CED: cumulative energy demand; LO: land occupation; WS: water scarcity.

emerged for CED in AQ-FISH (no significant effect of the diet in the case of two standard deviation decrease of the HI-LCA unitary impact) and WS in AQ-FEED model (significant decrease of the impact moving from H0 to H6 and H12 diets in the case of two standard deviation decrease of the HI-LCA unitary impact) (Supplementary Table S8).

4. Discussion

This study investigated the environmental impact of a partial substitution of fish meal, a conventional ingredient used in aquafeed formulations, with HI meal, an innovative and promising ingredient (van

Huis and Gasco, 2023), in the context of rainbow trout reared in aquaponics. A comprehensive assessment encompassed various impact categories, addressing both emissions and resource exploitation. This broad spectrum of impact categories enables a more accurate evaluation of the overall environmental impact, facilitating the identification of both positive and negative effects resulting from production changes. Such a holistic approach is crucial for obtaining insights that may be overlooked in a single-category assessment (McClelland et al., 2018).

When evaluating changes in diet formulation for mitigation purposes, three key aspects must be considered: 1) the impact of these changes on the environmental footprint per unit of diet; 2) the

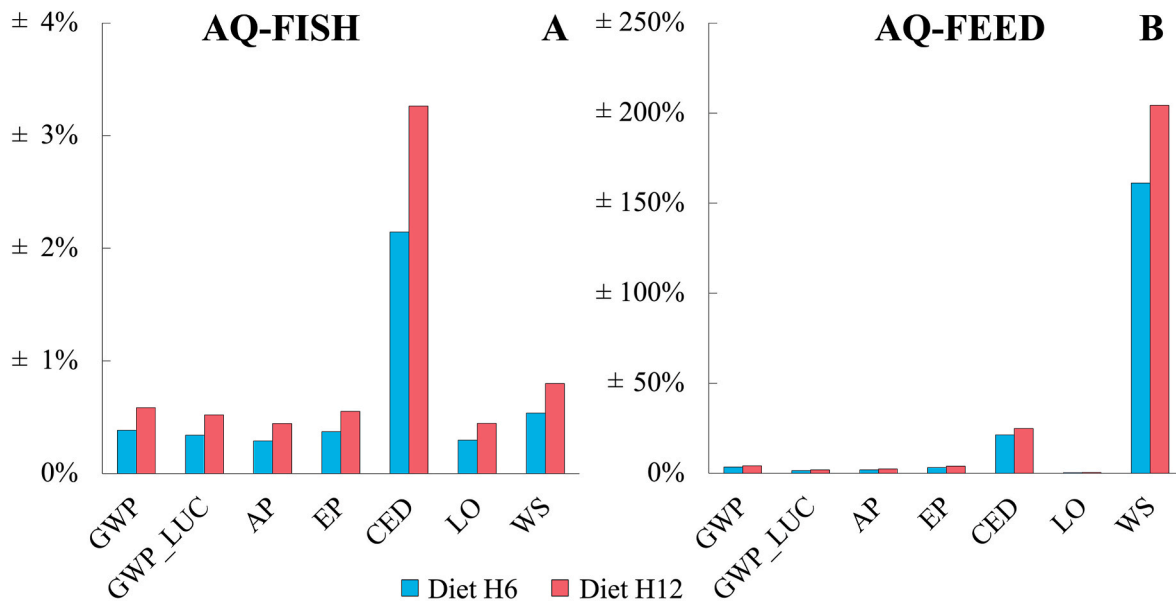


Fig. 8. Sensitivity difference (%) in the impact category values associated with 1 kg live weight increase of rainbow trout in the aquaponic system (whole system: AQ-FISH; only impact due to feed production: AQ-FEED) due to the ± 2 standard deviations increase/decrease of the unitary impact related to *H. illucens* (HI) meal production. Diet H6: experimental units with rainbow trout fed with diet including 6% HI meal; Diet H12: experimental units with rainbow trout fed with diet including 12% HI meal. GWP: global warming potential; GWP_LUC: GWP plus emissions due to land use change; AP: acidification potential; EP: eutrophication potential; CED: cumulative energy demand; LO: land occupation; WS: water scarcity.

operational parameters, including feed consumption and animal performance; and 3) their interactions regarding the overall environmental impact of the final animal product. These aspects will be discussed in the following sections.

