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Assessing environmental sustainability of substitute feeding formulas for gilthead seabream (*Sparus aurata*) using Life Cycle Assessment

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HIGHLIGHTS

- Innovative ingredients as alternative protein sources for gilthead seabream
- LCA used for exploring environmental impact of four aquafeed diet from cradle to market
- Critical issues for improvement are investigated by sensitivity analysis.
- Variations on insect rearing have been suggested toenhance sustainability.
- Encouraging the utilization of local and by-product-related materials is advisable.

GRAPHICAL ABSTRACT

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ABSTRACT

The rise in fish and seafood consumption driven by aquaculture comes with its share of challenges and controversies, notably the need for expanded feed production. The use of fishmeal and fish oil to raise carnivorous fish has caused environmental problems, including ecosystem imbalance and habitat destruction, as well as ethical issues like fishing forage fish for feed instead of human consumption. Thus, the industry has been actively pursuing alternative feed ingredients to reduce reliance on fish-derived components. This progress in the aquaculture feed sector has made selecting the best feed solution complex across various fronts. This study aims to assess the environmental impacts of three feed formulations, each with different protein sources (poultry by-products, PMB, *Tenebrio molitor larvae*, TM, or *Hermetia illucens larvae*, HI), tailored for the gilthead seabream (*Sparus aurata*), a prized species in European aquaculture. The environmental sustainability of these alternatives was evaluated against benchmarks of fishmeal and fish oil-based feed. Employing a cradle-to-gate approach and a FU of 1 kg of product, the study utilized OpenLCA software supported by the Ecoinvent ® v3.7.1 database.

The results focused on the production stages of each ingredient, including logistical and transportation aspects leading up to the final formulation. All alternatives to traditional feed demonstrated either comparable or

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superior environmental performance (i.e. - 66 % of PMB-f, −33 % of TM-f and − 29 % HI-f kgCO_2 eq) with few exceptions for TM-f. This investigation highlighted how integrating innovative ingredients could positively impact the environmental footprint of aquafeed production chains. Furthermore, the main hotspots in the alternative feed formulas life cycles have been identified and viable alternatives for improvement have been proposed, such as selecting different input materials or enhancing energy efficiency. This assessment allows to guide the selection of more environmentally friendly feed formulations before their integration into aquaculture chain processes.

1. Introduction

The global population growth and subsequent rise in demand for food, particularly animal proteins, have exacerbated food security concerns. These concerns are compounded by ongoing threats such as biodiversity loss, ecosystem degradation, and climate change (Wallner-Hahn et al., 2022). Most of the global food production and supply chain is underpinned by the existence of ecosystem services, which are the benefits and resources nature provides to humans (Carmona-Moreno et al., 2021). Thus, food security is interdependent with water, energy, and ecosystems, as highlighted by the concept of the Water-Energy-Food-Ecosystem (WEFE) Nexus (Carmona-Moreno et al., 2021). The seafood industry (wild fishery and aquaculture) also draws on these natural resources to provide healthy food. Likewise, as stated by the Food and Agriculture Organization (2024), seafood, whether captured or farmed, effectively supports human nutrition, income, and culture worldwide. To date, almost 51 % of edible fish species entering the global market (around 185 million tons) derive from aquaculture, and this percentage is forecasted to increase up to 60 % before 2030(FAO, 2024). Thus, aquaculture will directly or indirectly contribute to the achievement of several Sustainable Development Goals (SDGs), including Zero Hunger (SDG2) and Good Health and Well-Being (SDG3), and increase the environmental sustainability of oceans, water, land, and climate (Climate action - SDG13, Life Below Water - SDG14, and Life On Land - SDG15) through the design of a responsible production (Troell et al., 2023).

However, at the same time, anthropogenic exploitation of natural resources and ecosystem services causes impacts and externalities on the environment. More specifically, as regards environmental sustainability, global CO_2 emissions have continuously increased since 1970 (Le Quéré et al., 2021), being the overall food systems responsible for approximately a third/a quarter of the whole anthropogenic emissions (around 53.2 billion tons of CO_2 equivalents (Poore and Nemecek, 2018a; Crippa et al., 2021), mainly caused by livestock and fish farms. Nevertheless, when considering the two activities alone, farming aquatic animals contributed to <0.50 % of anthropogenic emissions in 2017, abundantly lower than farming terrestrial animals (MacLeod et al., 2020). This result might be due to some peculiarities of aquaculture, identifiable in the favorable feed conversion ratio, shorter production cycles, and the lack of the impacts derived from direct land use, in particular in Integrated Multitrophic Aquaculture (IMTA) where the integrated approach (Troell et al., 2003) stimulates sustainable processes eased by precise livestock farming (Barbaresi et al., 2022). However, the impacts caused by aquaculture, excluding macroalgae, can significantly vary according to the farmed species and the level of productive intensification, even if it is well established that the primary sources of emissions derived from the feed used, especially for marine finfish (Poore and Nemecek, 2018b; MacLeod et al., 2020).

Feeding formulas for carnivorous fish species contain high levels of proteins, which have been derived from marine fishmeal (FM), evaluated as optimal due to its balanced nutritional composition (Oliva-Teles et al., 2015; Gasco et al., 2018), high palatability, minimal anti-nutritional factors, and effortless digestibility (Qiu et al., 2023a). In fact, fishmeal (FM) and fish oil (FO) were so far essential components of aquafeeds, particularly for carnivorous fish species in intensive aquaculture systems (Geremia et al., 2024; Pinho et al., 2024)), due to their high protein and lipid content, which contribute to 40–70 % of production costs, needed for animal maintenance and growth (Henry et al., 2015). In 2022, 20 million tonnes of fish, shellfish, and crustaceans were globally landed and used for non-food purposes, primarily FM and FO production (FAO, 2024), which serve as ingredients for aquafeed. The EU contributes 10–15 % to global FM and FO production, producing 400,000–600,000 t of FM and 120,000–200,000 t of FO annually (European Commission, 2021). The production of FM and FO relies heavily on small pelagic fish industrial catches contributing significantly to environmental and social impacts. On one hand, this dependency has led to overexploitation of forage fish stocks, causing negative impacts on marine ecosystems, such as habitat destruction and biodiversity loss; on the other, 90 % of wild fish not used for direct human consumption are food-grade, leading to competition that challenges food security (Auzins et al., 2024). Moreover, the shortage of supply (mainly due to climatic events, such as El Niño), the consequent rising prices and the unsustainability aspects of FM utilization (FAO, 2024) have prompted a crisis in developing affordable, high-quality fish feed, leading to efforts to find alternative protein sources (Hossain et al., 2024; Moutinho et al., 2024), with nutritional values similar to FM and not intended for human consumption (Barroso et al., 2014). Efforts to find alternative protein sources are crucial to reduce dependency on FM and FO and they can be contextualized in the policy of the Blue Economy directives and innovative research into sustainable feed alternatives aim to mitigate these impacts, promoting sustainable ocean resource use, economic growth, improved livelihoods, and ecosystem health (Geremia et al., 2023).

In particular, the research interest has focused so far on sources that have similar contents of the essential amino acids, phospholipids, and fatty acids (especially, docosahexaenoic and eicosapentaenoic acids) that may support optimum animal growth, development, maintenance, and reproduction, which would allow aquaculture production to remain economically and environmentally sustainable over the long term (Aragão et al., 2022).

Alternatives to marine ingredients have been proposed during the last decades, from plant protein sources to animal proteins, such as poultry by-products and, lately, insect meals (Barroso et al., 2014; Gasco et al., 2018). Despite previous results indicating that plant protein-based aquafeeds, containing soybean and rapeseed meals, have a considerably lower impact on the environment as compared to the one with FM (Samuel-Fitwi et al., 2013), the use of these plant ingredients raises considerable concerns both for ethical reasons (deforestation, feed vs. food competition) and for the possible adverse effects on fish welfare (Pang et al., 2023), primarily triggered by the abundance of anti-nutritional elements, imbalance in amino acids, and limited utilization (Qiu et al., 2023b).

Hence, other alternatives to FM should be considered to promote a circular economy application and give second uses to wastes and added value to by-products, reintroducing them in the economic system for as long as possible (Campos et al., 2020), and helping to design Aquafeed 3.0 (Colombo and Turchini, 2021).

As introduced, the recent re-approval of non-ruminant processed land animal proteins for use in European animal feeds, particularly in aquaculture, has ignited a growing interest in exploring nutrient-rich sources within land animal by-products, which are abundantly produced and represent valuable biological resources (Campos et al., 2020). Hence, poultry by-product meal (PBM) meets the needs and enhancement requirements for re-using by-products and shows an overall nutritional index comparable to that of FM (Qiu et al., 2023b); therefore, this promising ingredient is being studied with interest. The poultry industry generates significant quantities of by-products that are not directly suitable for human consumption and, by processing these by-products, valuable sources of nutrients are extracted. Briefly, PBM can be defined as the ground, melted, and cleaned parts of slaughtered poultry carcass, such as the neck, head, feet, undeveloped eggs, gizzards, and intestines (provided their contents/chime are removed), excluding feathers (except in the quantities that could inevitably occur in good manufacturing practices) (Karapanagiotidis et al., 2019). This meal has high chemical-biological safety standards and a low environmental impact (Maiolo et al., 2020), as well as numerous nutritional qualities given by its high protein content and amino acid balance similarity close to the golden standard represented by FM and higher than plant protein sources (Chaklader et al., 2023).

Other innovative solutions consider the use of insect meal as a protein source for fish feeding (Iaconisi et al., 2019). The environmental benefits of insect farming might be attributed to the better feed conversion index, and to the lower use of soil to produce nutrients such as animal proteins and lipids (Doi and Mulia, 2021). Two to ten times less agricultural land use is required to produce 1 kg of insect protein when compared to 1 kg of protein produced from pigs and beef. Their high fecundity, rapid growth rates and ability to effectively convert organic substrates of various origins (vegetables, fish offal, bran, meat, etc.) make them valid candidates from the point of view of environmental sustainability (FAO, 2021).

