INTRODUCTION

Every responsible aquaculture sector focuses on reducing production costs, improving efficiency of the production systems and promoting environmental sustainability. Given that fish feeds are among the most expensive inputs in aquaculture production, it is therefore necessary to provide cost-effective, eco-friendly and nutritionally complete, fish feeds. Fish meal is the popular protein source in fish feeds thanks to its elevated protein levels, proper essential amino acid profile, minerals, vitamins, attractants, palatability and digestibility (FAO, 2017; Tacon, 1993). However, fish meal has progressively become scarce and expensive due to diminishing capture fisheries, high competition between human and animal industry, the global increase in the cost of energy and uncertain year-round supply (FAO, 2013). Therefore, the consistent use of fish meal in aquaculture has not only threatened the sustainability of fisheries ecosystems but has also increased fish demand, and thus affecting profit margins of the fish farmers (Munguti, Liti, Waidbacher, Straif & Zollitsch, 2006; Ogello, Munguti, Sakakura & Hagiwara, 2014).

Several prospects to produce cheap, reliable and eco-friendly alternative fish feeds are still debatable. The animal-based protein sources for fish feeds are limited by insufficient amino acids such as lack of methionine in meat and bone meal (Munguti, 2007), inadequate lysine in poultry by-products (Fasakin, Serwata & Davies, 2005), lower isoleucine levels and poor digestibility in blood meal (Mendoza et al., 2001). Moreover, the sustainability of the use of

Abstract

Use of fish meal in aquaculture is gradually becoming unsustainable due to competition, cost and ecological challenges hence the need to expand the alternative protein sources. The earthworm, *Eisenia fetida* is among the non-conventional protein sources, which have been tested with relatively promising results, thanks to its high protein levels, proper amino acid profile, high reproduction rate, low mortalities, fast growth and ease of production. The feasibility of using *E. fetida* for commercial fish feed production depends on the fundamental knowledge of its growth and reproductive biology, as well as the production methods. On the other hand, the nutritional suitability of *E. fetida* is determined by its amino acid composition and meal processing methods. Therefore, this study reviews the biological, biochemical composition as well as production and processing methods, as critical aspects for sustainable production and utilization of the earthworm in commercial fish feed production. Further, the study provides some recommendations and options to provide nutritionally complete and economically viable fish feed for efficient and sustainable aquaculture production systems.

KEYWORDS

*Eisenia fetida*, fish feed, fish meal, nutritional quality, protein source
animal-based protein sources is faced with the challenges of microbial contamination and potential transfer of diseases (i.e., from livestock to fish and human such as the mad cow disease) (Ogello et al., 2014). It is also difficult to quantify the amount produced, water pollution potential and fatty acid rancidity attributed to animal protein sources. Furthermore, there are religious and cultural restrictions that limit the use of animal protein sources in some communities, especially in the developing countries. On the other hand, the plant-based protein derivatives such as soybean is limited by low levels of methionine and inconsistency in availability and cost ineffectiveness (Gamboa-Delgado, Rojas-Casas, Nieto-López & Cruz-Suárez, 2013). Similarly, sunflower has inadequate lysine, phenylalanine, methionine, phosphorus, high fibre levels, low energy and poor palatability (Gallagher, 1994; Ogello et al., 2017). Generally, plant-based protein derivatives are limited by mismatching essential amino acids, presence of endogenous anti-nutritional factors that reduce their efficiency of utilization in fish (Hossain, Focken & Becker, 2003). Moreover, they have low palatability (Refstie et al., 2000), high ash and fibre contents (Olvera-Novoa, Olivera-Castillo & Martínez-Palacios, 2002), which if included at high levels reduces digestibility and protein conversion by fish as well as pellet quality of the feed (Drew, Borgeson & Thiessen, 2007; Mugo-Bundi et al., 2013; Ogello & Munguti, 2016).

All these attributes are known to reduce the bioavailability of nutrients to the fish, decrease efficiency of utilization thereby, increasing the feed conversion ratio, and thus reducing economic success (Hossain et al., 2003; Shao, Den & Gao, 2002). Indeed, the challenges associated with sustainability of protein feed ingredient sources in view of cost, nutritive value and resources have necessitated further research on viable animal protein replacers in fish diets (FAO, 2016). Therefore, non-conventional protein sources, such as earthworm, have gained interests to provide an alternative protein source thanks to its nutritional values that are close to that of fish meal (Fadaee, 2012; Rondón, Ovalles-Durán & León-Leal, 2003; Zhenjun, Xianchun, Lihiu & Chunyaing, 1997).

There are several earthworm species that have been tested for fish feed production (Dedeke, Owa, Olurin, Akinfe & Awotedu, 2013; Istiqomah, Sofyan, Damayanti & Julendra, 2009; Pucher et al., 2014; Tacon, Stafford & Edwards, 1983; Tam, Ngoan & Ogle, 2005; Vodounnou, Juste, Kpogou, Apollinaire & Didier, 2016). For example, Blue worm (Perionyx excavatus) and African night crawler (Eudrilus eugeniae) have comparable nutritional content with fishmeal that are within the recommended nutritional requirements of most fish (Pucher et al., 2014; Tam et al., 2005). However, the two earthworm species are not adaptable to wide range of climate and excess handling (Hasanuzzaman, Ahamed, Rahmatullah, Akhter & Rahman, 2010; Savala, 2007; Sinha, Herat, Valani & Krunalkumar, 2009; Tam et al., 2005). Perionyx excavatus resulted in similar or higher growth rate, protein efficiency and energy retention in Common carp (Cyprinus carpio) compared to fishmeal in the diet (Ngoc, Pucher, Becker & Focken, 2016). However, P. excavatus meal if not thermally treated might depress fish growth due to the presence of anti-nutritional factors (Pucher et al., 2014). Other species such as Lumbricus rubellus and Lumbricus terestris have also been studied with limited success to replace fishmeal due to incomparable nutritional contents. Lumbricus rubellus has low amino acid index (Hasanuzzaman et al., 2010; Istiqomah et al., 2009) while L. terestris has crude protein levels as low as 32.6%, which is below the dietary requirements of most carnivorous fish in intensive culture systems (Julendra, 2003; Tacon, 1987). Other commonly tested earthworm species for fish feed production include Libyodorlis violaceus (Dedeke et al., 2013), Allobophora longa (Tacon et al., 1983), Hyperidorulis africanus, Libyodorlis vilaceous and Alma mansoi (Dedeke, Stephen & Kayode, 2010). However, a majority of these species have resulted to depressed fish growth and poor feed utilization, and thus their utilization is limited in fish feed processing.

Studies have shown that Eisenia fetida has recommendable levels of protein, essential amino acids and lipids, which are similar to those found in fishmeal and, are in line with the nutritional requirements of many fish species (Vodounnou, Juste, et al., 2016). Other studies have recommended that E. fetida can be utilized to replace the conventional fish feed protein sources without compromising growth performance and reproduction (Stafford & Tacon, 1985). Eisenia fetida has superior growth rate thanks to its high feeding rate of up to 50% of its half body size (Tohidinejad, Madani & Jafari, 2011; Vodounnou, Juste, et al., 2016). It is adaptable to different organic materials with the ability to convert biodegradable matter up to five folds (Tripathi & Bhardwaj, 2004; Visvanathan, Trankler, Jospeh & Nagendran, 2005). Compared to most earthworm species, E. fetida has relatively high reproduction rate (i.e., three hatchlings per egg) that takes a short cycle to mature (Edwards & Fletcher, 1988). Moreover, E. fetida has low mortality compared to most earthworm species since it has the ability to balance and model its energy expenditure priorities (Rorat et al., 2015). This attribute enables it to survive in extreme conditions such as low temperatures, toxic and saline environments (Sinha et al., 2009). Besides, unlike Lumbricus terestris, which is an anaceic (deep burrowing) earthworm, E. fetida is epigeic (surface dweller). This phenomenon facilitates harvesting and lowers its production cost as it requires less human labour to feed and continuously turn its’ substrate to promote aeration. Moreover, E. fetida can efficiently be contained in great quantities within several levels of production units using simple technologies of vermicomposting, thanks to its hardy and adaptability nature (Apelhof, Webster & Buckerfield, 1996). Being a waste-borne organism, earthworms are cultured using organic wastes such as livestock manure, household remains and agro-industrial residues that are not often used for most farm activities. These attributes lower its production cost as it can be cultured in the backyard bins or in cheaply constructed holding units using locally available materials such as offcuts and stones. For this reason, the rural fish farmers with low income or urban dwellers with limited resources can be able to produce the earthworm with ease. Unlike fish and fish products, which often attract better prices when traded for human consumption than when sold to industries for fishmeal production, earthworms are principally utilized for organic fertilizers provision. In addition, Zakaria, Salleh, Mohamed, Anas and Idris (2012) found out E. fetida
protein to be economically viable compared to soybean and chicken waste meal in catfish (Clarias gariepinus) production. Therefore, they do not often compete directly for food with most domestic animals and human hence can be used as feed source.