4.1. Environmental impact of insect meal

Overall, our results on HI meal impact values (per kg HI meal) ranged within the values reported by Tran et al. (2022) across all impact categories. In addition, the uncertainty analysis evidenced that most of the impact values were minimally influenced by the uncertainty linked to inventory data, particularly secondary data retrieved from the literature. Consequently, our impact values demonstrated sufficient stability for their use in evaluating diets, including HI meal. However, the CED and WS categories showed a higher degree of uncertainty compared with GWP, GWP_LUC, AP, EP and LO. The higher uncertainty in CED was expected, given the significant uncertainty range applied to electricity and heat inventory data ($\pm 25\%$, from Smetana et al., 2019). Regarding WS, its high uncertainty was explained by the high uncertainty related to the WS factor obtained from the inventory sheet of electricity grid production in the Ecoinvent database (CV: 2810%). To enhance the accuracy of the impact category values, by reducing uncertainties, improvements should be made in the collection of inventory data on energy consumption for insect production at manufacturer level, as well as in reducing uncertainties in the Ecoinvent database. Such improvements are essential to increase the reliability of WS as an impact category for future environmental assessments.

In comparison with the baseline scenario, the sensitivity analysis evidenced a notable impact on the choices operated in the LCA model related to HI meal production. Regarding SENS_OM, the decreased values found with respect to the baseline were due to the quantity of frass and HI meal derived from insect production. Indeed, as the organic matter from frass was higher than that from HI meal, the partition factor related to frass was greater (54%) than that of HI meal (32%), resulting in a lower impact associated with HI meal. Conversely, the economic value of HI meal was greater than that of frass, resulting in a higher partitioning of the entire impact of HI meal, i.e. the target product. The comparable impact values between economic allocation and system

expansion can be attributed to similarities in the partitioning methodology, except for WS, the decrease of which with respect to the baseline scenario could be related to the significant water scarcity associated with mineral fertilisers (substituted by frass in the system expansion allocation method). In perspective, as a standard methodology for managing this multi-functionality is lacking at the present day, advancements in this area would be needed. As an example, the differences between partitioning methods based on internal references (organic matter) and external references (economic value and alternative systems associated with co-products) offer interesting perspectives for future research on LCA standardisation in insect production and utilisation.

Regarding the sensitivity analysis of the wheat bran inclusion level (SENS_WB), only the AP, EP and LO categories showed a notable deviation from the baseline scenario. This discrepancy was probably due to the ratios of the impact value per unit of input between WB and the brewery grains, which were notably higher in AP, EP and LO (over 1500 times higher) compared with the others such as GWP, GWP_LUC, CED and WS (from nearly 0 to 37 times higher).

4.2. Environmental impact of aquafeeds

Our results showed that the inclusion of HI meal at 6% and 12% generally resulted in a significant increase in the environmental impact values per 1 kg of diet. This increase could be attributed to the higher impact values of HI meal compared to fish meal (see Supplementary Table S1), which aligns with previous research on HI (Salomone et al., 2017; Smetana et al., 2019) and with other studies on insect meals (Tran et al., 2022). Conversely, in AP, EP and LO, the small but statistically significant differences between the control diet H0 and the two alternative diets H6 and H12 could be partially attributed to the following: a) the relatively low contribution of HI meal to AP (6%–11%), EP (4%–8%) and LO (2%–4%) (see Supplementary Figs. S1–S3), b) the minimal differences between the unitary impacts of fish meal and HI meal with respect to these categories (Supplementary Table S1) and c) the proportional reduction from H0 to H6 and H12 of ingredients with high unitary impact values, such as gelatinised starch (Table 1 and Supplementary Table S1). These results highlight the importance of considering all changes in the diet formulation determined by the inclusion of a

new ingredient to minimise potential trade-offs. In this regard, future efforts in formulating diets, including novel ingredients, should also consider the indirect effects on other production systems by applying consequential LCA models. In fact, previous consequential LCA studies on insect production (Smetana et al., 2019) or fish oil inclusion in aquafeeds (Bordignon et al., 2023) evidenced complex cascades of effects that can have both positive and negative environmental consequences. In this study, HI meal was obtained from insects reared on a substrate mainly based on fruit and vegetable leftovers. As these substrate ingredients were not originally intended for feed or food production, their use did not affect food production systems. In addition, the diversion from its original destination (composting) was counterbalanced by the frass obtained in insect rearing. However, a possible constraint may arise from the European Union policies that aim at minimising waste and food waste production (Waste Framework Directive; 2008/98/EC), which could limit the scalability of the system because of the low availability of leftovers to be used for insect production. Nevertheless, in such a context, insect production could help in valorising raw materials with low current values, thus sustaining feed and food supply.

