Earthworm as an alternative protein source in poultry and fish farming: current applications and future perspectives

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Abstract

Among the different agricultural activities, the livestock is one of the most impacting on the environment. The feeding of animals is often the main responsible of the adverse environmental impact related to animal productions. Above all for intensive production, the consumption of protein feed is a key aspect for the achievement of sustainable production processes. The actual consumption of soybean meal and fish meal is not sustainable due to the related environmental impact and to the increasing prices. Among the different alternative protein sources, in the last 20 years, the attention of research centres and private companies focused on insects, algae and other invertebrates but, up to now, little consideration was paid to the use of fresh earthworm or earthworm meal as a protein feed for monogastric animals.

The use of earthworms as an alternative protein source for fish and poultry feeding is an opportunity for providing environmental services via cleaner technologies. Thanks to earthworms, organic wastes and by-products generated by livestock activities can be valorised and become a resource for animal feeding in a circular perspective

In this context, this manuscript was designed to summarize the productivity, suitability and effectiveness issues connected with the utilisation of earthworms as alternative protein feed in poultry production as well as in aquaculture. The studies investigating the earthworm meal use are quite old above all those carried out in Europe; however, some general indications can be drawn: both for broiler and fish, the parameters usually evaluated are body weight gain, growth rate, feed intake and feed conversion rate, the acceptability level of earthworm meal in broiler diet is lower than 15% while in trout diet ranges between 25-30%. The inclusion of earthworm meal in diets with an inclusion level lower than the acceptability threshold allows good productive performances without affecting the quality of the final food products.

Keywords: Worms, animal feeding, circular economy, fish meal, soybean meal

1. Introduction

Food production is continuously increasing to sustain the incessant human demand. Animal-based food has high land-use and carbon footprint and the growing demand for meat and seafood induces remarkable pressure on terrestrial and marine ecosystems (FAO, 2017). Moreover, agro-food production systems and livestock activities produce a huge amount of organic wastes and by-products, whose management represents a serious concern both from economic and environmental points of view. Finally, considering that food consumption is centred mainly in urban areas, while agricultural production in rural ones, the current structure of the food production chain induces the accumulation of organic matter around cities, entailing logistic and management issues (Gerber et al., 2007; FAO, 2018).

The International Feed Industry Federation predicts that livestock production will be doubled by 2050 (IFIF, 2016). Meat production mainly comes from species such as cattle, pig, and poultry. The latter represents one of the most consumed animal foods and it is predicted to rise up over 90 % by 2050 (Alexandratos Bruinsma, 2012). In Europe, 14.6 Mtons of poultry carcass weight were consumed in 2017 (AVEC, 2018), and the per capita consume has been increased from 21 kg/year in 2007 to 24 kg/year in 2017. In Italy, 1.3 Mtons of poultry carcass weight was produced in 2017, making this Country as self-sufficient with regard to poultry meat.

Poultry has been recognized as one of the less environmentally impacting meats, mostly due to the absence of enteric fermentation and to the low Feed Conversion Rate (FCR) (Gerber at al., 2007). In fact, the constant improvement of the genetic potential of poultry and the careful knowledge of their nutritional requirements has allowed to achieve very high food efficiency (1.5 - 1.8 g feed/g weight gain). The productive performances (i.e., feed intake, FCR, live weight gain, mortality) are the main drivers of the broiler environmental impact (Bahadori et al., 2017).

In addition to livestock production, from 1960 to 2016, the global demand for edible fish products has increased from 9.9 kg to 20.0 kg per capita per year and reached 171 Mtons in