Due to the biological and economic attributes of *E. fetida*, several studies have reviewed its potential as fish meal replacer. This study reviews the biological, biochemical composition, production and processing methods as critical aspects for sustainable production and utilization of the earthworm, *E. fetida* in fish feed industry. Further, the study provides recommendations needed to overcome the challenges in production and processing of *E. fetida* as feed ingredient for sustainable aquaculture.

## 2 | MATERIALS AND METHODS

The study adopted the scoping review methodology (Arksey & O'Malley, 2005) and systematic reviews to generate a comprehensive literature review of the potential of the earthworm, *E. fetida* as an alternative protein source in fish feed production. The systematic review of literature related to the topic of this paper was restricted to peer-reviewed publications and grey literature from approved international bodies such as the Food and Agricultural Organization (FAO). The published online research was collected from Scopus, Web of Science, Science Direct and Google scholar databases. Published printed review papers, books, book chapters, doctoral dissertation/thesis and conference proceedings were collected from various libraries using the online catalogue. The collected literature database was organized in copies, excerpts and notes according to topics. Mendley software was used to organize and make quotations that filtered cited literature. This paper reviewed the general literature database to get the insights of the limitations and challenges in obtaining fish feed ingredients. The paper also presents a detailed assessment on the biology, production and processing methods of obtaining *E. fetida* as fish feed ingredient. There is an account of the nutritional properties of *E. fetida* and associated tests on its potential as fishmeal replacer for feeds of various aquatic organisms. Recommendations and intervention options for its commercial production and efficient optimization and utilization have been presented.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Reproduction biology of *E. fetida*

Understanding the biology, growth, survival and reproduction is fundamental for utilization of *E. fetida* as an ingredient for fish feed production. *Eisenia fetida* belongs to the family Lumbricidae and genus *Eisenia*. *Eisenia fetida* has striped dark red colour, slimy segmented body and yellowish abdomen. The segmentation helps it in turning and burrowing while the stripes are an adaptation for camouflage to avoid predation. The exoskeleton is composed of a nitrogenous polysaccharide chitin that helps the worm to move and burrow though hard substrates. This means the chitin is proportional to the age (the older worms have higher chitin levels and vice versa), and highly dependent on the texture of culture substrate. Its shape is maintained by a hydrokeleton coelom fluid found in the cavity between dermal layer and the internal organs, which consists of watery matrix, plasma and coelomocytes (Patil & Biradar, 2017). Besides, the coelom fluid allows movement of internal organs, nutrients, wastes, eggs and sperms (Dorit, Walker & Barnes, 1991). The presence of haemolytic, proteolytic and cytotoxic enzyme in the coelom fluid builds the innate immunity of the earthworm (Cooper & Roch, 2003; Patil & Biradar, 2017). Naturally, earthworms release the coelom to keep its skin moist and to ease movement. However, whenever disturbed the foul-smelling yellow fluid is ejected to confuse and repel predators. *Eisenia fetida* is highly sensitive to light and heat. This means that continuous exposure to direct sunlight is detrimental to its survival (Tohidinejad et al., 2011). This negative phototropism allows the earthworms to feed actively at night when it is dark and reduced incidental vibrations (Kostecka & Garg, 2015). Naturally, the worm habits humid environments, because their skin must stay moist always to allow gaseous exchange through the skin into the blood stream. *Eisenia fetida* is an epigeic feeder, as it dwells on the surface without burrowing below the soil. Therefore, being a waste-borne organism, it thrives in surface habitats of rich organic material such as manure, horticultural lands and thick forests that contain woody and leaves compost (Vodounou, Kpogue, Tossavi, Mensah & Fiojbe, 2016). In presence of rich organic matter, the worm can thrive and remain active in a wide range of climate conditions. The worm has loads of microorganisms along its gut and consumes between 35% and 50% of their body weight a day (Tohidinejad et al., 2011). They have a high reproduction rate due to their hermaphrodite nature, which allows each individual to produce 9 cocoons, each containing up to 20 eggs at least every 14 days (Tripathi & Bhardwaj, 2004). The worm has a higher growth rate of up to 19 mg worm$^{-1}$ day$^{-1}$ with the fertilized egg taking 4 months to reach the adult stage (Neuhauser, Hartenstein & Kaplan, 1980; Tripathi & Bhardwaj, 2004). In captivity, they have a potential life span of between 4 and 5 years (Dynes, 2003).

### 3.2 | Culture substrates for *E. fetida*

*Eisenia fetida* is a product of the vermicomposting, which is a process involving mutual action of earthworm and microorganisms to convert biodegradable organic matter to humus-like vermicast and vermiliquid (Amouei, Yousefi & Khosravi, 2017; Hussain & Abbasi, 2018). Given that biomass and nutritional quality are the superior attributes considered when producing *E. fetida* for fish feeds, the vermicomposting process can be engineered not only to treat waste, but also to produce nutritious earthworms and vermisoil as fertilizer.

The production, growth and maturation of the *E. fetida* cocoons are determined by the quantity and nutritional quality of culture substrate, food particle size, environmental conditions and stocking density (Dynes, 2003; Fadade, 2012; Rorat et al., 2015). Therefore, substrate selection is fundamental in the culture of *E. fetida* indented
for fish feed production. The quality of culture substrate affects the nutritional quality of *E. fetida* in numerous ways. Substrates with elevated oily levels translate to a worm with the high fatty acid profile, which subsequently lowers protein content of the worm because of the inverse relation of proteins and lipids in animal tissues (Mohanta, Sankaran & Veeratayya, 2016). Moreover, depending on the substrate nutritional quality, the gut contents in the worm have a significant effect on the protein levels during processing (Degefe, Mengistou & Mohammed, 2016). Apart from nutrition, for the substrate to promote the worm biomass, it should be metabolizable, palatable, hold nitrogen contents above 8.3% dry weight, have low levels of potential contamination of undesired substances and contain less growth retarding chemicals and non-assimilated carbohydrates (Bakar, Afzan, Gawi, Mahmood & Abdullah, 2014; Degefe et al., 2016; Misra, Roy & Hiraoka, 2003; Prasanthrajan & Kannan, 2011; Sharma & Garg, 2018). Besides, the substrate should have the ability to provide microbes because during vermicomposting, the presence of fungi community provides organic matter to the worms, and thus increasing the worm’s biomass (Pramanik & Chung, 2011; Pramanik, Ghosh, Ghosal & Banik, 2007). On the other hand, the substrate should be of right texture. The harder the substrate, the higher the chitin levels in the worm’s skin since the earthworm will need hard tissue to penetrate the hard substrate. This translates to depressed growth of fish fed on earthworms grown in hard substrates because chitin is an indigestible protein (Mukti, Mahapatra, Rao, Chakrabarty & Pal, 2012).