One mainly interesting species is represented by *Tenebrio molitor* (TM) which belongs to the Coleoptera order, and it is commonly called "mealworm" since it has been reckoned so far as a pest of grain, flour, and agri-food industry. This insect aroused considerable interest thanks to its nutritive characteristics, such as the high content of crude protein (47–60 % of dry matter, DM), lipids (31–43 % DM), relatively low content of ash (<5 % DM) and calcium (Gasco et al., 2018; Iaconisi et al., 2019). Furthermore, mealworm meal is characterized by an aminoacidic profile close to soybean meal profile, albeit with potential deficiencies in methionine, histidine, lysine, cysteine, and threonine, while it is rich in tyrosine and valine (Barroso et al., 2014). However, the insect's developing stages might affect its amino acid composition (Finke, 2002). Nowadays, the scientific evidence allows us to consider TM suitable for the partial replacement of fish meal in aquafeeds for various fish species.

Another species of insect is represented by *Hermetia illucens* (HI), popularly known as the black soldier fly, a Diptera of the family Stratiomyidae. It is believed to have a tropical origin, but international trade has allowed this insect to move and enter other ecosystems, easily adapting (Salomone et al., 2017). HI is one of the most promising agents for the bioconversion of poor-quality biomass and by now, its farming is spreading in Europe. The leftovers of fruit and vegetables represent a cheap and available substrate that can be used for the breeding of HI larvae, creating a virtuous circle of transformation of the processing by-products into insect meal allowed by the European Commission to be used as feed ingredient (European Commission, 2017). This bioconversion process allows food waste to regain value thanks to its reintroduction into the production chain, according to the perspective of the circular economy (Cappellozza et al., 2019; Ojha et al., 2020) and intervenes on the nutritional profile of larvae to fully meet the needs of farmed animals, creating values throughout the supply chain.

However, while incorporating these by-products or new protein sources into animal feeds appears advantageous, it is important to recognize that the processes involved in collecting and maximizing their value can have environmental consequences. To assess the true advantages of using these ingredients as alternatives to conventional FM and fish oil (FO) in animal feeds, it is crucial to evaluate their environmental impacts from a life cycle perspective (Campos et al., 2020). Indeed, as noted by Bohnes and Laurent, 2021 aquaculture presents serious environmental challenges. The rapid expansion of aquaculture highlights the need to address these environmental concerns urgently, with aquafeed production being a crucial area for intervention (Quang Tran et al., 2022), since the demand for aquafeeds expected to rise from 160 million to over 180 million metric tons by 2025 (Hossain et al., 2024). More precisely, its sustainability hinges on addressing the environmental impacts mainly related to aquafeed production, since feeding is identified as the main source of both environmental and financial costs for the aquaculture and livestock industries (da Silva Pires et al., 2022). García García et al., 2016 emphasize the potential of using alternative feed ingredients to mitigate these challenges, by demonstrating reductions in several impact categories, including the potential for global warming at level of aquacultural production of seabream. Thus, exploring alternative feed ingredients offers promise in mitigating these challenges and fostering a more sustainable future for the aquaculture industry. Understanding the interplay between sustainability practices is crucial for illustrating to decision makers the need to balance production yield and environmental performances, supported by Life-Cycle Assessment (LCA) analyses (Lourenço et al., 2024).LCA is an international standardized methodology (European Standard Commision, 2006a) for assessing the environmental and human health impacts associated with a product or a service (Vinci et al., 2022). Thus, it represents one of the most extensively employed methodologies for measuring and appraising the ecological repercussions of manufacturing commodities and services. Many LCA studies have been implemented to assess the valorization of by-products from various food production sectors (Curran, 2016; Arun and Shanmugam, 2020; Campos et al., 2020) and, to date, it has emerged as the established approach for assessing impacts, especially greenhouse gas emissions, linked to food and livestock production (Berton et al., 2023). In addition, in the dynamic context of feed formulation, the utilization of LCA methodology might be crucial for evaluating the sustainability of this productive sector (Basto-Silva et al., 2018, 2019). (Basto-Silva et al., 2019) assessed environmental impacts of four experimental diets to gilthead seabream with different dietary protein to carbohydrate ratios, quantifying fisheries-derived ingredients as the main contributors to environmental impact. Under this perspective, the researchers have considered possible alternative ingredients as protein source to fish derivate, in particular for carnivorous species (Maiolo et al., 2020; Vinci et al., 2022). Already, Maiolo et al., 2020 has evaluated environmental impact of microalgae biomass, insect meal (IM) from *Hermetia illucens* larvae and poultry by-product meal (PBM) as alternatives, limiting the study at the production processes of each ingredient, highlighting the best performances of the last two. However, each species in aquaculture should be considered an individual with specific farming parameters, feeding included. (Goglio et al., 2022) has evaluated the impact on salmon farming of a partially algal–insect-based diet compared to a conventional fish meal/fish oil-based diet, giving an idea of potential improvements in the algal insect value chain. Then, we chose the gilthead seabream (*Sparus aurata*) as the subject of our study because, like the European seabass (*Dicentrarchus labrax*), it is one of the most widely farmed and economically important species in Southern European aquaculture. By focusing on seabream, we aimed to assess the environmental impacts of three of the most promising innovative feed formulations, tailored for this species, with similar nutritional values, with different protein sources, compared to benchmarks of fishmeal and fish oil-based feed. A product-based approach, with cradle-to-gate models based on 1 kg of product as functional unit, have been chosen to focus on the production phase of the individual ingredients studied, the related logistics and transports up to the preparation of the final formulation. The aim of the study also is to provide insights for the choice of a more sustainable feed formulation before its use in the aquaculture chain processes, because it is the highest voice of environmental impacts in aquaculture production. Furthermore, considering the important role of secondary data choices, which involve uncertainty in an LCA study, a sensitivity analysis of the alternative feed formulas has been performed, based on different aspects, to identify the main hotspots, improvable choices and critical parameters for further improvements in the production chain, such as selecting different input materials or enhancing energy efficiency. The insights provided offer vital information that guides decision-making across environmental, financial, and operational aspects (GFI, 2023). This can be particularly valuable for emerging entrepreneurs and new factories looking to initiate the production of novel ingredients for animal feed or for aquaculture companies interested in reducing their environmental impact by starting with feed formulas.

2. Material and methods

The Life Cycle Assessment (LCA) was carried out according to the four main steps recommended by the ISO standards for LCA (European Standard Commision, 2006a, 2006b): (1) Goal and scope definition; (2) Life Cycle Inventory (LCI); (3) Life Cycle Impact Assessment (LCIA); (4) Results interpretation. Calculations were made using OpenLCA 1.11 software.

2.1. Goal and scope definition

The fish feed is the main factor, followed by operations related to feeding and emissions of N and P due to the metabolism of the fish, which make the greatest contribution to environmental impacts of fish farming systems. Then, the goal of the study is to evaluate the sustainability of different feed formulas, as a means of decision. In particular, this study was conducted to evaluate the environmental impacts associated with the production phase of various feed formulations designed for seabream (*Sparus aurata*), in preparation for market distribution. Traditional attributional life cycle assessment models have been applied for the defined case studies, with sensitivity analyses of key assumptions performed at system level. This work aimed to conduct a comparison following a “prospective attributional approach”, where inputs and outputs are assigned to the functional unit selected for the product system, by connecting and/or partitioning the unit processes within the system according to normative rules. It has been preferred this approach rather than establishing the consequences of their application towards the change of the agricultural and food systems (as it is for the use of consequential approach) (Hospido et al., 2010). The research entailed a comparative analysis between a conventional aquafeed formula and innovative fish diets that incorporated alternative ingredients, either partially or entirely replacing fish meal (FM). In particular, the alternatives to fish meal considered are poultry by-products or wastes, reported with the acronym PBM, and insect meal of two different species: *Tenebrio molitor* larvae, abbreviated as TM, and *Hermetia illucens* larvae, abbreviated as HI. The primary objective was to identify the fish feed formulation that exhibited the best performance in terms of environmental impact during the production phase, while also exploring criticalities and underlining potential enhancements in the production processes. A cradle-to-gate approach was chosen to model the production of these different, using as functional units 1 kg of marketable feed. Afterward, the system boundaries were set homogeneously, since in all cases they consider and include all the input materials to the feed processing procedures, the transportation, energy, and water consumptions, as schematized in Fig. 1. The models take into consideration the input ingredients until the pelletized products by extrusion process are ready for the aquafeed market.

Finally, we chose the gilthead seabream (*Sparus aurata*) as the subject of our study because, like the European seabass (*Dicentrarchus labrax*), it is one of the most widely farmed and economically important species in Southern European aquaculture. By focusing on seabream, we aimed to address specific nutritional needs, thereby contributing to a more tailored approach to aquafeed formulation. This approach addresses gaps in the existing literature and offers insights into more sustainable practices in aquafeed production for this fish species. This was made to minimize the number of assumptions in our study. Including the performance data would require making assumptions about various technical parameters that might widely influence the animals’ growth and welfare, which could vary significantly depending on farm conditions. Such assumptions could lead to inaccuracies in representing the actual farm conditions and, consequently, affect the reliability of the environmental assessment.

2.2. Life cycle inventory

Fishmeal (FM), poultry by-products or wastes (PBM), and insect meal of two different species (*Tenebrio molitor* larvae, TM, and *Hermetia illucens* larvae, HI) were chosen as the four different protein sources for seabream feeds to be analyzed in this study. Then, four diet formulations were extracted from literature studies (; Piccolo et al., 2017; Karapanagiotidis et al., 2019; Gai et al., 2023) in order to have grossly isoproteic, isolipidic, and isoenergetic fish feed formulas, with similar nutritional values (47 % crude protein content, 17.25 % crude lipid content, 21.7 MJ/kg on average, respectively). The lists of the ingredients considered in the four cases are presented in Table 1, where FM-f is a common recipe considering fishmeal as the main source of protein, PMB-f considers poultry by-product meal as the main source, and finally, TM-f and HI-f present a partial substitution of fishmeal with insect meal derived from *Tenebrio molitor* and *Hermetia illucens* larvae, respectively.