2016. This value outweighed population growth and even exceeded the value of food consumption deriving from meat from all terrestrial animals' production (FAO, 2018). The supply is no longer sustained by fisheries, which remained stable for more than 10 years at around 90 Mtons, but by aquaculture, which increased by an average of 8.8% per year (1980-2010) (Globefish, 2016). Considering that the 60% of fish stocks are overexploited and 90% is fully exploited, aquaculture can be an effective solution to reduce overfishing and to tackle the increasing demand of fish. Interestingly, in 2017 freshwater fish species accounted for 66 % of the production of all farmed aquatic animals worldwide (Zhou, 2019). Among these, the farming of trout, namely the rainbow trout (*Oncorhynchus mykiss*), the brown trout (*Salmo trutta*) and the brook trout (*Salvelinus fontinalis*), represents 2% of all thespecies reared in aquaculture (+8.2% from 2010 to 2016) while, in Europe, it yields 15% in volume of the farmed species and about 14% in value of aquaculture. In Italy, 27% of the revenue from aquaculture activities derives from trout farming and about 36,000 tons are farmed especially in Northern regions, making Italy the main European trout producer (FAO, 2018). Trout production (mainly the species *Oncorhynchus mykiss*) is characterized by high nutritional quality of the final product and very low values of FCR (with values closed to 1.2 that should even reach approximately 0.9 in the near future).

The increasing demand for animal products (meat and fish) means more pressure on feed resources, particularly in the face of rising fuel-feed-food competition. Feeding of animals, with regard to the protein components of feed, is a key aspect for the economic and environmental sustainability of livestock activities (Khan, 2016). For long term-sustainability of livestock production, the quest for alternative protein feed resources is essential, as the costs of conventional feed, such as soymeal and fishmeal, continue to be high. Proteins are provided by both animal and vegetable sources, depending on the Country. Fishmeal and soymeal provide popular protein sources for livestock of monogastric animals due to their amino acid profiles. The production of soybean is connected with deforestation, soil erosion, eutrophication, extensive use of pesticides, loss of biodiversity and a huge carbon footprint.

In recent years, the drastic increase of the market price for soybean and fishmeal has become a critical aspect of the economic sustainability of the poultry meat industry and of aquaculture. This has given rise to the demand for a new and more sustainable protein source (Veldkamp and Bosch, 2015).

Alternative protein sources of comparable value are therefore urgently needed in order to make poultry and fish from aquaculture production sustainable in the next future. Therefore, to meet the future requirements for proteins, new protein sources must be explored. For instance, the current increase in the content of terrestrial vegetable proteins in fish feed has serious implications for aquaculture from a technical, economic and environmental point of view (Burr et al., 2012). Although the digestibility of most vegetable proteins is generally similar to or higher than that of fishmeal, the amino acid profiles are lower than fishmeal. Consequently, amino acid supplementation is needed to maintain growth performance of fish fed diets containing high levels of plant-protein concentrates (Gaylord and Barrows, 2009; Lim et al., 2008). In addition, another issue affecting the feed used in livestock and aquaculture concerns the presence of diverse environmental contaminants, which can cause deleterious consequences to animal health, reduce rearing performances and represent a potential risk for humans due to the bioaccumulation of lipophilic contaminants in tissues of farmed organisms. For these reasons, modern feed mills use energy-intensive processes to remove polychlorinated biphenyls (PCBs) from wild fish and so-called FAN (Anti-nutritional factors) from terrestrial plants, in order to achieve an acceptable feed digestibility in farmed fish. It should also be noted that some fishmeal and fish oils are made from wild fish containing high levels of heavy metals, dioxins and PCBs, which are considered unsuitable for processing. It is technically possible to decontaminate fish oil, but this, of course, increases its price (Le Gouvello et al., 2017).

Among the alternative protein sources, in the last 20 years, the attention of scientific research and private companies has focused on insects (Smetana et al., 2016, Barbi et al., 2020), algae (Shields and Lupatsch, 2012; Ibekwe et al., 2017; Ansari et al., 2020) and other invertebrates (Cayot et al., 2009; Brown et al., 2011; van der Poel et al., 2013). In particular, despite the concerns due to the potential toxicity of insect meal due to bioaccumulation of toxic contaminants, the deficiencies in some amino acids, the content of chitin and saturated fatty acids, as well as palability and digestibility, insects are considered as an interesting protein source. . However, to date little consideration was addressed to the use of fresh earthworm or earthworm meal as a protein feed for monogastric animals.

The study about the use of earthworms as an alternative protein sources are limited to the '80 years. Earthworms can break down organic wastes (e.g., cattle and pig manure) rapidly under controlled conditions to provide valuable horticultural composts and high-grade protein suitable for animal feed (Edwards, 1985) and they are a valuable potential source of animal feed able, on one side, to develop an efficient management of agricultural waste and by-products and, on the other side, to reintroduce in the food production process organic matter whose traditional management involves serious environmental concerns (Hardy, 1996).