The earthworms’ diet preference is primarily of fungi, protozoa and bacterial. In nature, earthworms dwell in grounds laden with rich organic material such as thick forests containing mass wooden and leave wastes. Therefore, *E. fetida* are cultured using a good number of animal and plants residues that are known to be high in organic matter and readily available (Sharma & Garg, 2017; Vodounnou, Juste, et al., 2016). A mixture of kitchen wastes comprising fruits, peelings, vegetable wastes and supermarket remains are among the commonly used culture substrates for *E. fetida* production due to their elevated organic matter levels (Bhat, Singh & Vig, 2016; Edwards, 1985; Pigatin, Atoloye, Obikoya, Borsato & Rezende, 2016; Tripathi & Bhardwaj, 2004). Acidic fruits and vegetables such as lemons, garlics, tobacco leaves and onions are not recommended as they might lower the pH of the substrate to suboptimal levels for the worms. Oil and greasy waste materials should be avoided not only because of the inverse relation of proteins and lipids in animal tissues (Mohanta, Sankaran & Veeratayya, 2016). Moreover, depending on the substrate nutritional quality, the gut contents in the worm have a significant effect on the protein levels during processing (Degefe, Mengistou & Mohammed, 2016). Apart from nutrition, for the substrate to promote the worm biomass, it should be metabolizable, palatable, hold nitrogen contents above 8.3% dry weight, have low levels of potential contamination of undesired substances and contain less growth retarding chemicals and non-assimilated carbohydrates (Bakar, Afzan, Gawi, Mahmood & Abdullah, 2014; Degefe et al., 2016; Misra, Roy & Hiraoka, 2003; Prasanthrajan & Kannan, 2011; Sharma & Garg, 2018). Besides, the substrate should have the ability to provide microbes because during vermicomposting, the presence of fungi community provides organic matter to the worms, and thus increasing the worm’s biomass (Pramanik & Chung, 2011; Pramanik, Ghosh, Ghosal & Banik, 2007). On the other hand, the substrate should be of right texture. The harder the substrate, the higher the chitin levels in the worm’s skin since the earthworm will need hard tissue to penetrate the hard substrate. This translates to depressed growth of fish fed on earthworms grown in hard substrates because chitin is an indigestible protein (Mukti, Mahapatra, Rao, Chakrabarty & Pal, 2012).

Animal-based residues, such as urine free livestock manure (except for poultry and rabbit manure, which are known to contain uric acid), have proven to be suitable substrate for earthworms. In vermicomposting, livestock manure is the most preferred because apart from providing organic matter, it stimulates biodegradation and raises pH within the culture substrate (Sharma & Garg, 2017, 2018; Vodounnou, Juste, et al., 2016). Horse manure has shown to produce *E. fetida* with higher (19 mg worm⁻¹ day⁻¹) specific growth rate than cow manure (7 mg worm⁻¹ day⁻¹) (Neuhauser et al., 1980; Reinecke, Viljoen & Saayman, 1992). Khomami, Mammadov, Chokami, and Sedaghathoor (2016) obtained a close-range maximum weight gain of 34 mg/g, 39 mg/g and 37 mg/g when *E. fetida* was fed with sugarcane bagasse, cow manure alone and when mixed with sugarcane respectively. A mixture of sewage and cow manure produced *E. fetida* with relatively high specific growth rate of 11.90 mg worm⁻¹ day⁻¹ (Li et al., 2016). This shows suitable organic wastes can either be used separately or in combination with other organic materials. Hog, sheep and goat manure have also been found to be suitable substrates for *E. fetida*. However, when using sheep or goat manure alone it is necessary to add additives to increase their C:N ratio (Munroe, 2007).

Other substrates that can be utilized for cultivating *E. fetida* are plant-based agro-industrial wastes such as rice straw and paper waste (Sharma & Garg, 2018), wheat straw, rice, barley bran (Chauhan & Singh, 2013), household and paper industry waste (Amouei et al., 2017), orange peels and filters (Pigatin et al., 2016), cake, sugar cane waste (Bhat et al., 2016) grass, pruning waste and flower wastes (Abbasi, Nayeem-Shah & Abbasi, 2015). Nevertheless, when culturing earthworm for fish feed production, livestock manure and sewage substrates should be used with caution to avoid transferring toxic compounds to fish and eventually human beings. Generally, earthworms bioaccumulate heavy metals from substrates therefore, food materials for culturing the worms should not be in contact with polluted soils or sludge, which can lead to contamination of diets and consequent impaired fish growth performance (Ireland, 1983; Kostecka & Pączka, 2006; Stafford & Bacon, 1985; Suleiman et al., 2017).

It is recommended that substrates for culturing *E. fetida* should be pre-composted for at least 10 days before use to increase acceptability, since the worm do not consume fresh vegetable wastes and livestock manures (Gunadi & Edwards, 2003). Moreover, pre-composting reduces anaerobic conditions, toxic/acidic compounds and salinity that might affect the earthworms (Gunadi & Edwards, 2003; Malińska, Zabochnicka-S’wia, Cáceres & Marfà, 2016; Nair, Sekiozoic & Anda, 2006). Additionally, when using plant-based materials as new substrates, it is advisable to include at least 10% of livestock manure when pre-composting to provide nitrogen, stimulate biodegradation and increase their pH levels (Bhat et al., 2016; Vodounnou, Kpogue, et al., 2016). Nonetheless, the quantity of food supplied to the earthworms should be controlled as overfeeding causes shrinking or death of worms as a result of substrate fermentation. Moreover, the rotting of the excess substrate attracts mites, which irritate the earthworm and competes with the worms for food (Loh, Lee, Liang & Tan, 2005).

### 3.3 Production systems of *E. fetida*

Vermicomposting uses the mutual action of earthworms and microorganisms to bio-transform organic matter into safe and stable materials. Therefore, earthworms are usually a by-product of vermicomposting alongside vermicast (solid phase) and vermiliquid (liquid phase) fertilizers. Producing earthworms for fish feed production
is depended on the intensity of the aquaculture system demand. In intensive aquaculture system, aquatic organisms are usually stocked at high densities and depend on high quality complete feeds, and thus earthworm should be produced in masses to meet the feed production demand. In Japan, there are over 3,000 vermicomposting plants that provide earthworm for processing fish feed for intensive aquaculture systems such as eel (Anguilla japonica) fish farming (Deolalikar, Hasan, Khan & Quibria, 1997; Ghosh, 2004).

On the other hand, in semi-intensive aquaculture, artificial feeds are required to supplement the naturally occurring food, earthworms are usually produced depending on primary production of culture systems. Beg, Mandal and Moulick (2016) demonstrated that E. fetida meal can supplement natural feeds at 50% replacement level of fishmeal in Indian carp (C. carpio) semi-intensive farming. In addition, vermicompost is utilized to supply organic fertilizer for improved primary production in semi-intensive farming. Several studies have shown that the vermicast fertilizer has more or less same impact on primary productivity in semi-intensive fish ponds as livestock manure (Ghosh, 2004). Kaur and Ansal (2010) recommended vermicast fertilizer application rate of 15,000 kg ha⁻¹ year⁻¹ in semi-intensive ponds. The vermiliquid too can be utilized in formulating feed fish. Zhenjun et al. (1997) found the E. fetida vermiliquid to contain nutritional qualities superior to most basal fish feed ingredients such as corn. Ramesh Guru and Govindarajan (2011) obtained a 36% increased yield on Oreochromis mossambicus fish fed diet containing 25% of vermiliquid. The authors related the increased fish yield to improved consumption, conversion and assimilation of the vermicast-enriched feed.

Therefore, vermicomposting is practiced in various intensities (either large scale or small-scale) depending on desired processing capacity (Adhikary, 2012; Kostecka & Garg, 2015). The nutritional content of E. fetida depends on culture methods (nutrient/medium), environment, stacking density, age of the worm, handling, harvesting and processing protocol (Kobayashi, Ohtomi, Sekizawa & Ohta, 2001; Mukti et al., 2012; Zakaria, Rahayu, MohdSalleh & Siti, 2013). Therefore, every production unit should provide optimal parameters for earthworm’s growth and development. Eisenia fetida requires aerobicity and on moist matter; moisture content ranging between 80% and 90%; pH within 5–9; ammonia levels less than 0.5 mg/kg; salt content not more than 0.5% and temperature between 20°C and 30°C (Edwards, 1985; Fadaee, 2012). Given that E. fetida is both epigeic and sensitive to light. It should be cultured in a dark, well ventilated and humid environment (Apelhof et al., 1996; Gunadi & Edwards, 2003).