Moreover, the rearing and larvae production processes of the insects have been modeled based on (Oonincx and de Boer, 2012) for TM and on (Salomone et al., 2017) for HI. Particularly, the rearing substrate for TM consisted of potato residues and a mixed grain feed (wheat bran, oats, soy, rye, and corn supplemented with beer yeast at the same

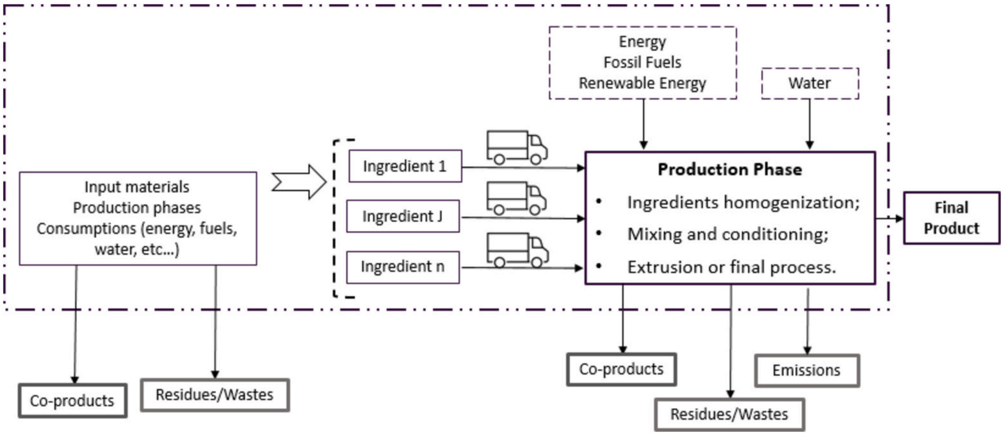

Fig. 1. System boundaries of the scenario analyzed, for the feed alternatives considered.

Table 1
Composition and percentage of inclusion for each ingredient in each feed involved in the LCA assessment.

Diets							
FM-f	%	PBM-f	%	TM-f	%	HI-f	%
Fishmeal	30	Poultry by-products meal	54	Insect meal *(Tenebrio molitor)*	50	Insect meal *(Hermetia illucens)*	18
Fish oil	12	Fish oil	9	Fish oil	6	Fish oil	12
Soybean meal	15	Corn gluten	18	Fishmeal	13	Fishmeal	15
Soy protein concentrate	8	Wheat meal	18	Corn gluten	13	Soybean meal	15
Wheat meal	8	Mineral and vitamin pre-mix	0.3	Starch	15	Soy protein concentrate	8
Corn gluten	18	Monocalcium phosphate	0.3	Mineral mix	1	Wheat meal	5
Starch	6	Vitamin E	0.1	Vitamins mix	1	Corn gluten	18
Mineral-mix	10	Vitamin C	0.1	Carboximethylcellulose	1	Starch	6
Vitamin-mix	10	Antimoulting agent	0.2			Mineral-mix	1
Methionine	0.7					Vitamin-mix	1
Lysine	0.8					Methionine	1
						Lysine	1
Proximate composition							
Crude Proteins (%)	47.4	Crude Proteins (%)	50.23	Crude Proteins (%)	43	Crude Proteins (%)	47.1
Crude lipids (%)	17	Crude lipids (%)	15.53	Crude lipids (%)	19.4	Crude lipids (%)	17.1
Gross Energy (MJ/kg)	22.13	Gross Energy (MJ/kg)	21.43	Gross Energy (MJ/kg)	21.1	Gross Energy (MJ/kg)	22.38

percentages). For HI, the growth substrate was defined as a mixture of 75 % of vegetables and 25 % of butchery waste, following Addeo et al. (2021).

Some assumptions for each specific feeding case were made for materials in input and for the preparation and production process, as reported below.

- In the cases of insect meal production, the emissions related to the rearing phase of the animals were considered as reported in some literature studies (Oonincx et al., 2010; Salomone et al., 2017).
- The outcomes of the insect-rearing process are two (frass and larvae), significantly different in quantity but also in economic value on the market (Scala et al., 2020; Van PhI et al., 2020). The frass produced is significantly higher in quantity than the produced larvae, but it has a similarly low value in the market compared to the insect larvae. In fact, the value of 1 kg of frass is two orders of magnitude smaller than larvae. For this reason, the economic value of the fertilizer is negligible compared to the larvae for which no economic allocation was performed. Alternatively, in accordance with the suggestions of the ISO standards, two different choices were evaluated: the physical allocation, which however tended to unbalance the impact values towards the co-product (frass) compared to the main product (larvae), and the system expansion approach, which has been preferred. In this case, the frass usage has been considered as organic digestate for fertilization.
- The first phase of starting the insect colonies was not considered since it is usually just a one-time operation. Then, the colonies self-regenerated during the production process; however, the phases of egg deposition and growth were considered in terms of the use of cardboard for the oviposition and the energy consumption for the control of the environment.
- The substrate materials for HI growth were reckoned as waste inputs, free of environmental burdens, and not as waste avoided. This modeling choice was evaluated as more adherent to the production reality, considering that the enhancement of these materials transforms them from waste to end of waste materials. However, it was considered the avoided waste scenario in the sensitivity analysis. Moreover, for these input materials are waste produced on the national territory and a distance of 100 km was considered for their transportations as was done for the PMB and other feed formulas ingredients (see Table 2).
- The processes of production of insect meal can be assumed to be similar to the ones for the production of fishmeal or soybean meal, with the treatment of the insects before and at the mill (Salomone et al., 2017), leading to the production of two products: insect meal and insect oil, see Fig. 2. Therefore, a physical allocation method was implemented for these co-products, considering the lack of data and thus the resulting degree of uncertainty of another type of allocation method. The ratio between meal and oil has been defined as 1 t meal: 0.35 t oil (Van PhI et al., 2020).
- The four feeds were assumed to be extruded to be sold in the market. Considering this, an estimation of energy consumption related to mixing, conditioning and extrusion processes have been performed, as presented below in Table 2, based on productive flows and secondary data about these capital goods.
- The aspects of transport and distances related to the different origins of the raw materials were covered, as well as energy and water consumption, derived from literature and other secondary data, referred to European area.
- A sensitivity analysis on the alternative case studies (PMB-f, TM-f, and HI-f) was performed based on the parameters evidenced in the detailed results analyses and the main hotspots have been identified.

Table 2
Life cycle inventory for common processes of 4 feed cases production.

Data	Amount	Unit
Fish meal origin (Perù/ Chile)	12,000	km
Fish oil origin (Perù/ Chile)	12,000	km
Soy and soy derivate (Brasil)	10,000	km
Wheat and corn derivates (France)	1200	km
Other Ingredients [Mineral-mix, vitamins etc] (Italy)	100	km
Poultry by-products meal (Italy)	100	km
Energy consumption (feed ready for market)	0.125	kW/kg
Intercontinental transports	Bulk carrier for dry goods	kg*km
Continental transports	Lorry	kg*km

Then, the several datasets of life cycle inventory of the feed cases are visible in Tables 2, 3 and 4. In the first table (Table 2), common data related to diet ingredients such as distance of origins and transports have been reported.

In Tables 3 and 4, some detailed data collected about specific processes of *Tenebrio molitor* and *Hermetia Illucens* rearing phase and feeds productions have been presented. In particular, detailed data related to

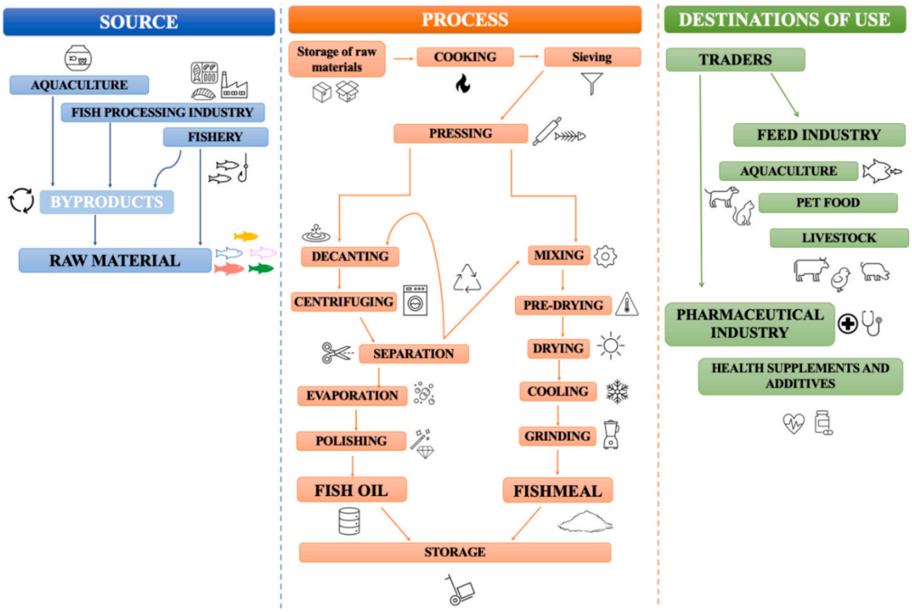

Fig. 2. Schematic representation in blocks of the production process of meals and oils (Tacon et al., n.d.; IFFO – The Marine Ingredients Organisation, 2020).

Table 3
Life cycle inventory for TM-f production considering: rearing, larvae production and insect meal process. The data refer to 1 kg of larvae produced or processed.

Rearing process		
	Amount	Unit
Mixed grain substrate	1.75	kg
DDGS from corn	0.4375	kg
Vegetable waste	3.125	kg
Tap water	2.54	kg
Electricity consumption	2.25	MJ
Paperboard	0.00315	kg
Larvae	1	kg
Insect meal production		
Insect larvae	1	kg
Heating for processing into meal and oil	0.61	MJ
Energy consumption	53.6	Wh
Chemical - hexane	4	g
Water consumption	0.0002	M^3
Insect meal	0.804	kg
Insect oil	0.18	kg

Table 4
Life cycle inventory for HI production considering rearing process.