The use of earthworms as an alternative protein source for fish and poultry feeding is an opportunity for providing environmental services via cleaner technologies. Thanks to earthworms, organic wastes and by-products generated by livestock activities can be valorised and become a resource for animal feeding in a circular perspective, reducing the use of high-impacting traditional protein and, at the same time, the impact related to the management of these matrices. Therefore, where intensive agricultural and livestock activities take place and, consequently, a huge amount of organic waste is available, exploring new and alternative feed resources able to fully exploit and reutilize this biomass is urgently needed.

In this context, this manuscript aimed at summarizing productivity, suitability and effectiveness issues connected with the use of earthworms as an alternative protein feed in poultry and fish production. The main novelty of the present review is to provide a holistic assessment of earthworm use in a circular economy perspective, pointing out its role in the transformation of organic waste to high value protein feed, as well as current trends and environmental challenges. The paper is structured as follow. On the one hand, we strived to report the current situation concerning the earthworm growing conditions and performances as well its chemical characteristics (see 2. *Earthworms rearing)*. On the other hand, we reported the results of the literature review carried out about previous studies about the use of earthworms as protein feed (see 3. *Previous experience about earthworm meal use in poultry and fish farming*). The literature review was carried out in the scientific databases Scopus® and Web of Science® combining the following keywords: "earthworm", "protein source", "animal feeding", "aquaculture" and "poultry". Finally, we summarized the main gaps and future perspectives of earthworm use in poultry and aquaculture.

2. Earthworms rearing

2.1 Earthworm growing and characteristics

Overall, rearing of earthworm species require a temperature ranging between 15 and 25 °C, soil moisture content ranging between 60 and 85%, pH of 6.8 to 7.2 and can survive in a relatively low O_2 and high CO_2 condition or in dissolved O_2 within water (Sherman, 2003). The conversion of animal wastes into earthworm tissue is very efficient: a ton of suitable animal wastes produces up to 100 kg of worms, equivalent on a dry-mass basis to a conversion efficiency in the order of 10% (Edwards, 1985). Similar results were found by Hennuy and co-authors (1986), showing that 450 kg of vermicompost and 40 kg of earthworms (6.5 kg of worm meal with 70% protein content for animal feed) were produced from 1 ton of cattle manure.

Moreover, on cattle and goat manure, in a 5-weeks-long trial, the Tiger worm *Eisenia foetida* increased its biomass by 57% and 25%, respectively (Loh et al., 2005).

Besides animal manure (mainly cattle manure), different organic substrates can be used for earthworm rearing. For example, Barcelo (1988) reared earthworms on a mixture of fresh manure from cattle, swine, goats and chickens, leaves, sawdust, rice hull and rice bran, while Conti and co-authors (2018) used fruits and vegetables wastes as feedstock.

Earthworms dry matter (16-20% of fresh matter) contains from 55 to 70% of proteins (Mohanta et al., 2016), with a higher content of essential amino acids, such as lysine and methionine, compared to meat or fishmeal. The other constituents of earthworms are 6-11% fat, 5-21% carbohydrate, 2-3% minerals and a range of vitamins, including niacin and vitamin B12. Sogbesan and Ugwumba (2008) reported that earthworms contain (on dry mass basis) 63.0% crude protein, 5.9% crude fat, 8.9% ash, 0.43% Na, 0.53% Ca, 0.62% K, 0.94% P and 1476 kJ/100g of metabolizable energy. Furthermore, they determined the essential amino acid composition of earthworm meal and found that it contained arginine 2.83 g/kg, histidine 1.47 g/kg, isoleucine 2.04 g/kg, leucine 4.11 g/kg, lysine 6.35 g/kg, phenylalanine 6.26 g/kg, tryptophan 4.43 g/kg and valine 4.43 g/kg on a protein basis. Similar results were reported by Finke (2002), who showed that earthworm meal included 10.5% of crude protein, with arginine 0.61%, methionine 0.19%, lysine 0.66%, threonine 0.47%, tryptophan 0.09%, crude fat 1.2% and ash 0.6%.