### 3.3.1 Large-scale earthworm production

Industrial/commercial vermicomposting is done principally for the management of municipal, agricultural and industrial bio-solids (Adhikary, 2012; Ghosh, 2004; Sinha et al., 2009). Since the inception of vermicomposting in 1970 at Canada, biotechnological advancement has seen the adoption of various production systems in most advanced countries such as USA, Italy, Australia, Cuba, Philippines and India. Commercial vermicomposting is broadly categorized into windrow and flow-through systems.

Windrows systems are a simple technology commonly used for composting crop waste materials for fertilizer production but, has been modified in South America, parts of Europe and Asia for large scale/industrial vermicomposting (Hanc, Castkova, Kuzel & Cajthaml, 2017; Savala, 2007; Singh & Singh, 2014). In this system, the bio-degradable materials are placed either vertically or horizontally up to one meter high then inoculated with earthworms. The beddings are watered once in a while to keep the bedding moist and depending on the weather, the windrows can be kept open or covered. It is however advisable to use a concrete floor to prevent earthworm predators and ease the collection of the vermiliquid (Munroe, 2007). Mass harvesting of cast-free earthworms from this system is done using the commercially available mechanical centrifugal harvesters (Munroe, 2007; Singh & Singh, 2014).

The open bed (flow-through system) is another type of large scale vermiculture. Unlike in windrows where there are no physical borders, in the open-flow system the earthworms are cultured indoor using large rectangular wooden beds (Hanc et al., 2017). In this vermicomposting system, cast-free earthworms are normally harvested using the fresh bait method (Kostecka & Garg, 2015). This method involves slightly starving the earthworms for at least 1 week, then by adding new food or bait (preferably cattle manure mixed with nettle, valerian or flaxseed) at the surface, prompts the hungry worms to move upwards to the new feed (Kostecka & Garg, 2015). The used vermicast is henceforth removed from a breaker at the base of the meshed bed. This technique takes the advantage of epigeic nature of earthworms to feed on the surface and is suitable procedure when transferring the worms.

Large-scale vermicomposting is usually integrated in semi-intensive fish farming to supplement limiting nutrients from natural feeds (Pucher et al., 2013, 2014). Earthworm biomass from large-scale vermicomposting has been used to reduce cannibalism in African catfish (C. gariepinus) at high stocking densities (Chakrabarty, Das, Das & Biswas, 2009). In addition, apart from direct consumption of earthworm biomass by fish, this vermicomposting system provides nutrition to semi-intensive aquaculture by applying the vermicompost as organic fertilizer to increase pond primary production (Pucher et al., 2013). Besides, vermicompost fertilizer promotes physio-chemical properties of water and pond sediment thanks to its rich organic matter, total nitrogen and exchangeable cations (Kaur & Ansal, 2010; Sujatha, Mahalakshmi & Shenbagarathai, 2003).

### 3.3.2 Small-scale earthworm production

Small-scale vermiculture is commonly done at backyards, balconies, basement, offices and schools, principally to produce organic fertilizer and earthworms for potted plants and aquariums fish respectively (Adhikary, 2012; Hanc et al., 2017; Kostecka & Pączka, 2006). The culture units usually include ecological boxes or containers made using wooden beds layered with polythene paper (Kostecka & Pączka, 2006; Singh & Singh, 2014). A control tap is normally fitted
at the base of the culture boxes to collect the vermiliquid. To prevent the earthworms from suffocating in the leachate, a layer of gravel is placed below the feed materials. Small-scale vermicomposting is also done in commercially available plastic culture bins, which are fitted with the drainage tap, a lid and perforated basin that is placed at the base of the container to separate substrate from leachate. The plastic bins have perforated holes near the surface; therefore, they can either be left open to permit maximum ventilation or closed to prevent predators. When selecting materials for constructing culture bins, it is advisable to avoid using metal and styrofoam materials because they can release heavy metal to earthworms and chemicals to organic substrate respectively.

Earthworms in small-scale semi-intensive production are normally fed on locally available phytomas such as kitchen and market wastes, biogas slurry, urine free cow dung, wheat straw, leaf litter and saw dust that are known to be high in organic matter (Abbasi et al., 2015; Li et al., 2016). Harvesting earthworms for fish feed in this system can be done by pouring the content from culture bins to a polythene paper under bright light (Adhikary, 2012). Due to the sensitivity of earthworms to light, they crawl away from the vermi‐cast and are then hand collected, cleaned and fed to fish (Munroe, 2007; Singh & Singh, 2014). Nevertheless, container or box vermicomposting is considered to be labour-intensive due to removal of batches whenever new feed or water is to be added (Savala, 2007). Besides, the small containers coupled with periodic handling negatively affect the living conditions of the earthworms (Hanc et al., 2017).

Small-scale earthworm production systems have for long been used to economically improve fish yields in semi-intensive farming systems. Müller, Pucher, Tran, Focken and Kreuzer (2012) demonstrated how small-scale vermicomposting can improve fish production in a semi-intensive aquaculture system in North Vietnam. By producing on dry matter, between 6 and 36 kg of earthworms, the authors could supplement natural feeds and improve common carp (C. carpio) yield by up-to 75%. Likewise, in India, Ghosh (2004) designed a low cost commercially and environmentally viable model of integrating vermiculture and pisciculture. The author utilized vermicomposting to provide both earthworm biomass and vermicast as organic fertilizer in catfish (Clarias batrachus) semi-intensive ponds (stocking rate of 15,000 fish/ha) and, obtained a mean individual weight gain of 1.64 g/day. The author related the improved fish growth in the vermicast fertilized ponds due to increased primary production and improved water retention capacity.

For these reasons, the simple biotechnology of vermicomposting coupled with the best aquaculture management practices can promote fish productivity and improve feed utilization, and thus increasing financial benefit to small-scale rural farmers who represent the greatest population living with poverty in developing countries (Pucher et al., 2013). More economic benefits can be achieved through ecological balance which is achieved through the autonomous agro-ecosystems that promotes recycling (Pucher et al., 2014).

### 3.4 | Processing techniques for E. fetida

Earthworm harvesting, handling, processing and preservation are fundamental processes because they not only affect the nutritional quality, but also the palatability, microbial risks and possible toxicity of the worm meal. Handling and processing of E. fetida has been one of the main limitations to its commercial production due to the worm’s slimy, sticky and moist nature (Dynes, 2003; Edwards, 1985).

Some handling and harvesting techniques such as the conventional hand-harvesting and centrifugal mechanical handling irritates and stresses the earthworms and prompts them to release the coelomic fluid. Coelom is known be toxic to vertebrate because lysenin protein, which binds sphingomyelin and other cellular membrane phospholipids, causes contraction of smooth muscles and lysis of red blood cells hence killing vertebrates’ spermatozoa (Kobayashi, Ohta & Umeda, 2004; Ohta, Sekizawa & Kobayashi, 2000). Therefore, when processing E. fetida for fish feeds, it is advisable to thoroughly clean the earthworm so as to remove the coelom, which apart from being toxic, it reduces palatability of worm meal to fish due to its foul garlic smell (Kobayashi et al., 2001; Ohta, Aizu, Kaneko, Sato & Kobayashi, 2003; Vodounnou, Kpogue, et al., 2016). In addition, since lysenin is a heat-labile protein, blanching E. fetida in hot water or cooking earthworm meal reduces the coelom fluid toxicity to fish (Kobayashi et al., 2001; Pucher et al., 2014; Tacon et al., 1983).