4.3. Environmental impact of rainbow trout rearing in aquaponics

When considering not only the production of a unit of diet but also its use in the aquaponic system, the partial substitution of fish meal with HI meal in the rainbow trout diet has shown promising results. The results published by Bordignon et al. (2022a) and used for calculations in the present study (reported in Table 1) showed no significant effects on fish growth performance and feed conversion ratio of rainbow trout fed the H0 vs. H6 diet and only minor negative effects were observed when feeding the H12 diet. Consequently, the use of HI meal as an innovative feed ingredient for formulating aquafeed for rainbow trout could potentially mitigate the environmental impact while maintaining production performance and ensuring economic profits. In fact, production performance and economic viability are essential for farmers when selecting and adopting a new mitigation option (Vellinga et al., 2011).

We found that dietary treatment had no significant effect on the overall environmental impact of the production of rainbow trout in an aquaponic system (AQ-FISH), except for the CED category. Considering that all inputs, except diet formulation, remained constant during the experimental trial, the significant differences in CED could be related to the notable CED values associated with HI meal production. Indeed, when considering only feed-related impacts (AQ-FEED), an increased level of HI meal inclusion (H6 and H12 diets) gave increased CED values compared with the insect-free diet (H0). In addition to the above-mentioned need to enhance the availability of data regarding energy consumption in insect production, the high CED value for insect production is related to the current low production scale. This limitation diminishes the potential benefits derived from economies of scale and improved production efficiency (Smetana et al., 2019; Wade and Hoelle, 2020). For these reasons, the scaling-up of insect production significantly contributes to the reduction of CED and the overall impact of insect products.

In addition to CED, the AQ-FEED model showed a significant increase in GWP for the H12 diet compared to the H0 diet. First, this result suggests that the inclusion of HI meal up to 6% (H6 diet) could be feasible without detrimental effects on the environment, as observed in other impact categories such as GWP_LUC, AP, EP, LO and WS. Second, the contrasting results found for GWP and GWP_LUC, i.e. excluding or including the emissions due to LUC, suggest that the a priori exclusion of some emission sources could alter the impact assessment. LUC emissions result from activities such as clearing forest and grassland in favour of pastures and crop production, such as soybean (e.g. Caro et al., 2018), and play a significant role in anthropogenic greenhouse gas emissions, particularly within food systems (IPCC, 2022). Incorporating LUC-related emissions into LCA-based analyses can be complex because

of spatial-temporal considerations and associated emission with a specific product (Audsley et al., 2010; Donke et al., 2020; Brandão et al., 2022). Nevertheless, the reduction of emissions related to LUC, along with other GHG sources, is essential to align with the global mitigation goals (IPCC, 2019). Therefore, their inclusion in LCA analyses is of notable importance.

The combination of the results obtained from AQ-FISH (whole aquaponic system) and AQ-FEED (impacts due to production of feeds consumed in the aquaponic system) proved the feasibility of substituting fish meal with moderate inclusion of HI meal in diets fed to rainbow trout reared in aquaponics. This substitution not only allows a reduction in the negative effects of aquafeed production on natural fish stocks (the primary source of fish meal) but also emphasises the importance of evaluating this dietary change across a broad set of impact categories.

Furthermore, this combination suggests that future reductions in the environmental impact related to the aquaponic system would need interventions at multiple levels, considering the results obtained from the hotspot analysis of the system. Indeed, the hotspot analysis evidenced that aquafeed production significantly influenced LO whereas GWP, GWP_LUC, AP and CED were primarily affected by electricity consumption in the aquaponic system. Conversely, EP was linked to nutrient release in the water and WS was substantially influenced by the rainbow trout hatchery phase, in line with previous studies (Wu et al., 2019; Greenfeld et al., 2022; Bordignon et al., 2022b). In this context, CED reduction would require interventions in both the energy efficiency of insect production and the increasing energy efficiency of the aquaponic system, with consequent positive effects also on GWP and GWP_LUC (as energy production is still largely based on fossil fuels) and AP. Conversely, although feed production greatly contributed to LO, HI meal contribution was relatively low (Supplementary Figs. S2–S3). This suggests the need to focus interventions on the other feed ingredients (see also Section 4.2). In addition, efforts to address WS should be focused on the rainbow trout hatchery phase, rather than on the aquaponic system or HI meal production.