Table 1 reports the main composition of earthworm tissue, soybean meal, fishmeal and insect tissue while **Table 2** summarizes the main strengths and concerns related to substituting conventional and insect protein source with earthworm meal (EWM).

Compared to insects, which are the main alternative protein source studied for animal feeding(Byambas et al., 2019, Thévenot et al., 2018), earthworms do not require heating during their rearing and can be efficiently grown on substrates that are waste or by-products owinga very low or null economic value. Furthermore, besides the production of earthworm tissue, the earthworm rearing involves the production of vermicompost, a valuable amendment that can be used as organic fertiliser and soil improver. Compared to insect, EWM has not deficiencies in amino acids profile, shows a better fatty acids profile and a negligible content of chitin, which in insect meal reduces the digestibility and palatability (Musyoka et al., 2019; Bohnes et al., 2019). On the other hand, concerning the regulatory aspects, it should be highlighted that, to date, the use of earthworm as an animal feed for monogastric animal and cattle is not admitted if earthworms are reared on wastes (e.g., animal manure, organic fraction of municipal solid waste), despite some preliminary findings showed that the safety of this procedure (Conti et al., 2019). On the contrary, insect meal can be used in aquaculture (Commission Regulation (EU) 2017/893).

Table 1 around here

Table 2 around here

2.2 Sustainability of earthworm rearing

Vermicomposting is recognised as a sustainable way to manage organic waste (Wu et al., 2014; Lim et al., 2016). Nevertheless, considering the three pillars making the sustainability concept (Pope et al., 2004), studies on economic and environmental analysis of vermicomposting process are scarce, while the social issues have not been analysed yet.

Regarding the economic sustainability, usually, only the selling of vermicompost is considered, although the price of earthworm meal is high (e.g., 15 €/kg of dry matter according Tedesco et al., (2019)). Lalander and coauthors (2015) estimated a profit of 100-280 USD per year and a ROI (Return of Investment) equal to 170-200% for a vermicomposting system for urban small-holder farmer with a productive capacity of 0.6-1.2 t/year of vermicompost, considering a life time of the plant of 5 years. Edwards et al. (2010) estimated an annual profit of about 2 million of USD for a medium size (36500 t/year) continuous-flow reactor vermicomposting system. To the best of our knowledge, no economic analysis was performed on the cost evaluation of earthworm rearing system specifically dedicated to earthworm selling. However, some general concerns can be highlighted. Concerning the costs, the production cost should encompass capital, manpower, processing energy, repair and maintenance costs (Blumenstein et al., 2012). Concerning revenues, the main product of vermicomposting is the vermicompost and the economic potential of a vermicomposting system depends on the initial costs, as well as vermicompost and earthworm revenues at a particular location, making the vermicomposting system not completely feasible under certain scenarios. Whatever the earthworms were used as an animal feeding (i.e., meal), the economic valorisation of meal would be affected by the cost of the other protein feed, such as fishmeal and soybean. Additional economic benefits could arise to the earthworm rearing through the improvement of the management of organic substrates, whose conventional management represents a non-negligible cost. For example, vermicomposting carried out on animal manure might reduce the nutrient content of the substrate (in particular of nitrogen; Busato et al., 2012), reducing the cost related to the manure treatments in order to fulfil the European rules (i.g., the Nitrate directive). Moreover, in urban areas, the earthworm rearing on organic fraction of municipal solid waste might benefit of disposal credits.

Regarding the economic benefits related to EWM use in animal feeding Djissou et al. (2016) assessed the economic consequences related to the substitution of fish meal by a mixture of earthworm and maggot meals. Experimental diets were tested during 42 days on catfish fingerlings and found that with alternative diets the growth performances and feed utilization of fingerlings are improved, and the diet cost reduced up to 50%.