Given that the E. fetida consumes up to half of its body weight, the worm should be kept in a bowl for at least 3 hr to enable it to evacuate the undigested residual contents in its guts (Akpodiete & Okagbere, 1999; Edwards, 1985). After harvesting and gut evacuation, the worm is usually washed repeatedly, and then killed either by lyophilization, osmotic shocking or blanching the worm in hot water (Medina, Cova, Vielma, Pujic & Carlos, 2003; Velásquez, Herrera & Ibáñez, 1986). Blanching is a more preferred mode of killing the worm than lyophilization and osmotic shocking because it preserves the nutritional value (Edwards, 1985). Moreover, the blanching enhances the palatability and reduces toxicity associated with the heat liable lysenin and haemolytic factors found in the coelom fluid (Kobayashi et al., 2004; Kostecka & Pączka, 2006; Medina et al., 2003; Tacon et al., 1983).

To prolong the shelf life and lower storage and transportation costs, the worms are usually dehydrated by either sun-drying, oven-drying or freeze-drying (Bou-Maroun et al., 2013). Several authors have got varying crude protein levels of E. fetida under different drying methods. Vodounnou, Kpogue, et al. (2016) got a crude protein of 66.2% and 59.7% dry matter when the earthworm was frozen and oven-dried respectively. Therefore, in order to preserve more protein contents of the worms during the drying process, freeze-drying is the most ideal, followed by oven-drying then sun-drying (Bou-Maroun et al., 2013; Gunya et al., 2016).
According to Gunya et al. (2016), freezing ensures long-term stability of proteins and preserves macro-nutrients. Moreover, freeze-drying method ensures longer shelf life of the worm and it reduces the loss of volatile chemicals and heat-labile nutrients, which translates to better growth performance on fish fed on the earthworm (Bou-Maroun et al., 2013; Khairnar, Kini & Harwalkar, 2013). However, more fatty acids are obtained when the worm is oven-dried unlike when freeze-dried (Bou-Maroun et al., 2013; Gunya et al., 2016). Nevertheless, oven-drying process is still excellent because, equally, it has minimal loss of dry matter content, it reduces the odour and off-flavour in worms that reduces unpalatability to fish and humans as well (Bou-Maroun et al., 2013).

The choice of biochemical test procedure is equally vital in determining the nutritional quality of earthworm when formulating fish feed. Most studies often use the Kjeldahl method to estimate the crude protein contents in *E. fetida* (Bou-Maroun et al., 2013; Chiu et al., 2015; De Chaves et al., 2015; Dynes, 2003; Gunya et al., 2016; Mohanta et al., 2016; Mukti et al., 2012; Rondón et al., 2003; Vodounnou, Kpogue, et al., 2016; Zhenjun et al., 1997). Zakaria et al. (2013) involved the statistical software (full factorial design) to compare digestion time and the volume of acid (*H*₂SO₄) using the Kjeldahl method. The authors suggested the worm sample to be digested for over 60 min in 15 ml *H*₂SO₄ to produce the highest percentage levels of nitrogen contents in the worms. Nevertheless, the Kjeldahl method might be misleading since the procedure accounts for all the nitrogenous compounds produced endogenous or by interaction between microorganisms in the earthworm gut during vermicomposting (Degefe et al., 2016; Gunadi & Edwards, 2003).

Besides, the Kjeldahl procedure also captures indigestible nitrogenous compounds such as chitin. Chitin is a modified polysaccharide unable to dissolve in most solvents, and thus indigestible by most fish species (Mukti et al., 2012; Shiau & Yu, 1999). Undigested chitin absorbs lipids in fish gut hence lowering its digestion and absorption (Tharanathan & Kittur, 2003). Besides, the chitin elevates fibre levels in earthworm meals and lowers energy availability in fish (Ringø, Zhigang, Rolf & Seong, 2012). It has been cited as one of the reasons behind the depressed growth recorded in most fish when earthworm meal inclusion level increases beyond certain level while replacing fishmeal (Mohanta et al., 2016). A chitin inclusion level of as low as 2% causes depressed growth, reduces feed digestion and poor absorption in fish and in return have financial implications due to poor feed utilization and food conversion ratio (Shiau & Yu, 1999). Therefore, it is recommendable to use analysis procedures that capture the true protein content in earthworms such as the Lowry (fol-lin) method. Otherwise, Ringø et al. (2012) recommended enzyme chitinase and chitobiase to breakdown the chitin. However, the authors cautioned that the incorporation of the two enzymes does not guarantee complete digestion of the chitin.

After analysis, *E. fetida* can be processed into fish feed by mixing it with molasses to form a paste or by air-drying to create the dry meal (Edwards, 1985). Processing of earthworm either whole or pelleted determines the feed intake and utilization in fish.

Mohanta et al. (2016) obtained a specific growth rate of 4.21 g/day and 3.38 g/day when *Labeo rohita* fish was fed on pelleted and whole *E. fetida* meal respectively. The authors also observed that the pelleted earthworm digestion and absorption on the fish as reflected in protein efficiency ratio, protein retention efficiency and energy retention efficiency of 1.26%, 23% and 18.6% was superior to that whole meal, which had 0.84%, 14.34% and 11.93% respectively. Therefore, it is advisable to process the worm meal in pelleted form to avoid leaching of protein and lipids that could have been assimilated in the body of the cultured fish (Fagbenro & Jauncey, 1998). It is prudent to thermally treat the final formulated earthworm meal in order to inhibit anti-nutritional factors in the worm (Rouelle, L’Huisser & Pussard, 1987). The thermal treatment of the worm meal is also necessary to potentially reduce the coelom-related toxicity and unpalatable species as well (Kauschke, Mohrig & Cooper, 2007; Kobayashi et al., 2004; Tacon et al., 1983).

### 3.5 Biochemical composition of *E. fetida* in relation to fish feed formulation

The feasibility of protein, essential amino acids, essential fatty acids and phospholipids are the main attributes considered when selecting protein sources for fish meal (Stanković, Đulić & Marković, 2011). They are responsible for growth, tissue development, energy source and substrate for key metabolic pathways. Protein is the most expensive and crucial component for a nutritionally complete fish feed due to fish inability to synthesize the indispensable amino acid, and thus they often remain inadequate (Tacon & Metian, 2008). Crude protein levels of *E. fetida* varies depending on culture substrate, environment, production system and processing method particularly on gut contents removal from the earthworm before analysis. Other studies have recorded good crude protein levels of *E. fetida* meal after evacuating gut contents. Gunya et al. (2016), Rondón et al. (2003) and Mohanta et al. (2016) evacuated the gut contents of *E. fetida* before biochemical analysis and got crude protein contents of 66.2%, 62.28% and 52% dry weight respectively. On the other side, Zhenjun et al. (1997) reported the inclusion of gut contents during earthworm analysis can lower crude protein levels up to 30% dry matter. The authors obtained crude protein content of 39.9% and 59.11% dry matter in *E. fetida* with and without gut contents respectively. Equally, Mukti et al. (2012) obtained relatively low crude protein levels of 40.43% dry matter when they did not evacuate the gut contents. On the contrary, Zakaria et al. (2013) achieved a high crude protein reading of 76.5% dry matter on *E. fetida* without evacuating gut contents. Other authors who did not evacuate gut contents and obtained relatively high crude protein of 70.42%, 56.9%, 70.3% and 60.65% dry matter are Bou-Maroun et al. (2013), Vodounnou, Juste, et al. (2016), De Chaves et al. (2015) and Chiu et al. (2015) respectively. Studies by Medina et al. (2003), Tacon et al. (1983) and Dynes (2003) were not clear if they evacuated the gut contents of *E. fetida* before crude protein contents but recorded recommendable levels of 61.8%, 58.78% and 55% dry matter respectively.
Apart from the gut contents, the crude protein in E. fetida is determined by the amount of temperature used during the drying of the worm. Alcívar-Cedeño, Dueñas-Rivadeneira, Sacon-Vera, Bravo-Sánchez and Villanueva-Ramos (2016) obtained crude protein contents of as low as 23.14% dry matter when they dried E. fetida at 120°C without air circulation. The authors got the highest crude protein of 62.5% followed by 61.5% dry protein after drying E. fetida at 60°C and 90°C respectively. Gunya, Muchenje and Masika (2019) obtained a crude protein of 51.62% dry matter after drying E. fetida at oven at 90°C. Nevertheless, all the above crude protein levels are higher than the minimum average 30% dry matter required for growth and reproduction by the most cultured fish species (Li, Kerogo, Munguti & Chorn, 2005; Takeuchi, Watanabe & Ogino, 1979; Wang, Takeuchi & Watanabe, 1985). Therefore, the earthworm can be cleaned and fed alive to the fish by rural farmers who have limited resources to formulate fish feeds.