Rearing process		
	Amount	Unit
Vegetable waste	25.5	kg
Butchery waste	8.5	kg
Rearing boxes extruded made of polyethylene	0.05	kg
Tap water	2.06	kg
Electricity consumption	0.44	kWh
Larvae	1	Kg
Frass	11.3	kg

the rearing process, larvae production and insect meals are collected. The data refers to a kg of larvae or to 1 kg of insect meal produced.

The mixed grain substrate used for the *Tenebrio molitor* growths has been considered made by an equal mixture of soy, rye grain, oats and wheat bran. Similarly, the Table 4, presents the inventory regarding the rearing process of *Hermetia illucens*. About the insect meal production, initially two different processes were taken in consideration: mechanical and thermal process. Considering the quality of the final product in terms of protein content for feeding purposes, the thermal process has been selected and it is common to both the insect species. In the meal processing, the capital goods considered is the operating mill considered with a lifespan of 25 years.

2.3. Life cycle impact assessment (LCIA)

The analysis was performed with the environmental assessment methodology EF 3.1 method, the most updated method available and no LCA in this sectors present results obtained with it, and Cumulative Energy Demand (CED), supported by primary data and secondary data from databases Ecoinvent 3.7.1.

The EF 3.1 method is an Impact Assessment methodology that evaluates and expresses the environmental performances of a product throughout their entire life cycle. EF3.1 method collects and uses scientifically robust assessment methods that are internationally recognized. These methods addresses 16 environmental impacts: Acidification (AC), Climate Change (CC), Eutrophication Freshwater (EUT_f), Eutrophication marine (EUT_m), Eutrophication terrestrial (EUT_t), Ecotoxicity Freshwater (ECO_f), Human toxicity cancer (TOX_h_c), Human toxicity non-cancer (TOX_h_nc), Ionising radiation (IR), Ozone depletion (ODP), Particulate Matter (PM), Photochemical Ozone Formation (POF), Resource use fossils (R_foss), Resource use minerals and metals (R_mm) and Water use (WU). Moreover, in synergy with the EF 3.1 method, the Cumulative energy demand (CED) has been taken in consideration in order to assess the primary energy usage. In particular, this method is considering the contribution of different energy sources whether they are non-renewable or renewable.

3. Results

Global fish production has increased steadily in recent decades, primarily due to aquaculture development. As a result, rising interest in the environmental implications of farmed fish has recently been observed, as evidenced by the increasing number of LCAs applied to aquaculture industries. Our results for theEF 3.1 assessment method are presented, in Table 5 and in Fig. 3, where three impact categories have been highlighted (CC, ECO_f and PM).

All the alternatives to traditional fishmeal, in Table 5, shows better

Table 5
Results of EF 3.1 method for the four feed formulas under study based on FU of 1 kg of product.

Impact category	Reference unit	FM_f	PBM_f	TM_f	HI_f
Acidification	mol H+ eq	0.02	0.01	0.01	0.01
Eutrophication freshwater	CTUe	21.5	9.5	0.001	0.002
Eutrophication marine	kg N eq	0.01	0.003	0.01	0.01
Eutrophication terrestrial	mol N eq	0.04	0.02	0.05	0.02
Human toxicity cancer	CTUh	2.1E-09	1.2E-09	2.2E-09	1.8E-09
Human toxicity non-cancer	CTUh	2.5E-08	1.2E-08	1.4E-07	1.5E-08
Ionising radiation (human health)	kBq U235 eq	0.2	0.06	0.17	0.13
Land use	dimensionless (pt)	80.2	47.6	95.9	60.4
Ozone depletion	kg CFC11 eq	2.1E-07	1.1E-07	1.1E-07	1.4E-07
Photochemical ozone formation (human health)	kg NMVOC eq	0.01	0.003	0.005	0.01
Resource use fossils	MJ (net calorific)	20.7	9.6	12.3	12.7
Resource use minerals and metals	kg Sb eq	4,7E-05	2,9E-05	3,8E-05	3,8E-05
Water use	m3 world eq	2.1	2.3	1.8	1.0

or comparable results than FM-f, with few exceptions. Considering the PBM-f, this product presents a small increase in the water use of around 0.20 m^3 world eq (+10 %). Similarly, the TM-f presents an increased water use of 21 %. Moreover, this type of feed shows a higher impact in terms of Land use due to the crop cultivation used as substrate for the insect growth. In general, PBM-f shows only moderate mitigation on the impact categories. Worst results for TM-f are visible also in Eutrophication marine and terrestrial (+ 53 % and + 15 %) and Human toxicity non-cancer, for more than an order of magnitude. In all the three impact voices, the main contributors are related to the rearing phase of the insects, in particular, the growth substrate compounds production, with their operations on fields.

In Fig. 3, three impact categories have been highlighted considering their importance: Climate Change, Ecotoxicity Freshwater and Particulate Matter. The first impact shows the carbon footprint of the production chain of these feeds; the second is useful to understand the impact of the feed on the water compartment; then, the last one underlines the air pollution of the production processes, which are significantly affected by the length of the supply chain and transport connections.

The Climate Change has characterized by significant decreases of CO_2 eq. emissions for the alternatives under study, starting from a reduction of 29 % of HI-f, 33 % of TM-f to 66 % of PBM-f. Similar results are detectable in Ecotoxicity Freshwater where are accountable reductions of 18 % for HI-f, and TM-f and 56 % of PBM-f. Then, the Particulate Matter results are slightly different because the TM-f is the one showing a lower improvement (– 24 %); on the contrary PBM-f and HI-f have similar results of around – 43 % and – 35 % respectively.

In terms of the energy demand of the processes, Fig. 4 shows CED results for the feed cases, highlighting the contribution of renewable energy sources or not. All alternative feed formulas showed lower (PMB-f and HI-f) or comparable (TM-f) consumption of energy compared to FM-f. Moreover, TM-f demonstrated to have a slightly higher energy demand compared to the FM-f (77.2 MJ vs 83.1 MJ, respectively), but it had a significant positive contribution of renewable energy sources in the total energy demand which was almost double the contribution in FM-f. The high CED here obtained for the TM-f could be attributable to two different aspects of this process. The first hypothesis is rooted in the growth substrate, primarily comprised of various grains originating from an energy-intensive production chain; otherwise, the second theory revolves around the energy consumption associated with raising TM larvae, which necessitates precise environmental control for optimal growth. Like aquatic and terrestrial animal rearing, the “feed” factor – in this case, the growth substrate – was demonstrated to be the largest contributor to the environmental impact categories in insect farming; thus, the formulation of the growing substrates is pivotal to reach the environmental sustainability of the production. Considering the presented results, all the alternative feed formulations appear promising; however, we proposed hereunder the detailed analysis of the process contribution level, considering the highlighted impact categories (CC, ECO_f and PM) impact categories plus Eutrophication Marine (EUT_m) impact, to highlight which are the processes that are affecting the environmental performances of these products. The processes have been reported considering a “cut-off” for the single contributions of 2 %, which have been incorporated in the voice “others”.

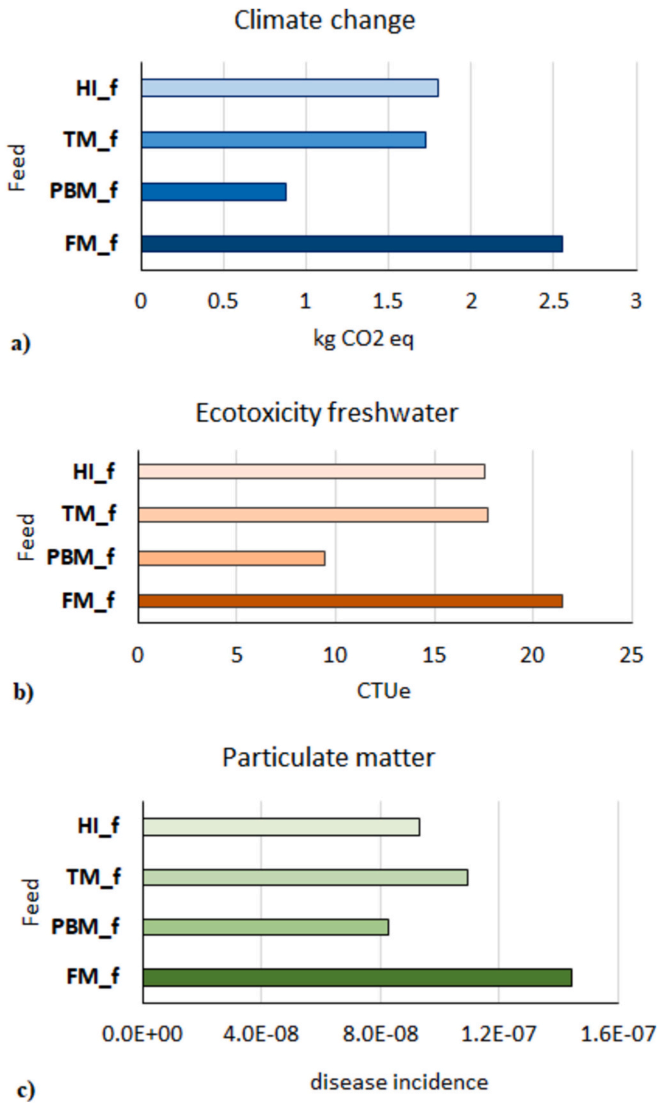

Fig. 3. EF 3.1 results for the feed cases under study: a) Climate Change, b) Ecotoxicity Freshwater and c) Particulate Matter.