From an environmental point of view limited attention was currently paid to the consequences related to the use of EWM as a substitute of soybean and, mainly, fishmeal. LCA fishmeal studies (Freon et al., 2017) have found that the environmental profile of this feed is low, at least in terms of conventional LCA impact categories, respect to other protein sources (Papatryphon et al., 2004; Davis et al., 2010). For example, compared to soybean meal and other vegetable protein sources coming from crops, fishmeal presents lower impact for acidification and eutrophication (Samuel-Fitwi et al., 2013, Silva et al., 2018), although traditional LCA does not account for biotic depletion of fisheries (Bohnes et al., 2019). This calls for comprehensive studies to assess the environmental trade-offs between crop-based and marine-based ingredients with coverage of a large spectrum of environmental impacts to avoid burden-shifting. The Life Cycle Assessment (LCA) approach was applied by Tedesco et al. (2019) and Conti et al. (2019) to quantify the environmental impact related to earthworm meal production considering vegetable and fruits waste as a rearing substrate. The emissions of methane and N-compounds during the vermicomposting was identified as the main environmental hotspots while the impact related to fresh earthworm processing to meal has a lower impact except than for lyophilization process.

3. Previous experience about earthworm meal use in poultry and fish farming

The protein content in poultry diet is an important parameter to guarantee high productive performance and to satisfy nutritional animals' requirements. The most important protein source used in broiler diet is soybean meal, characterized by high protein concentration, great digestibility and equilibrate amino acid profile.

Fish also needs high quality and quantity of protein in the diet, supplied largely from fishmeal but also from vegetable sources, mainly soybean meal.

The use of these ingredients is no longer sustainable and thus becomes necessary to search new protein sources with an optimal amino acid profile.

Earthworm use has already been analysed for poultry, being earthworms already part of their natural diet.

The studies focused on the use of earthworms for poultry feeding have started about 30 years ago and, especially the recent ones, were carried out in developing countries.

In contrast, there is a dearth of information on the use of EWM in trout diets. However, some experiences were carried out with other fish species. **Table 3** and **4** summarize the results of previous studies about EWM use in poultry and fish production, respectively.

3.1 Poultry farming

Barcelo (1988), in Philippines, evaluated six iso-protein (21% of crude protein) and iso-energetic diets including 0%, 6% and 14% of EWM. The results showed that increasing the level of EWM in the broiler diet from 6% to 14% and concurrently decreasing levels of fishmeal from 14% to 6% the FCR improves.

Loh et al. (2009), in Malaysia, evaluated the effect of EWM as a replacement of soymeal and fishmeal in the broiler diet. Five different diets (including 0%, 5%, 10%, 15% and 20% of EWM) were tested as partial replacement of soybean and fishmeal for 6 weeks. The final body weight, growth rate and feed efficiency measured in broiled fed with a diet including 10% and 15% of EWM were better than that of the control group, while no effect on feed intake was noted.

Prayogi (2011) conducted a study in Indonesia on quails. Four diets were tested (0% of EWM and 15% of fish meal - control, 5% of EWM and 10% of fishmeal, 10% of EWM and 5% of fishmeal and 15% of EWM and 0% of fishmeal). An increase of EWM amount in the diet decreased feed consumption, although supplementation with 10% earthworm meal returned good growth performance in terms of FCR and body weight gain. Thus, the inclusion of EWMcan be considered as a valuable solution to replace a large portion of the fishmeal in the diet with no adverse effects. However, in the study feed intake was reduced at a 15% inclusion level.

Son and Jo (2013) supplemented with EWM the diet of to 7-days old broilers for 6 weeks to assess its effect on growth performance and nutrient digestibility. Three diets containing 0%, 0.2% and 0.4% of EWM were tested. High feed intake, weight gain and nutrient digestibility was observed when the broiler were fed diets containing 0.4% EWM.

Son (2009) reported that supplementation of EWM ranging between0.2 and 0.6% in laying hens diet improves laying performance and egg quality, especially the ratio of egg yolk n-6/n-3 fatty acids. Furthermore, the supplementation of 0.2 to 0.6% EWM was effective in improve digestibility of crude protein of diet resulted improved broiler performance.