Essential amino acid profile is crucial in formulating fish diets because they are known to promote growth, increase reproduction rates as well as improve disease resistance and enhance proper behavioural activities of the fish (Andersen, Waagbo & Espe, 2016; Wu, 2009). However, essential amino acids are often unbalanced in the common fish feeds. *Eisenia fetida* has comparable essential amino profile to that of fish meal and within the recommended dietary requirement of *Oreochromis niloticus*, which is one of the mostly cultured fish globally (Table 1). Besides, the worm has satisfactory lipid levels that can supplement insufficient protein levels thanks to the energy trade-offs between the metabolism of the two (Takeuchi et al., 1979). Some of the documented lipid contents on dry matter of *E. fetida* are 7.34% (Zhenjun et al., 1997), 11.3% (Medina et al., 2003), 6.6% (De Chaves et al., 2015) and 18% (Mohanta et al., 2016). Likewise, the worm has a high and a recommendable ratio of polyunsaturated fatty acids (linolenic ω-3 and ω-6) that are critical for formulating diets of many fish species (Hansen & Czochanska, 1975; Nehra, 2018; Istiqomah et al., 2009).

Earthworms have shown to contain essential elements and vitamins recommended for fish physiology, an attribute suitable for small-scale farmers in rural areas where vitamin/mineral premix is scarce and expensive to obtain (Halver, 1979). *Eisenia fetida* contains recommendable levels of essential micronutrients that are critical for fish growth and development (De Chaves et al., 2015) as shown in Table 2. It has ten times more iron content than fish meal and soya bean meal thanks to presence of haemoglobin in their blood plasma that boosts oxygen transport in fish (Zhenjun et al., 1997). Zhenjun et al. (1997) reported the worm to contain vitamin A (13.46 mg/L), B₁ (54.65 mg/L), B₂ (83.06 mg/L), E (31.64 mg/L) and C (292 mg/L/L) that are within the recommended dietary requirement of most fish (NRC, 1993). The earthworm is highly metabolizable and has gross energy of about 13.6 MJ/kg (Bahadori et al., 2017) and 3.85 Kcal/g (Mukti et al., 2012) appropriate for fish metabolic requirements. Moreover, *E. fetida* produces bioactive lumbricin and the coelom fluid contains strong antibacterial properties suitable for fish defense mechanism (Bansal, Gupta & Nehra, 2018; Istiqomah et al., 2009).

### 3.6 Utilization of *E. fetida* in fish feed processing

Owing to the high reproduction rate, low feeding cost, ease of production in captivity and the ability to thrive in a wide range of climates, *E. fetida* has attracted many biotechnological applications. Research and technological advancements have seen an earthworm being widely used in feed formulations, fertilizer production, pharmaceutical, bio-indicator, physiology, cosmetics, sanitary, ecotoxicology and genetics studies among others (Fadaee, 2012; Medina et al., 2003; Tohidinejad et al., 2011; Nehra, 2018; Istiqomah et al., 2009).

### TABLE 1  Amino acid composition (g/100 g crude protein) of *Eisenia fetida*, other worms and recommended requirement for Nile tilapia (*Oreochromis niloticus*)

| Amino acids | *Eisenia fetida*<sup>A</sup> | *Eisenia fetida*<sup>B</sup> | *Eisenia fetida*<sup>C</sup> | *Eisenia fetida*<sup>D</sup> | *Eisenia fetida*<sup>E</sup> | Other worms<sup>F</sup> | Peruvian fish meal<sup>G</sup> | *O. niloticus* requirements<sup>H</sup> |
|-------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Threonine   | 1.76            | 2.72            | 5.2             | 2.99            | 3.6/3.2         | 0.8–8.66        | 2.8             | 3.75            |
| Valine      | 1.32            | 2.39            | 4.7             | 3.22            | –               | 3.96/1.12       | 2.8             | 2.8             |
| Methionine  | 0.76            | 1.1             | –               | 1.2             | –               | 2.08–2.24       | 1.65            | 3.21            |
| Isoleucine  | 1.16            | 2.4             | 4.3             | 2.95            | 6.2/5.3         | 4.5–5.5         | 2.42            | 4.2             |
| Leucine     | 3.12            | 3.94            | 7.2             | 5.02            | 16.6/13.8       | 6.05–7.02       | 4.28            | 3.39            |
| Phenylalanin| 1.84            | 2.12            | 3.8             | 2.72            | 3.53/2.9        | 4.05–4.52       | 2.68            | 1.79            |
| Histidine   | 1.36            | 1.36            | 2.6             | 1.74            | 2.5/2.3         | 2.8–3.36        | 1.66            | 1.72            |
| Tryptophane | 0.12            | 1.73            | –               | –               | 0.92/0.5        | –               | 2.12            | 1.79            |
| Lysine      | 2.68            | 4.26            | 6.8             | 4.4             | 4.3/3.1         | 4.95–5.7        | 4.35            | 5.2             |
| Arginine    | 2.84            | 3.27            | 6               | 4.41            | –               | 8.01–8.66       | 3.87            | 4.2             |

Note: A - Vodounnou, Juste, et al. (2016), B - Zhenjun et al. (1997), C - Dynes (2003), D - Bahadori et al. (2017), E - Rondón et al. (2003), F - Dedek et al. (2010), G - Vodounnou, Juste, et al. (2016), H - Santiago and Lovell (1988).
Zakaria et al., 2013). In the recent past, there have been several studies on the use of *E. fetida* to replace conventional meals in fish nutrition. The increased interest in the use of *E. fetida* in animal feed specifically in fish diets is principally due to the superior nutritional attributes of having high protein contents, good amino acid profile and suitable compounds as discussed previously in this article. Many studies have reported the efficacy of *E. fetida* (alone or in combination with other ingredients) in promoting fish growth performance, increase reproduction, enhance digestibility, reduce stress, improve survival, lower feed conversion ratio as well as feed utilization and assimilation efficiency (Table 3). The least replacement level of 10% was obtained

**TABLE 2** Contents of mineral elements of *Eisenia fetida* and recommended requirements by fish

| Minerals     | *Eisenia fetida*<sup>A</sup> | *Eisenia fetida*<sup>B</sup> | *Eisenia fetida*<sup>C</sup> | *Eisenia fetida*<sup>D</sup> | Requirements by fish<sup>E</sup> |
|--------------|-------------------------------|-----------------------------|-----------------------------|-------------------------------|---------------------------------|
| Calcium      | 0.82% dry weight              | 0.3 g/L                     | 4.6 g/kg                    | 5.03% dry weight              | 5 g                             |
| Magnesium    | 0.3%                          | 0.11 g/L                    | 0.2 g/kg                    | 0.25% dry weight              | 500 mg                          |
| Potassium    | 2.2%                          | 0.9 g/L                     | 6.5 g/kg                    | 2.04% dry weight              | 1-3 g                           |
| Phosphorous  | 1.2%                          | -                           | 8.6 g/kg                    | 1.21% dry weight              | 7 g                             |
| Zinc         | 317 mg/kg                     | 6.9 mg/L                    | 1.2 g/kg                    | 183 mg/kg                     | 30-100 mg                       |
| Coper        | 812.1 mg/kg                   | 1.08 mg/L                   | 0.01 g/kg                   | 420.91 mg/kg                  | 1-4 g                           |
| Iron         | 1498 mg/kg                    | 0.33 g/L                    | 2.8 g/kg                    | 73,425 mg/kg                  | 50-100 mg                       |
| Manganese    | 116.6 mg/kg                   | 3.27 g/L                    | 1.8 g/kg                    | -                             | 20-50 mg                        |

Note: A - Gunya et al. (2016), B - Zhenjun et al. (1997), C - De Chaves et al. (2015), D - Gunya et al. (2019), E - NRC (1977).