4.4. Sensitivity analysis of environmental impact related to rainbow trout reared in aquaponics

The present study underscores the critical importance of considering the sensitivity of production systems to obtain more robust LCA results (ISO, 2006).

Although remarkable variations could be observed in the impact values related to HI meal due to the different sensitivity scenarios (Fig. 5), the results found regarding AQ-FISH and AQ-FEED were generally slightly affected by the sensitivity analysis regarding the choices related to the LCA model of HI meal production (allocation method – SENS_SE and SENS_OM – and wheat bran inclusion in the insect substrate – SENS_WB). This was probably due to the low contribution of HI meal to the different impact categories in the overall AQ-FISH results. Conversely, AQ-FEED exhibited higher sensitivity, particularly in GWP and CED, the specific categories where a significant effect of dietary treatment was observed. This suggests the importance of separately considering the whole impact and the impact specifically associated with the aspect undergoing a change (protein ingredient in the diet), as implemented in our study. Nevertheless, the ANOVA results related to AQ-FISH and AQ-FEED with SENS_SE, SENS_OM and SENS_WB did not show changes compared to those obtained in the baseline scenario, suggesting robustness in our results. This robustness is a crucial aspect for ensuring comparability with other studies and facilitating their utilisation in future research.

In addition, ANOVA results related to UNCERT_HI generally remained consistent with the baseline scenario, except for CED and WS. These findings highlight two distinct aspects. The first aspect pertains to the aforementioned uncertainty of HI meal impact concerning CED and WS, which should be minimised to enhance the accuracy of the final results. At the same time, this uncertainty needs to be considered also in

the whole aquaponic system, given the substantial contribution of other processes to the overall CED and WS impacts. Notably, a similar high uncertainty related to WS was also identified by Goglio et al. (2022), suggesting that this issue is not isolated to our study. The second aspect involves exploring the potential effect of a predicted decrease in the impacts associated with HI meal production. The consistently stable statistical results reinforce the conclusion that mitigation strategies should address both HI meal production and aquaponic production. At the same time, the lack of a significant effect observed for CED when the HI-related impact decreased by two standard deviations indicates that efforts to reduce the environmental footprint of HI meal could effectively support its inclusion in aquafeed. This finding alleviates concerns regarding potential negative effects on cumulative energy.

5. Conclusion

Conventional protein ingredients such as fish meal are facing limited supply, production caps, economic challenges and environmental concerns. In this context, the use of insect meals as innovative protein-rich diet ingredients for aquafeeds replacing conventional ingredients such as fish meal has been increasingly explored from different perspectives, i.e. nutritional value as feed, animal performance and environmental impact. The results of this study showed that the production of the 1 kg live weight increase in rainbow trout reared in aquaponics was associated with 15.6 kg CO₂-eq (GWP), 18.3 kg CO₂-eq (GWP_{LUC}), 67 SO₂-eq and 55 g PO₄-eq and used 354 MJ, 3.5 m²/y and 311 m³-eq. These impact values were not significantly affected by a moderate inclusion of HI meal (6%–12% of the diet) in aquafeed, except for the CED (+3%–5%). This lack of notable differences is due to the predominant influence of other factors, such as electricity consumption, nutrient release in the water and water consumption in the hatchery phase. When focusing only on the environmental impact from aquafeed production, the inclusion of HI meal negatively affected GWP (+13%–26%) and CED (+34%–68%) because of the high energy demands associated with insect meal production. Overall, our findings suggest that general improvements to reduce the environmental impact associated with rainbow trout production in aquaponics should be concurrently directed towards both the system setting and management and towards the HI meal production process, with a particular emphasis on enhanced energy efficiency. To minimise potential trade-offs, especially from an environmental perspective, future studies should prioritise the investigation of energy use and greenhouse gas emission associated with aquafeed production. This requires a thorough evaluation of all changes in the diet formulation resulting from the inclusion of a new ingredient.

CRedit authorship contribution statement

Francesco Bordignon: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Angela Trocino:** Writing – review & editing, Supervision, Conceptualization. **Laura Gasco:** Writing – review & editing, Validation. **Sara Bellezza Oddon:** Writing – review & editing, Validation. **Gerolamo Xiccato:** Writing – review & editing, Funding acquisition. **Marco Berton:** Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization.

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

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jclepro.2024.142901>.

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