Bahadori et al (2017) evaluated, over a 7-weeks period, the effect of varied amount of EWM and vermi-humus (VH) on the growth performance of broilers. Besides the control, 4 experimental diets (containing 10 g VH/kg of dry matter supplemented with 0, 10, 20, or 30 g EWM/kg of dry matter) were evaluated. A greater feed intake was observed in broilers fed the control diet than those fed the diets containing EMW. This parameter decreased linearly as the amount of EWM supplementation increased. Weight gain was increased as supplementation of EWM was increased.However, FCR was slightly lower and it decreased linearly as dietary EWM supplementation increased. Similar results (i.e., 2% and 3% of EWM in the diet improved the FCR of broiler) were achieved in a previous study (Bahadori et al., 2015).

Zang et al. (2018) tested diets with 1%, 3% and 5% of EWM on broiler pullets for 1 month and found that FCR were improved by 12.6% and 22. 5%, when 3% and 5% EWM, respectively.

Table 3 around here

3.2 Fish farming

In the last 35 years, some experiences were carried out with different fish species. Yaqub (1991) successfully produced earthworms from waste to feed catfish (*Heterobranchus isopterus*). Ghosh (2004) used fresh earthworms (*Eisenia foetida*) as feed for catfish (*Clarias batrachus*) in India and observed a higher weight gain respect to the control fed with a traditional diet without EWM. Vodounnou et al (2016) tested, for a 6-weeks period, EWM (from *Eisenia foetida*) as substitute of fishmeal on *Parachanna obscura*: higher growth rate and FCR were obtained with the fish diet containing 50% of EWM.

Mohanta et al. (2016) evaluated weight gain, growth rate and FCR in rohu (*Labeo rohita*) using earthworm (whole, custard and pellet from *Eisenia foetida*) and highlighted how the pellet achieves the best performances for all the evaluated parameters.

Concerning the use of EWM in trout rearing, some tests were performed 20-30 years ago. Most of them, carried out using high levels of EWM (e.g., 100% of the diet in Tacon et al. (1983), from 25 to 75% in Pereira and Gomes (1995) or substituting from 50 to 100% of fishmeal in Stafford and Tacon (1984)), concluded that a high inclusion of EWM in trout diets adversely affects growth rate and FCR. On the contrary, a lower amount of EWM in trout diets (max 30% of the diet weight) had no adverse effect on the growth performance and FRC of fish (Stafford and Tacon, 1984 and 1985). EWM from *Eisenia foetida*, when used to replace 25 and 50% of the fish meal component in the trout diets, gave higher growth rates compared to the control diet with fishmeal (Velasquez et al., 1991).

Table 4 around here

4. Discussion and future perspectives

The use of earthworm and EWM as an alternative protein feed for the rearing of monogastric animals has been studied occasionally in the last 30-years in different geographic areas. However,in the last years, more attention has been paid to the use of earthworm asan alternative protein sources in poultry and aquaculture. Despite the increasing price of traditional protein sources, the literature review highlights that several aspects should be furtherly investigated.

Future research activities should be addressed to:

1) the identification of the best substrate(s) where earthworms can efficiently grow. In fact, to date there is a lack of information regarding the growing performances of the different earthworm species in single and mixed substrate (e.g., waste and by-products from agriculture and organic fraction of municipal solid waste);

2) the identification of the best species of earthworms to be reared and used to be used in feed for poultry and fish. To date, although different earthworm species were evaluated as alternative protein source, *E. foetida* was by far the most used but it could not be suitable on specific substrates;

3) the characteristics of earthworm and earthworm meal (e.g., amount of crude protein and fats, amino acid profile, levels of potential toxic contaminants) reared on different substrates, both independently and in mixture, as well as about their digestibility and palatability for animals;

3) the potential adverse effects due to the inclusion of EWM in feed towards the health status of reared poultry and fish is needed;

4) the productive performance expected for broiler and fish when soybean meal or fishmeal are replaced by EWM. To date, it is unclear if the partial substitution of traditional protein sources with EWM allow to reach better results;

5) the economic, environmental and social benefits related to the use of EWM rather than the use of soybean and fishmeal. Concerning economic issues, a limited information is

available about the cost of EWM but some preliminary studies reported a price considerably higher compared to traditional protein sources. Regarding the environmental sustainability as protein source there is a need for comprehensive studies to assess the environmental trade-offs between crop-based, marine-based ingredients and earthworm with coverage of a large spectrum of environmental impacts to avoid burden-shifting;

6) the consumer perception and the willingness-to-pay about poultry meat and fish produced using EWM as a local and less environmental impacting protein source.