**TABLE 3** Replacement level of fish meal using *Eisenia fetida* meal in fish diets

| Fish tested on          | Attribute/element tested                              | Replacement levels | Author(s)                                                                 |
|-------------------------|-------------------------------------------------------|--------------------|--------------------------------------------------------------------------|
| Rainbow trout (*Salmo gairdneri*) | Growth performance and feed utilization efficiency | Below 50%          | Stafford and Tacon (1985)                                                |
| Rohu (*Labeo rohita*)   | Growth and nutritional gains of fry                   | 40%                | Mohanta et al. (2016)                                                    |
| Nile tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*) | Growth performance and assimilation efficiency of post larvae | 339 g/kg dry diet | De Chaves et al. (2015)                                                  |
| Rainbow trout (*Onchorhynchus mykiss*) | Growth performance                           | 25%                | Tacon et al. (1983)                                                     |
| Wynaad mystus (*Mystus montanus*) | Growth performance and feed utilization           | 40%                | Sakthika, Ronald, Siva Kumar and Felicita (2014)                         |
| Eel (*Anguilla anguilla*) and Carp (*Cirrhinus mirgala*) | Growth performance                             | 25%                | Knipesh, Mohan, Subha and Vijayalakshmi (2003)                           |
| Guppy (*Poecilia reticulata*) | Reproduction and survival                          | 100%               | Kostecka and Paczkza (2006)                                             |
| Goldfish (*Carassius auratus*) | Growth and performance                           | 10%                | Popek et al. (1996)                                                     |
| Nile tilapia (*Oreochromis niloticus*) | Growth performance and feed utilization           | 32.2%              | Mukti et al. (2012)                                                     |
| Catla (*Catla catla*), Rohu (*Labeo rohita*), Mrigal (*Cirrhinus mirgala*) | Growth performance and feed utilization          | 50%                | Beg et al. (2016)                                                       |
| Obscure snakehead (*Parachanna obscura*) | Growth performance and feed utilization on fry | 25% and 50%        | Vodounnou, Juste, et al. (2016)                                          |
| African catfish (*Clarias gariepinus*) | Growth performances and feed utilization in fingerlings | Ratio of 2:5 mixed with maggots | Djissou et al. (2016)                                                   |
| Rainbow trout (*Onchorhynchus mykiss*) | Growth performance                              | 25% and 50%        | Veláquez, Ibanez, Herrera and Oyarzun (1991)                            |
| Common carp (*Cyprinus carpio*) | Growth performance and feed efficiency            | 1:1 with *Musca domestica* larvae | Coroian et al. (2015)                                                  |
| Chinese shrimp (*Penaeus chinensis*) | Growth and survival                               | Liu (2006)          |                                                                          |
| White shrimp (*Penaeus vannamei*) | Growth performance and muscle nutritional        | Chiu et al. (2015)  |                                                                          |
| Catfish (*Clarias gariepinus*) | Growth and survival                                | Zakaria et al. (2012)|                                                                          |
| Tilapia (*Oreochromis mossambicus*) | Fry growth                                      | 36% vermilion       | Rameshghuru and Govindarajan (2011)                                      |
by Popek, Łuszczek and Rościszewska (1996) on Carassius auratus while the highest (100%) was achieved by Kostecka and Pańczka (2006) on Gupy (Poecilia reticulata). Nevertheless, the 10% inclusion of E. fetida meal by Popek et al. (1996) doubled the reproduction of the C. auratus. With the 100% replacement of fish meal with E. fetida meal Kostecka and Pańczka (2006) observed a higher survivability, increased biomass and improved reproduction of the aquarium fish (P. reticulata). The authors showed the small-scale production of E. fetida at households and in school using the ecological boxes has the possibility of reducing costs of fish production in backyard ponds and aquariums. Vodounnou, Juste, et al. (2016) obtained a high specific growth rate of 2.11 g/day in Parachanna obscura fingerlings when fed on E. fetida meal at 25% inclusion levels in fish meal that had 1.5 g/day.

Despite the high protein content and the comparable nutritional properties in E. fetida, on average, most of the above summarized studies showed that earthworm can only replace fish meal up to 50%. Most of the studies observed reduced fish growth when E. fetida meals were included beyond 25%, citing the indigestible chitin and foul-smelling coelom fluid that are known to lower digestibility and palatability (Dedeke et al., 2013; Tacon et al., 1983). Common carp (C. carpio) and Nile tilapia (O. niloticus) had lower specific growth of 2 g/day and 1.3 g/day, respectively, when fed on E. fetida meal compared to 2.2 g/day and 2 g/day obtained when fed on fish meal (De Chaves et al., 2015). Furthermore, despite the several research studies on the earthworms, still worm meal is not commonly produced commercially nor traded locally particularly in developing countries. Therefore, the success of E. fetida in fish feed production can be improved through innovative techniques to overcome the various limitations associated with the earthworm application in the animal diet formulation.

There is a need to develop harvesting technologies, which can recover coelom and substrate free worms to reduce unpalatability, avoid the toxicity and unnecessary continuous washing. Research should provide simple harvesting technologies such as the use of fine meshed net to calmly harvest substrate and coelom free worms. By taking the advantage of E. fetida sensitivities to light, a net can be placed in the opposite direction of the source of light. The light can prompt the worms to escape through the net to collecting container. The same net technology can also be used to move the worms to the new substrate. By placing the net above the old bedding, the worms can move comfortably through the net to the new food, this can avoid unnecessary handling that stresses them. With the knowledge that the chitin level in worms is directly proportional to the age of the worms, the net can be engineered to allow harvesting medium aged worms only. Besides, separating chitin from the earthworm can greatly improve digestion and absorption of its meal in fish. Research should provide a nutritionally complete and soft textured culture substrate. A nutritious substrate would eliminate the need for gut evacuation because their inclusions during analysis or feed formulation might not significantly affect the nutritional quality of the worm meal. Likewise, a nutritious and soft textured substrate would mean the worm will have reduced development of the indigestible chitin in the worm's exoskeleton. This will further reduce unnecessary movement and burrowing in search of food materials. Also, when formulating fish feed, mixing the worm with other feed ingredients such as soy bean or barley can be an option to balance the essential amino acid profiles. Djissou, Adjahouinou, Koshio and Fiogebe (2016) mixed E. fetida with maggot and were able to completely replace fish meal. The authors obtained the best growth performance and feed utilization in C. gariepinus from the mixture compared to the fish meal. Since the worm is being used in several regions, principally for vermicomposting, the process can be utilized to provide fish nutrition; directly through the earthworm protein biomass and indirectly by increasing primary productivity in semi-intensive fish pond consequently reducing the usage of conventional diets (Boaru, Georgescu, Struți & Ladoși, 2017; Dynes, 2003). In addition, the vermicomposting process has various ecological benefits because the worm transforms organic waste, which reduces landfill, avoids secondary pollution, and saves energy resources (Pérez-Godínez, Juan & Martha, 2017). Moreover, the worm's ability to convert organic materials can be cultured using less economically viable but readily available agro-industrial residues such as coffee husks to produce low cost and ecologically friendly fish feeds.

4 | CONCLUSION

Understanding the biology, production and processing methods of E. fetida in this study are prudent for the mass production of nutritionally complete earthworm meal. There is potential of commercial production of nutritionally complete E. fetida meal if, and when proper technological innovations to overcome the various limitations associated with worms are put in place. Combined with proper feed management practices, the worm can efficiently replace the unsustainable conventional animal and plant protein sources without compromising growth and having major economic and sustainability concerns. Together with the other vermicomposting by-products (i.e. vermicast and vermiliquid), E. fetida can economically and ecologically benefit the small-scale fish farmers who often have under-utilized organic wastes and lack technological know-how in fish feed formulation. Therefore, there is a need for more research on simple technological advancements to promote the commercial production of E. fetida meal to formulate a low-cost practical and environment friendly nutritional feeds for sustainable production. This will make the fish feed affordable and easily available to both resource-poor farmers (i.e. small-scale) and large-scale farmers and eventually overcome the fish feed-related challenges derailing the aquaculture development and ecological woes.