Fig. 5 shows the results related to FM-f, where the main contributor to all impacts is FO production and soy derivates production. About the first specific voice, its environmental impact goes from a minimum of 26.7 % in CC to a maximum of 41 % in PM and it relies on the heating consumption in the production and in the management of the fish residues (transports and disposal). Then, the soy derivate contributes for a maximum of 32.9 % in the ECO_f to a minimum of 5.2 %% in the PM category. Its strong contribution in all selected category might be due to

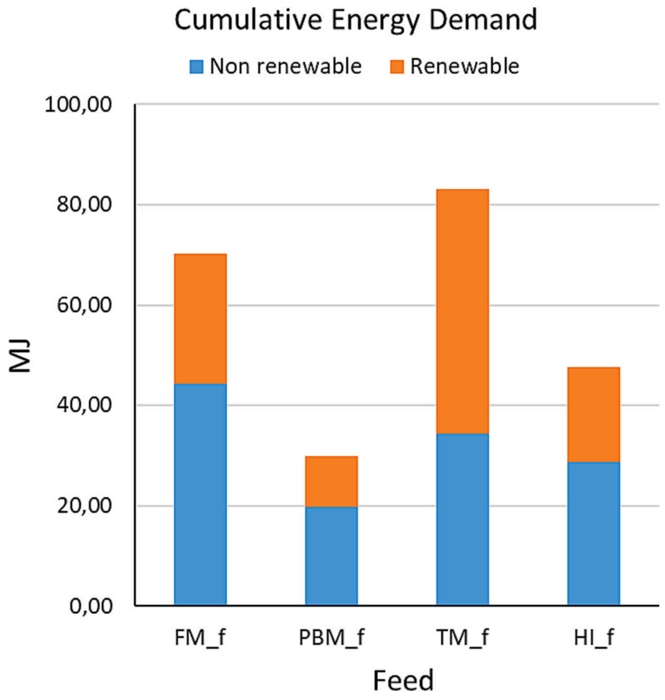

Fig. 4. Cumulative Energy Demand (CED) results for the feeds under study, with the distinction between renewable and not contributions.

the intensive crop production phase, with field operations, fossil fuel consumption, and market transports.

Another important contribution is related vitamin and mineral mix used in the feed diet. In particular, it affects the Climate Change and the Particulate Matter in high percentages, 23.9 and 20.9 respectively. In the other two impacts, it is settled under the between the 10 and 5 %. Taken a look to the contributions inside this process too, the main aspects of impact are due to the energy consumption and to the input compounds for the production processes. On the contrary, other contributions have differentiated results based on the impact category under analysis. For example, fish meal and transports have a limited role in CC and ECO_f, with <5 %, but higher values in PM and EUT_m, from 5.8 to 6.8 %. Focusing on the last one, the transports can play an important role due to the long distances that must be performed by displacing all the raw materials and ingredients from the geographical area of production to the Italian market or production site.

Regarding PBM-f results (see Fig. 6), the highest impact contribution is due to the FO production and then wheat meal ranks in second place in all four categories (CC, ECO_f, PM and EUT_m) with percentages from 13.4 % to 31.4 % of the total impact. Then, PBM production show very limited contributions in the categories selected. In particular, in poultry meal production, the main impacts are due to heat and electricity consumption.

In this case, similarly to the soy derivate for the case of FM-f, the presence of wheat grain meal in the formula represents a heavy impact source and, this might be ascribable to its production itself since it is characterized by intensive crop cultivation and fossil fuel consumption. Similarly, also the corn gluten production is characterized by significant impacts, mainly in ECO_f and EUT_m, 15.5 % and 8.5 % respectively. As seen in the results of TM-f (see Fig. 7), the contributions present a distribution of the main impacts on three or four main voices: TM larvae production and FO, firstly, and FM and maize starch productions. Notably, it is highly appreciable how the rearing process of the insects is impactful compared to other contributors, fluctuating between 49.4 % of total PM impact to 78.3 % of total EUT_m impact. This condition may undergo alterations, as demonstrated in the sensitivity analysis section, through adjustments to the insect rearing process. A difference of CC results compared to the other categories is the numerousness of contributors to the impact value.

Differences can be appreciated in HI-f compared to the other options and, particularly, compared to TM-f. In fact, in Fig. 8, it is visible a greater diversification of significant items in terms of contributions. More specifically, HI meal production had a limited contribution in all the selected categories from a minimum of 3.1 % in EUT_m to a maximum of 8.9 % in PM, compared to TM-f results. Instead, FO production demonstrated the highest impact in terms of Ecotoxicity freshwater, Particulate Matter and Ecotoxicity Marine, and a relevant impact also in CC (27.8 %). On the contrary, the soy derivate showed the highest impact in Climate Change (32.4 %) but also meaningful contributions in ECO_f (40.0 %), and in EUT_m (38.5 %). It has only a limited role in terms of PM, around 6.5 %. Similarly to the other case studies, for the same reasons previously explained, the vitamin and mineral mix in the formula shows relevant values in Climate Change (21.4 %) and Particulate Matter (18.7 %) and a lower impact in Eutrophication marine (10.9 %).

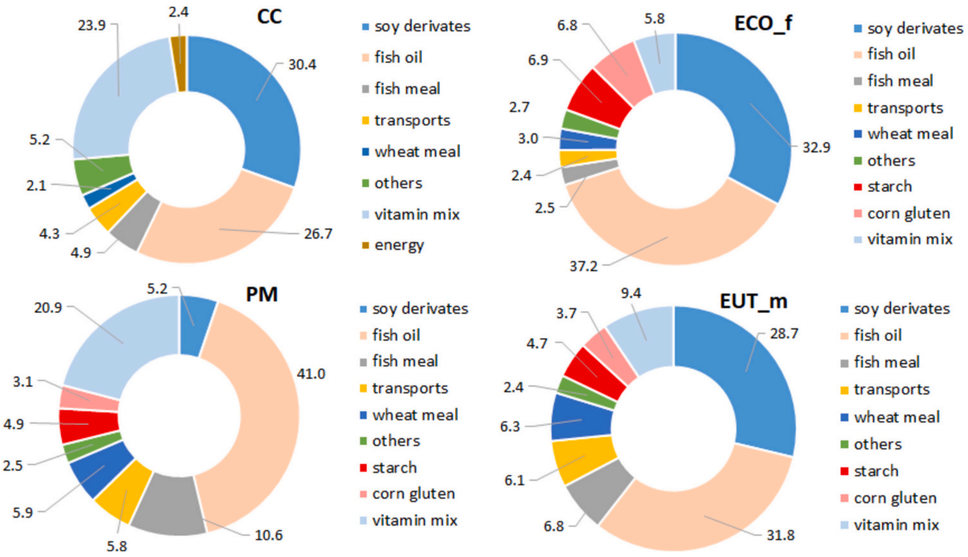

Fig. 5. Specific contributions of the process of FM-f, involved in the value of the impacts for Climate Change, Ecotoxicity Freshwater, Particulate Matter and Eutrophication Marine.

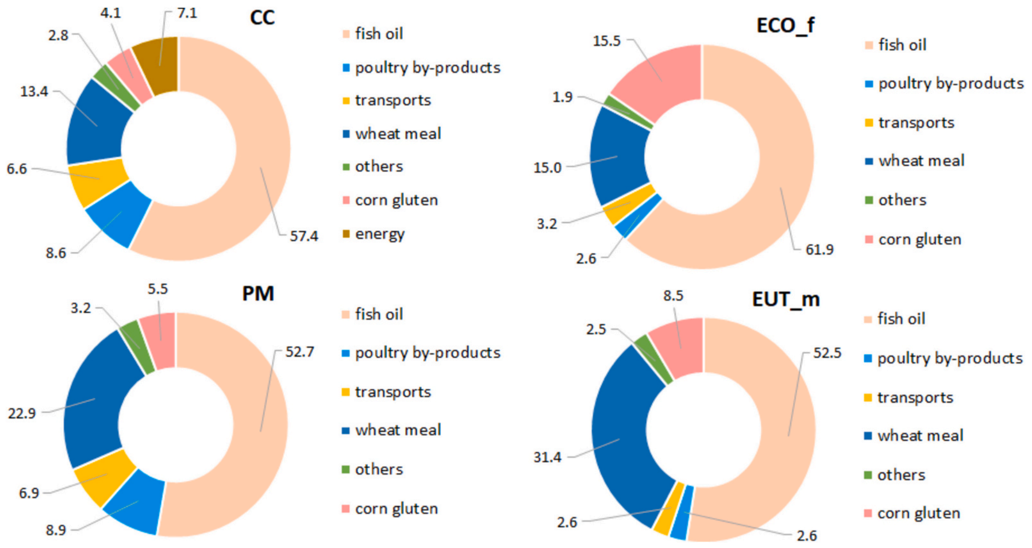

Fig. 6. Specific contributions of the process of PBM-f, involved in the value of the impacts for Climate Change, Ecotoxicity Freshwater, Particulate Matter and Eutrophication Marine.

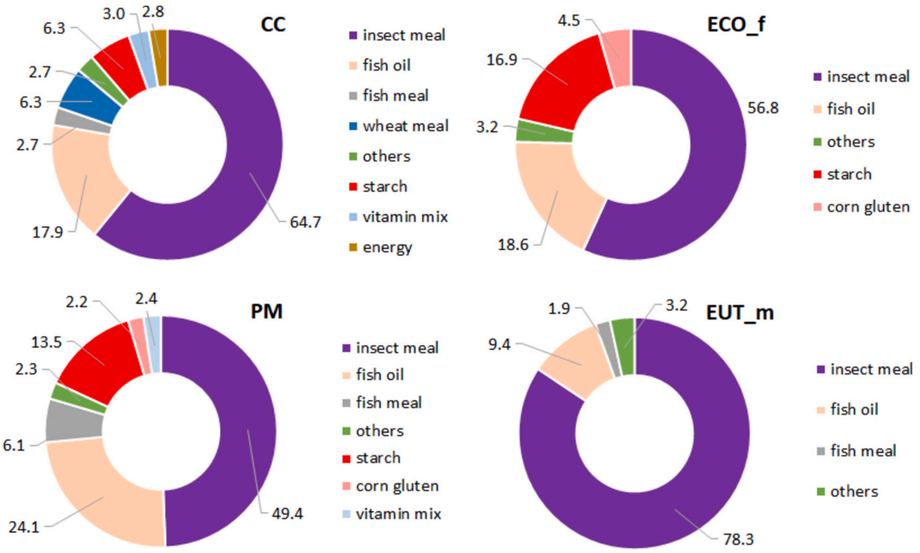

Fig. 7. Specific contributions of the process of TM-f, involved in the value of the impacts for Climate Change, Ecotoxicity Freshwater, Particulate Matter and Eutrophication Marine.