Lastly, the regulatory aspect is one of the main issues currently limiting the use of EWM. To date, there is a dearth of information on the effect due to the use of EW and EWM through the food supply chain, from rearing substrate (e.g., OFMWS) to fish and meat. This calls for researches focused on quality, safety and security evaluation of animal fed with earthworm. Furthermore, when earthworm rearing takes place on manure and other organic waste the bioaccumulation of organic and inorganic contaminants, as well as the contamination due to pathogens, must be evaluated in order to provide useful information for the development of an updated regulatory framework.

5. Conclusions

This review highlighted the ample scientific and technical scope to propose earthworms as a suitable solution to valorise organic agricultural waste, to produce alternative protein sources for animal feeding.

The literature review carried out pointed out the state of the art regarding the earthworm use as alternative protein feed for monogastric animal summarizing the main results achieved in the last 30 years regarding the best growing conditions of earthworm, its biochemical characterisation and regarding the productive performances of poultry and fish produced using earthworms and EWM as substitutes of soybean and fish meal. The knowledge about these issues is still fragmented in term of geographic area and timing where the trials were performed, species of earthworms and fish and poultry taken into account and evaluated parameters.

For fish and poultry farming, the studies investigating the EWM use are quite old above (80's and 90's) all those carried out in Europe.However, some general indications can be drawn: 1) both for broiler and fish, the parameters usually evaluated are body weight gain, growth rate, feed intake and feed conversion rate (FCR), 2) the acceptability level of EWM in broiler diet is lower than 15% while in fish diet ranges between 25-30%. The inclusion of EWM in broiler and trout diets with an inclusion level lower than the acceptability threshold allows good productive performances without affecting the quality of the final food products.

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Table 1 - Composition of the traditional protein sources used for poultry and fish rearing and of earthworm meal (EWM)

PROTEIN SOURCE	DRY MATTER (% of fresh matter)	CRUDE PROTEIN (% of dry matter)	CRUDE FAT (% of dry matter)	LYSINE (% of crude protein)	DIGESTABLE ENERGY (MJ/kg)	SOURCE
Soybean meal	94%	40-42%	18-22%	2.65%	22.6	Pucher et al. (2016)
Fish meal	92%	56-62%	6-7%	4.08%[a]	16.3	Mohanta et al. (2016)
Earthworm meal	90%	58-71%	5-7%	4.04%[b]	12.46[b]	Bahadori et al.(2015) Khan et al. (2016)

Table 2 - Strengths and concerns of different protein sources from alternative (i.e., earthworm meal - EWM) and conventional protein sources.

	Earthworm meal (EWM)	Conventional protein sources
Cost	Still expensive compared to conventional protein sources but improvements could be achieved to make it more attractive. Can be reared on low-grade bio-waste and can turn bio-waste into high-quality proteins. Beside earthworm biomass also the produced vermicompost can be sold Companies rearing earthworms could benefit from subsidies because of the reduction of nitrogen in manure.	The cost is increasing year by year due to increasing demand of protein for animal rearing.
Composition	Well-balanced nutrient content (above all amino acid and fatty acid profile); they have the same or an even better amino acid profile compared to soybean meal and fishmeal. Earthworms while ingesting organic waste and soil, consume heavy metals through their intestine and skin, wherefore concentrating heavy metals in their body	Imbalances between essential and nonessential amino acids in soybean meal often require the addition of exogenous amino acid sources in monogastric diets. Low palatability and antinutritional factors in soybean meal when used in fish diets. Polychlorinated biphenyls (PCBs) from wild fish and so called antinutrients from terrestrial plants, in order to achieve an acceptable feed digestibility in farmed fish.
Sustainability	In a circular perspective, it can be an effective solution to re-valorise agricultural organic wastes and by-products while reducing the environmental impact related to traditional management of these matrixes. It can be produced locally	Poor environmentally sustainable vegetable or animal-derived sources. Protein crop production (e.g. protein from legumes seeds, soybeans, etc.) currently occupies only 3% of the EU's arable land. Large amounts of vegetable protein sources are imported in EU, largely originating from South America

Table 3 - Preliminary data about EWM use in poultry livestock.