3.1. Uncertainty and sensitivity analysis

Secondary data typically pertain to resources and emissions associated with a particular process, technology, and production equipment and are obtained from literature that is cited. In ideal situations, metadata is added to secondary data to offer qualitative details about system boundaries and allocation rules, for example, and to determine whether the data can adequately characterize the system under investigation (Reap et al., 2008; Cellura et al., 2011). As underlined by Huijbregts et al. (2001), an important aspect to consider for the correlation between data used in the LCA study and data needed to represent the examined system is the temporal correlation. In specific, the degree of agreement between the study year and the year of the available data is represented by the “temporal correlation.” The results of current studies can be greatly distorted by using secondary data from earlier, faster-developing industrial technologies. The authors have performed not only an uncertainty analysis on secondary data available but also a sensitivity analysis in order to assess the effects of processing aspects (specific ingredients, energy consumption, and origins of raw materials) on feeds environmental profiles.

Focusing on the uncertainty analysis, the aspect taken into consideration is the level of updating of the database considered, comparing the results obtained by version 3.7 (Base scenario) and the ones obtained by 3.10 (New scenario), for all the four feed formulas. Table 6 shows the results of New Scenario, using EF 3.1 method, and has to be compared to the ones in Table 5 and Fig. 3. If we also compare the feeds in this case, the comments and the analysis that was conducted in the previous section remain valid. Instead, the aspect to evaluate is how the results of each individual case change based on the database used. The comparison highlights the absence of a general trend that unites all the impact categories. In fact, there are several environmental impact results that are limitedly affected from use of an updated database. It is the case of acidification where, in the first three cases (TM_f, PMB_f and TM_f) the variations remain below 10 % and in HI_f reaches 22 %.

Fig. 9 shows the environmental impact categories grouped according to a similar trend of the variation of the results based on the database, for

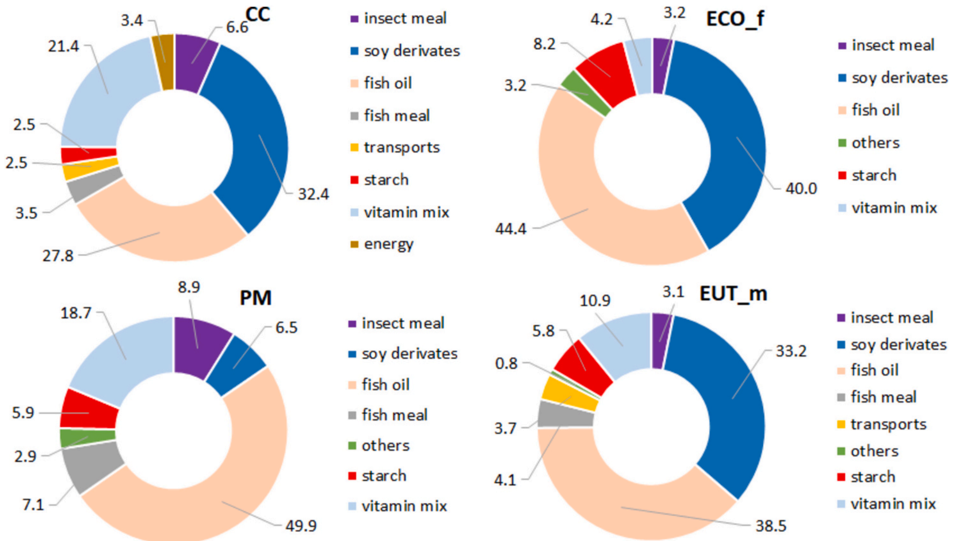

Fig. 8. Specific contributions of the process of HI-f, involved in the value of the impacts for Climate Change, Ecotoxicity Freshwater, Particulate Matter and Eutrophication Marine.

Table 6
Results of EF 3.1 method for the four feed formulas under study based on Ecoinvent 3.10.

Impact category	Reference unit	FM_f	PBM_f	TM_f	HI_f
Acidification	mol H+ eq	0.015	0.01	0.016	0.01
Climate change	kg CO2 eq	2.34	0.8	1.84	2.10
Ecotoxicity freshwater	CTUe	287.14	10.6	182.1	243.9
Eutrophication freshwater	kg P eq	0.0026	0.002	0.0015	0.003
Eutrophication marine	kg N eq	0.0067	0.002	0.012	0.01
Eutrophication terrestrial	mol N eq	0.041	0.02	0.06	0.03
Human toxicity cancer	CTUh	7.2E-09	4.1E-09	5.9E-09	6.7E-09
Human toxicity non-cancer	CTUh	3.6E-08	1.07E-08	3.3E-08	3.3E-08
Ionising radiation (human health)	kBq U235 eq	0.12	0.04	0.14	0.10
Land use	dimensionless (pt)	48.7	37.9	78.2	36.05
Ozone depletion	kg CFC11 eq	7.9E-08	3.7E-08	5.9E-08	7.5E-08
Particulate matter	disease incidence	1.5E-07	8.6E-08	1.2E-07	1.3E-07
Photochemical ozone formation (human health)	kg NMVOC eq	0.009	0.004	0.006	0.008
Resource use fossils	MJ (net calorific)	22.4	9.3	16.04	21.3
Resource use minerals and metals	kg Sb eq	2.03E-05	1.40E-05	8.8E-06	1.9E-05
Water use	m3 world eq	1.97	2.07	2.45	1.10

the four feed cases considered. In Fig. 9 (a) the impact categories are reported whose variations in the result dependent on the database are overall <50 % of the value itself. Similar behavior is detectable for Climate change, Eutrophication terrestrial, Land use, Particulate matter, Photochemical ozone formation and Water use. Resource use fossils category can be attributed to this group except for the result related to HI_f which has a result variation over 50 %. In Fig. 9 (b), the group of categories demonstrates higher results diversities that range from 40 % to 80 %, apart from Eutrophication marine in FM_f, which is limited to a variation of 13 % (+0,0002 kg P eq). Another exception, attributable to this group, is the Human toxicity non-cancer, which respect the range of variations for all the cases except for HI_f. In this last scenario, the results showed an increase of $1,8E^{-08}$ CTUh, compared to Base scenario. Finally, two impact categories lead to significantly different results in terms of magnitude: Ecotoxicity freshwater and Human toxicity cancer.

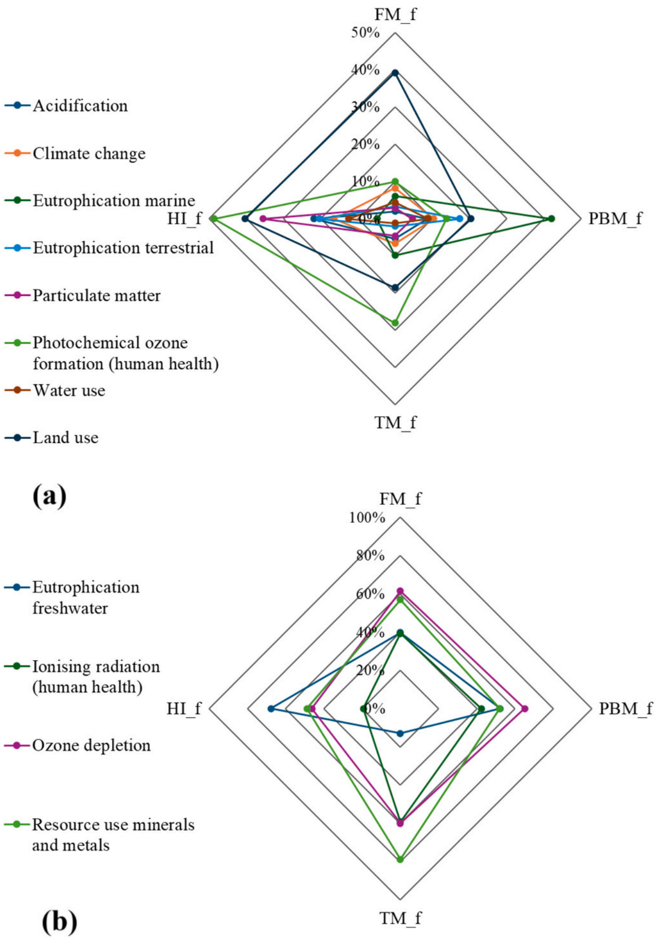

Fig. 9. Trends of results based on the two different databases.

In the first impact category, except for the PMB_f (+12 %), the results differ by an order of magnitude from those of the Base scenario. Observing the contributions, the highest value is attributable to the soy elements present in the recipes. Instead, in the other category, the differences are similar in the four feed formulas, two times the Base scenario values. Focusing on the Ecosystem freshwater category, the strongest difference consists in the contribution of soy elements. Observing the soybean processes in the two databases, the raw material production has increased massively its impact, due to a more impactful emission to environment of Chlorpyrifos, an organophosphate pesticide. This element is present in significant quantity (around 0.0018 kg/kg meal), with a high value of characterization factor for the indicator. Overall, the new results obtained give us a picture of how the impacts have varied over time, considering the reference years of the secondary data in the two databases.

Not only the secondary data can affect the variability of an CA results but also the modeling choices.In this case, the main contributions in the four feeds under study were investigated, allowing us to consider actions to possibly improve their environmental burdens. Considering FM-f as a benchmark case study, the analysis was focused on the alternative cases to classical feed. Firstly, two different parameters were considered for the sensitivity analysis of PBM-f: heat and energy reduction, and wheat meal alternatives. Then, for each impact category, the system's responses to the variations were assessed by calculating the ratio between the system's output in the base case (original LCA system) and the various scenarios, as showed in the Figs. 10 and 11 below. Subsequently, the percentage changes in the overall life cycle assessment results were calculated and analyzed.

A reduction from 10 to 30 % of heat and energy consumption in the PBM was considered. Next, we examined the inclusion of barley in the feed formula as a partial (50 %) and full replacement for wheat grain, owing to their closely matched nutritional attributes. The results of the first parameter variation are shown in Fig. 10 and the results of impact variations are expressed units based on the reference value of original PBM-f model (equal to 1). The reduction of the environmental impacts was limited, with all the decreased percentages in heat/energy consumption. The results underline the negligible effect of heat/energy variations at poultry by-product processes.

Considering the partial substitution of wheat grain with barley, it did not show a homogenous trend for impact variations. In particular, the substitution at 50 % of the wheat grain was characterized by a visible reduction in several impact categories, no sensible variation in others and increase impact values in two. The use of barley instead of wheat grain (substitution 100 %) seemed strengthening the results found with the partial substitution of 50 %. In particular, the sensible reductions are visible in Acidification (− 9 % and 19 %), Eutrophication Terrestrial (−13 % and 26 %), Land Use (−8 % and − 17 %) and Particulate Matter (−6 % and − 12 %). On the contrary, the results show an increase of impact for Eutrophication marine (+12 % and 23 %) and Human Toxicity non cancer (11 % and 22 %). In the other categories, the variations are negligible. This intensified impact might concern the open field cultivation practices or the use of specific chemical compounds. In fact, going into the LCI of barley production (from database), it is possible to observe significant differences of quantities of chemical substances used for this cultivation. This is likely due to the specific characteristics of the crops that can be more delicate and sensible to environmental conditions.

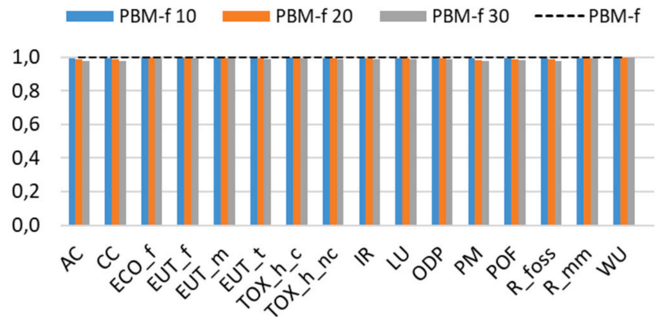

Fig. 10. Sensitivity analysis normalized results of the energy/heat consumption parameter for PBM-f model.

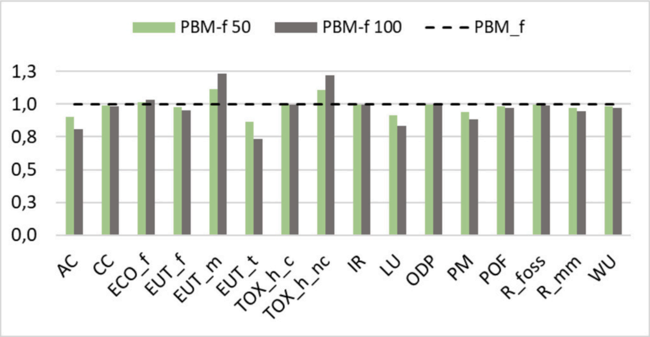

Fig. 11. Sensitivity analysis normalized results of the wheat grain substitution parameter for PBM-f model.

A better quantification of the degree of reaction of the LCA systems to these parameters have been given by Sensitivity indicators (Sis), categorized into five types (Lewandowska et al., 2004): VHS (Very High Sensitivity) over 10.5 %, HS (High Sensitivity) ranging from 4.5 % to 10.5 %, LS (Low Sensitivity) ranging from 1.0 % to 4.5 %, LIS (Low Insensitivity) ranging from 0.3 % to 1.0 %, HIS (High Insensitivity) ranging from 0 % to 0.3 %, VHIS (Very High Insensitivity) at 0 %.

The Sensitivity indicators are shown in Table 7 with the share of the input data in the environmental impact (in percent) in the final environmental impact.

Then, the energy consumption can be considered a parameter characterized by a very high insensitivity for the PMB-f, whereas the wheat grain substitution a parameter characterized by a sensitivity that is closer to high sensitivity limit for PBM-f.

Considering these results, the combined scenario of 30 % energy reduction and 50 % barley grain, to understand a possible positive combined effect, was not modeleddue to the negligible role of the energy consumption. Globally, the average percentage of impact reduction was lower compared to the reached average percentage in the case of heat/energy reduction of 30 %. Then, for improving the environmental impact of this type of aquafeed, a different solution to the wheat grain meal could affect in poultry by-product meal production should be the first choice. Barley can be a possible substitute and other cereals could be assessed if they have similar nutritional role.

For the TM-f case, the sensitivity analysis involved two different parameters, namely the modeling of the substrate of growth and the heat/energy consumption, similarly to PBM-f, categorized also based on the Sensitivity Indicators. Under the prospective of valorizing wastes or processing organic residues, a sensitivity analysis on the substrate origin for insects' growth was performed to investigate the possible variations

Table 7
Share of the input data in the environmental impact (in percent) and the results of the sensitivity analysis, sensitivity indicators for PBM-f.

Input	On the level of the whole life cycle		Range of the changes (%)	Criterion [SI]
	Mean (%)	Median (%)		
Energy consumption	0.9	0.9	0.3–1>	LIS
Wheat grain substitution	6.7	3.1	4.5–10-5>	HS

in environmental impact assessment for feeds with TM meal.

In the case of TM, an alternative scenario was performed considering the potatoes' residues as avoided wastes, diverted from the disposal chain, and the mixed grain feed as not derived from dedicated crop cultivation but as a by-product of the same production chain.

The results of the new scenario, visible in Table 8, demonstrated a significant decrease in whole impact categories. The main effects involved Photochemical ozone formation (human health), Human toxicity non-cancer, Land use and Eutrophication marine which decreased by one order of magnitude, −97 %, −88 % and –85 % respectively. In terms of Sensitivity Indicators, the results demonstrated that is a parameter characterized by (VHS) Very High Sensitivity, as visible in Table 9. The possibility of growing the *Tenebrio molitor* on a substrate composed of end-of-waste ingredients would lead to a significant improvement in the environmental footprint of this type of feed.

Moreover, similarly to the approach performed in the PBM-f investigations, the heat/energy consumption in the insect-rearing phase and meal preparation varied from −10 % to −30 %. In the case of TM production, the performed energy decreases did not lead to significant environmental impact reduction.

Even in the scenario with −30 % of energy/heat consumption, the percentage of reduction was lower than 5 % in most of the categories, closer to 10 % for the environmental categories strictly related to fossil fuels and related emissions (Resource use fossil fuel, Climate Change and Resource use minerals and metals). Finally, the only categories characterized by a decrease of ≥10 % were Human toxicity and Ecotoxicity freshwater. The heat and energy consumption reduction until 30 % did not have any appreciable effect on the environmental impact assessment. In terms of the whole lie cycle, the energy consumption is a parameter characterized by a Low sensitivity (LS) for the environmental impact (see Table 8).

Following the same method, the alternative scenarios for HI-f were based on variation of two ingredients of the feed, which were shown to be more impactful: soy derivate and fish oil. In fact, an assumption considered the substitution of common soy products with soy products not associated with deforestation processes (HI-f$_{\_no\_def}$). The second assumption took into consideration the substitution of the fish oil from fresh fish and anchovy with fish oil produced from animal residues (HI-f$_{\_fo\_residues}$). The results of these different model variations are presented in Table 10.

Table 8
Sensitivity analysis results for TM-f based on alternative modeling procedure for input materials for insect growth substrate.

Impact category	Reference unit	TM-f	TM-f (new scenario)
Acidification	mol H+ eq	0,01	0,004
Climate change	kg CO2 eq	1,7	0,60
Ecotoxicity freshwater	CTUe	17,7	4,6
Eutrophication freshwater	kg P eq	0,001	0,0008
Eutrophication marine	kg N eq	0,01	0,0016
Eutrophication terrestrial	mol N eq	0,05	0,0091
Human toxicity cancer	CTUh	2,2E-09	7,5E-10
Human toxicity non-cancer	CTUh	1,4E-07	4,5E-09
Ionising radiation (human health)	kBq U235 eq	0,17	0,1078
Land use	dimensionless (pt)	95,9	11,7
Ozone depletion	kg CFC11 eq	1,1E-07	6,7E-08
Particulate matter	disease incidence	1,1E-07	4,1E-08
Photochemical ozone formation (human health)	kg NMVOC eq	0,005	-0,013
Resource use fossils	MJ (net calorific)	12,3	8,4
Resource use minerals and metals	kg Sb eq	3,8E-05	1,6E-05
Water use	m3 world eq	1,8	0,6

Table 9
Share of the input data in the environmental impact (in percent) and the results of the sensitivity analysis, sensitivity indicators for TM-f.

Input	On the level of the whole life cycle		Range of the changes (%)	Criterion [SI]
	Mean (%)	Median (%)		
Growth substrate origin	69	65	(>10.5)	VHS
Energy consumption	1.7	1.3	1.0–4.5>	LS

The implementation of the "no deforestation" soy derivate compounds would lead to a general reduction of the whole impacts, with exceptions of unvaried values for Ionising radiation (human health) and Water use, or slight flexions (<5 %) of the indicators Acidification, Eutrophication freshwater, Ozone Depletion, Particulate Matter and Resource use fossils. More specifically, significant reduction in terms of Climate Change, as −30 % kg CO_2 eq., Land use, as −38 % dimensionless (pt), of Ecotoxicity freshwater and of Eutrophication marine, as −28 % CTUe and − 27 % kg N eq respectively would be reached. In particular, the sustainable origin of soy derivate compounds can be considered a VHS parameter for the environmental impact of HI-f (see Table 11). The usage of fish oil from residues led to similar but more evident results of soy derivate substitution. In fact, the reductions go from the minimum of Acidification (−11 %) to the maximum of Eutrophication freshwater (−84 %). Then, the fish oil origin can be categorized as a Very High Sensitivity parameter for the environmental impact of HI-f, more than soy derivate in the formula.

Then, it was considered the combined scenario, which showed cumulative results in line with the sum of the values obtained in the two individual scenarios. Therefore, the combination of different origins of fish oil and soy derivate would lead to very high impact reductions.

Table 10
Sensitivity analysis results of the scenarios HI-f with EF 3.1 method.