Reference	Country/ Species	Amount of inclusion of EWM in the diet	Investigated parameters	Main results
Barcelo (1988)	Philippines/ Broiler	0%, 6% and 14%	FCR	Increasing EWM from 6% to 14% (while FM decreases from 14% to 6%) FCR improves
Loh et al (2009)	Malaysia/ Broiler	0%, 5%, 10%, 15% and 20%	body weight, growth rate and feed efficiency, feed intake	6-weeks trials. With 10% and 15% of EWM body weight, growth rate and feed efficiency are improved. No effect on feed intake.
Son (2009)	South Korea/ laying hens	0.4% and 0.6%	egg quality, digestibility	Higher ratio of egg yolk n-6/n-3 fatty acids, improved digestibility
Prayogi (2011)	Indonesia/ Quails	0%, 5%, 10%, 15%	Feed intake, FCR, body weight	10% of EWM improves FCR and body weight. Feed intake is reduced at a 15% inclusion level
Son and Jo (2013)	South Korea/ Broiler	0%, 0.2% and 0.4%	Feed intake, weight gain and nutrient digest.	During a 7-weeks tests, 0.4% of EWM improves feed intake, weight gain and nutrient digestibility.
Rezaeipour et al. (2014)	Iran/ Broiler	7.7% and 15.4%	FCR, breast muscle weight	FCR and breast muscle weight were improved
Bahadori et al., 2015	Iran/ Broiler	3.1%, 4.2% and 5.3%	FCR	2% and 3% of EWM improved the FCR. Besides EWM also 1% of vermicompost
Bahadori et al (2017)	Iran/ Broiler	1% vermi-humus + 1%, 2% and 3%	Feed intake, Weight gain, FCR. Pathogenic microbiota	In a 7-weeks test, when EWM increases feed intake and pathogenic intestinal microbiota decreases, FCR is slightly lower and weight gain increases.
Zang et al. (2018)	China Broiler	1%, 3% and 5%	FCR, heavy metals level	FCR improves by 13% and 22%, whit 3% and 5% of EWM.No difference for heavy metals
Note: FCR = Feed conversion rate, EWM = earthworm meal, FM = Fish meal				

607 **Table 4** – Results about EWM use in fish rearing (Scientific name of the species is reported

608 when available)

Reference	Country/ Species	Amount of inclusion of EWM in the diet	Investigated parameters	Main results
Tacon et al. (1983)	UK/ Trout	100%	Growth rate and FCR	Growth rate and FCR are negatively affected by EWM
Stafford and Tacon (1984)	UK/ Trout	from 50 to 100% of FM	Growth rate and FCR	Growth rate and FCR are negatively affected by EWM
Stafford and Tacon, 1985)	UK/ Trout	30%	Growth rate and FCR	No adverse effect on the growth performance and FRC
Yaqub (1991)	India/ Catfish	100%	FCR, Weight gain	EW and maggot meal. FCR improves, no differences in weight gain.
Velasquez et al., 1991	UK/ Trout	25 and 50% of the FM	Growth rate	Higher growth rates compared to the control diet with fishmeal
Pereira and Gomes(1995)	Portugal/ Trout	from 25 to 75%	Growth rate and FCR	Growth rate and FCR negative affected by 75% of EWM
Ghosh (2004)	India, Catfish	100% Fresh EW	Weight gain	Higher weight gain
Mohanta et al. (2016)	India/ rohu (*Labeo rohita*)	40%, 60%, 100%	Weight gain, growth rate and FCR	40% as pellet, 60% as custard, 100% as fresh earthworms. Best results for all the evaluated parameter for the pellet
Vodounnou et al (2016)	Benin/ *Parachanna obscura*	50%	growth rate and FCR	higher growth rate and FCR were obtained
Ngoc et al (2016)	Vietnam, Carp	30%, 70%, 100% of FM	Growth rate, FCR and feed digestibility	Compared to the control, protein digestibility was higher, growth rate was higher only with 70% of EWM, lipid conversion was lower with 100% EWM
Note: FCR = Feed conversion rate, EWM = earthworm meal, EW = earthworms, FM = Fish meal				