Impact category	Reference unit	Result		
		HI-f	HI-f$_{\_fo\_residues}$	HI-f_no def
Acidification	mol H+ eq	0.009	0.008	0.008
Climate change	kg CO2 eq	1.8	1.4	1.3
Ecotoxicity freshwater	CTUe	17.6	10.3	12.6
Eutrophication freshwater	kg P eq	0.002	0.0002	0.001
Eutrophication marine	kg N eq	0.005	0.004	0.004
Eutrophication terrestrial	mol N eq	0.02	0.02	0.022
Human toxicity cancer	CTUh	1.8E-09	7.9E-10	1.6E-09
Human toxicity non-cancer	CTUh	1.5E-08	9.4E-09	1.2E-08
Ionising radiation (human health)	kBq U235 eq	0.09	0.05	0.09
Land use	dimensionless (pt)	60.4	42.3	37.8
Ozone depletion	kg CFC11 eq	1.4E-07	7.7E-08	1.4E-07
Particulate matter	disease incidence	9.3E-08	7.3E-08	9.0E-08
Photochemical ozone formation (human health)	kg NMVOC eq	0.006	0.005	0.005
Resource use fossils	MJ (net calorific)	12.7	8.3	12.5
Resource use minerals and metals	kg Sb eq	3.8E-05	1.9E-05	3.4E-05
Water use	m3 world eq	1.0	0.4	1.0

Table 11
Share of the input data in the environmental impact (in percent) and the results of the sensitivity analysis, sensitivity indicators for HI-f.

Input	On the level of the whole life cycle		Range of the changes (%)	Criterion [SI]
	Mean (%)	Median (%)		
Soy derivate origin	12.4	7.8	(>10.5)	VHS
Fish oil origin	37.1	36.4	(>10.5)	VHS

4. Discussion

This study evaluates different feed formulas carefully and in detail with a view to environmental sustainability. In fact, regardless of the farmed finfish species, most studies agree that feed is the main hotspot in most of the impact categories (Parker, 2018; Sanchez-Matos et al., 2023; Zoli et al., 2023). Recent literature works on LCA of fish diets were including alternative proteins or oils to partially substitute FM and FO (fish oil); specifically, authors primarily focus on microalgae (McKuin et al., 2022, 2023), cyanobacteria (Napolitano et al., 2022), brewery by-products (Iñarra et al., 2022), plant/vegetables (Samuel-Fitwi et al., 2013; Smarason et al., 2017; Basto-Silva et al., 2019; Goyal et al., 2021; Bordignon et al., 2023), insects as HI (Smarason et al., 2017; Goyal et al., 2021) or TM (Le Féon et al., 2019), poultry by-products (Basto-Silva et al., 2018) and others (Ghamkhar and Hicks, 2020). The results of this study give more details on possible improvement of feed formulas for the gilthead seabream rearing and production. In particular, all the evaluated alternatives could positively affect the environmental performances of aquafeed diets, without losing nutritional properties, In particular, the results confirmed not only a lower water footprint but also a lower land use of the feed formula containing *Hermetia illucens* meal, such as reported in Goyal et al. (2021) where it was considered as ingredient for tilapia feed (*Oreochromis niloticus*). Moreover, this analysis show better results for HI-f on the whole environmental indicators, compared to Smarason et al. (2017) where HIM feed (included at 41 % corresponding to a complete FM substitution) lowered most of the impact categories, based on CML-IA method of impact assessment, but presented eutrophication and energy demand higher than a marine ingredients-based feed. Considering the TM-f instead, the results confirmed the fact that the use of TM, compared to FM-based feed, increased eutrophication at different levels, and energy use, as presented in (Le Féon et al., 2019). On the contrary, the use of TM in a feed formula lead to less Climate Change impact (−0.8 kg CO_2 eq for 1 kg of feed) and reduce the several aspects such as the water footprint and the use of fossil fuel resources. However, the disparities in data sources and diet composition create challenges when attempting to draw direct comparisons between these data. The results that have been obtained in terms of strong impact of the rearing process of the insects are confirmed from other results available in literature, obtained with other methods. Maiolo et al., 2020 showed that both Eutrophication and CED were higher for HI grown on wheat bran and rye meal than for the same insect farmed on wheat bran, alfalfa hay, and corn meal due to the presence of rye, a highly-impact material. Hence, the best performance shown by HI-f compared to the TM-f can be explained by the different substrates here hypothesized (grains for TM and organic wastes for HI). As shown by Smetana et al. (2016), the best scenario for insects is rearing HI on municipal organic wastes. This solution would reduce considerably the environmental impacts of TM production, as confirmed from the sensitivity analysis. In particular, this solution would lead to a production of 1 kg of HI proteins with a CC and CED reduced by 30 and 50 %, respectively, compared to the production of the same amount of TM, farmed on a substrate based on a mixed grain recipe.

An important consideration regards the feed formulas containing insect meal, in particular related to feed conversion ratio (FCR). In fact, considering updated studies on gilthead seabream diets on feed conversion ratios (FCR), Gai et al., 2023 reported FCR ranges of 1.64–1.74 for HI-f, while Piccolo et al., 2017 found narrower ranges of 1.02–1.28 for TM-f. This highlights differences in feed efficiency linked to HI meal and TM meal. The use of HI meal entails about 540 kg more feed per ton of fish than TM meal, impacting economic feasibility and environmental sustainability in aquaculture. According to (Sogari et al., 2023), factors influencing insect-based meal efficiency include insect species, developmental stage, meal type, and processing methods. The same authors also indicated variability in growth performance with insect meals in aquafeed, influenced by fish species and meal composition from different breeding substrates. Despite potential benefits like sustainability and nutrient-rich feed production, optimizing HI meal use in aquaculture diets is crucial for improving feed conversion efficiency and overall sustainability. Based on the above results, general considerations can be drawn. Firstly, as the researches available in literature corroborate, transportation plays a pivotal role in determining the environmental impacts associated with fishmeal production, which is in contrast with the three alternative ingredients explored. The alternatives are more environmentally sustainable in this aspect, due to the fact that they often involve shorter production and supply chains. Additionally, there is a concerted effort to promote local production, changing and reducing the imports, limiting economic and environmental costs associated with them. This principle can also be applied, even if with more complexity, to the production of fishmeal. To make fishmeal production more sustainable, one well-known approach is obviously to reduce the quantity of fishmeal used in animal feeds, but also to encourage the establishment of local fishmeal and fish oil production, possibly derived from processing waste (trimmings) within domestic facilities. This multifaceted strategy seeks to align fishmeal production with the principles of sustainability and minimize its environmental footprint. Secondly, on one hand vegetable ingredients, such as soybean, soy derivates, and wheat meal, significantly contribute to the whole impact of the feed and the choices on the diet formulas could be crucial, on the other hand animal-derived products, both from marine and terrestrial origins, notably affect positively the environmental performances of aquafeeds. The results confirm what was previously observed by Samuel-Fitwi et al. (2013) and Bordignon et al. (2023), who proposed that the use of plant ingredients to substitute FM might be a valuable strategy to mitigate the impacts, excepted eutrophication, as we found. Additionally to this statements, according to Basto-Silva et al. (2018) and Campos et al. (2020), meals and oils obtained through by-product processing, instead of original raw material, can improve the environmental impact of feed, especially in terms of climate change, energy consumption and use of resources. However, this evaluation, while conceptually acceptable, does not consider the economic and environmental value of resource recovery, so much that the authors themselves highlight the limitation of including the poultry production phase within the LCA boundaries of PBM and suggest reducing the use of fish offal to obtain FO.

Finally, this study relies on data available in the literature and the best available data have been used, but it would be desirable that true empirical studies, involving primary data gathering on key parameters, should be undertaken to corroborate the results.

5. Conclusions

The study investigated and compared the environmental impact assessments of different feed formulations for a carnivorous marine species, namely Gilthead seabream (*Sparus aurata*). The investigations underscored how implementing and incorporating three innovative ingredients, as protein sources, could significantly and positively change the environmental impact associated with the production chain of aquafeeds. The proposed fish feed formulas, serving as alternative to a standard fish meal-fish oil formula, exhibited similar nutritional characteristics for the gilthead seabream diet. The main conclusions of the paper can be summarized as in the following:

- The total or partial substitution of FM with insect meal or poultry by-products lead to more sustainable feed formulas, under different aspects. *Hermetia illucens* meal and Poultry by-products demonstrated improvements across global impact categories. On the contrary, *Tenebrio molitor* meal showed some criticalities but can be considered a possible impact reduction solution for the highlighted impact categories;
- Environmental sustainability improvement of feed formulas can be performed also by reducing fish oil (FO) content, where it is present, or optimizing its production process (i.e. using fish residues, rather than fresh fish); similarly, a proper selection of the origin or a reduction in the use or a substitution of soy and wheat derivatives, commonly used in feed formulas, could significantly limit the environmental impacts of these products, on whole impact categories. This entails focusing on cultivation methods, origin, and logistics in the supply chain.
- Insect meal from insect rearing processes, carried out valorizing organic residues or wastes products, emerges as viable pathway for achieving more sustainable ingredients for aquafeeds.

While it is crucial to consider each species in aquaculture individually - considering their unique farming parameters, including specific dietary requirements and the feed provided - our study proposes a broader application. Our work might serve as a model for other research into diet formulation and production for various carnivorous, euryhaline finfish. Furthermore, the quest for suitable alternatives should also consider both fish welfare and fillet quality, given that several ingredients, such as fish oil, are crucial sources of long-chain polyunsaturated fatty acids pivotal for human nutrition. Then, the purpose for future studies regarding LCA analyses is to evaluate new ingredients for seabream diets using real data from fish farms, improving the reliability. Indeed, coupling feed formulation with feed conversion efficiency data would provide more accurate quantifications of impacts to design a more sustainable production at least for gilthead seabream, which is underrepresented in scientific literature despite its importance in European/Mediterranean aquaculture.

CRediT authorship contribution statement

Maria Vittoria Tignani: Writing – original draft, Validation, Data curation, Conceptualization. **Enrica Santolini:** Writing – original draft, Methodology, Data curation, Conceptualization. **Giulia Secci:** Writing – original draft, Supervision, Data curation, Conceptualization. **Marco Bovo:** Writing – original draft, Supervision, Methodology. **Giuliana Parisi:** Writing – review & editing, Supervision, Conceptualization. **Alberto Barbaresi:** Writing – review & editing, Supervision, 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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