ACKNOWLEDGEMENTS

We would like to acknowledge the Austrian Agency for International Cooperation in Education and Research (ÖeAD-GmbH) for the scholarship at the University of Natural resources and life sciences, Austria.
CONFLICT OF INTEREST

We hereby certify that there is no conflict of interests among the four authors whose names are listed in the Manuscript. The Authors have neither financial nor non-financial interests in the topic review of the manuscript.

ETHICAL STATEMENT

We certify that this is our original review work and it has not been neither submitted nor published elsewhere as a whole in part. The authors are responsible for all the content in the Manuscript.

ORCID

Sonnia Nizlani Musyoka https://orcid.org/0000-0001-8172-8105

REFERENCES

Abbsi, S. A., Nayeem-Shah, M., & Abbassi, T. (2015). Vermicomposting of phytoasses: Limitations of the past approaches and the emerging directions. *Journal of Cleaner Production*, 93, 103–114. https://doi.org/10.1016/j.jclepro.2015.01.024

Adihary, S. (2012). Vermicompost, the story of organic gold: A review. *Agricultural Sciences*, 3, 7–13. https://doi.org/10.4236/as.2012.37110

Akpodiete, O. J., & Okagbore, G. N. (1999). Feed accessories from animal production. In S. I. Omeje (Ed.), *Acompendium of ideas, facts and methods in the science and technology of Animal Agriculture Ran Kennedy; Animal Sciences*. 71–82.

Alcivar-Cedeño, U., Dueñas-RivadeneiraI, A., Sacon-Vera, E., Bravo-Moura, E., & Medina, L. (2013). Impact of preparation process on the protein structure and on the volatile compounds in *Eisenia fetida* protein powders. *Food and Nutrition Sciences*, 4, 1175–1183. https://doi.org/10.4236/fns.2013.460172

Boaru, A., Georgescu, B., Struţ, D., & Ladoş, D. (2017). The potential of *Eisenia fetida* usage in aquaculture. *ABAH Bioflux*, 9(2), 55–58.

Bou-Mourou, E., Loupiac, C., Loison, A., Rollin, B., Cayot, P., Cayot, N., ... Medina, L. (2013). Impact of preparation process on the protein structure and on the volatile compounds in *Eisenia fetida* protein powders. *Food and Nutrition Sciences*, 4, 1175–1183. https://doi.org/10.4236/fns.2011.33013

Bhat, S. A., Singh, J., & Vig, A. P. (2016). Effect on growth of earthworm and chemical parameters during vermicomposting of press mud sludge mixed with cattle dung mixture. International Conference on Solid Waste Management. *Procedia Environmental Sciences*, 35, 425–434. https://doi.org/10.1016/j.proenv

Boaru, A., Georgescu, B., Struţ, D., & Ladoş, D. (2017). The potential of *Eisenia fetida* usage in aquaculture. *ABAH Bioflux*, 9(2), 55–58.

Bou-Mourou, E., Loupiac, C., Loison, A., Rollin, B., Cayot, P., Cayot, N., ... Medina, L. (2013). Impact of preparation process on the protein structure and on the volatile compounds in *Eisenia fetida* protein powders. *Food and Nutrition Sciences*, 4, 1175–1183. https://doi.org/10.4236/fns.2013.460172

Beg, M. M., Mandal, B., & Moullick, S. (2016). Potential of earthworm meal as a replacement of fish meal for Indian major carps. *International Journal of Fisheries and Aquatic Studies*, 4(3), 357–361.

Bhat, S. A., Singh, J., & Vig, A. P. (2016). Effect on growth of earthworm and chemical parameters during vermicomposting of press mud sludge mixed with cattle dung mixture. International Conference on Solid Waste Management. *Procedia Environmental Sciences*, 35, 425–434. https://doi.org/10.1016/j.proenv

Boaru, A., Georgescu, B., Struţ, D., & Ladoş, D. (2017). The potential of *Eisenia fetida* usage in aquaculture. *ABAH Bioflux*, 9(2), 55–58.

Bou-Mourou, E., Loupiac, C., Loison, A., Rollin, B., Cayot, P., Cayot, N., ... Medina, L. (2013). Impact of preparation process on the protein structure and on the volatile compounds in *Eisenia fetida* protein powders. *Food and Nutrition Sciences*, 4, 1175–1183. https://doi.org/10.4236/fns.2013.460172

Bhat, S. A., Singh, J., & Vig, A. P. (2016). Effect on growth of earthworm and chemical parameters during vermicomposting of press mud sludge mixed with cattle dung mixture. International Conference on Solid Waste Management. *Procedia Environmental Sciences*, 35, 425–434. https://doi.org/10.1016/j.proenv

Bhat, S. A., Singh, J., & Vig, A. P. (2016). Effect on growth of earthworm and chemical parameters during vermicomposting of press mud sludge mixed with cattle dung mixture. International Conference on Solid Waste Management. *Procedia Environmental Sciences*, 35, 425–434. https://doi.org/10.1016/j.proenv

Bhat, S. A., Singh, J., & Vig, A. P. (2016). Effect on growth of earthworm and chemical parameters during vermicomposting of press mud sludge mixed with cattle dung mixture. International Conference on Solid Waste Management. *Procedia Environmental Sciences*, 35, 425–434. https://doi.org/10.1016/j.proenv

Cajika, P., & Damjanac, B. (2008). Effects of earthworm extract against pathogenic bacteria isolated from *Cyprinus carpio*. *International Journal of Microbiological Applied Science*, 7(04), 2329–2340. https://doi.org/10.20546/ijcmas.2018.704.267
Liu, S. L. (2006). The effects of fresh diet Eisenia fetida on growth and immunological parameters of shrimps. Master Thesis, Chinese Academy of Sciences.

Loh, T. C., Lee, Y. C., Liang, J. B., & Tan, D. (2005). Vermicomposting of cattle and goat manures by Eisenia fetida and their growth and reproduction performance. Bioresource Technology, 96, 11–114.

Malitiska, K., Zabochnicka-S’wia, M., Cáceres, R., & Marfà, O. (2016). The effect of precomposted sewage sludge mixture amended with biochar on the growth and reproduction of Eisenia fetida during laboratory vermicomposting. Ecological Engineering, 90, 35–41. https://doi.org/10.1016/j.ecoleng.2016.01.042

Medina, A. L., Cova, R. A., Vielma, P., Pujic, M. P., & Carlos, J. V. (2003). Immunological and chemical analysis of proteins from Eisenia fetida earthworm. Food and Agriculture Immunology, 15(3), 255–263.

Mendoza, R., De Dios, A., Vazquez, C., Cruz, E., Rique, D., & Aguilera, C. (2001). Fish meal replacement with feather-enzymatic hydrolyzates co-extruded with soya-bean meal in practical diets for the Pacific white shrimp (Litopenaeus vannamei). Aquaculture Nutrition, 7, 143–151. https://doi.org/10.1046/j.1365-2095.2001.00164.x

Misra, R. V., Roy, R. N., & Hiraoka, H. (2003). On-farm composting methods. Food and Agriculture Organization of the United Nations. Land and Water Discussion Paper 2.

Mohanta, K. N., Sankaran, S., & Veeratayya, S. K. (2016). Potential of earthworm (Eisenia fetida) as dietary protein source for rohu (Labeo rohita) advanced fry. Cognitive Food and Agriculture, 2, 1.

Mugo-Bundu, J., Kyoo-Okoth, E., Ngugi, C. C., Manguya-Lusega, D., Rasowo, J., Chepkirui-Boit, V., ... Njiru, J. (2013). Utilization of Caridina nilotica (Roux) meal as a protein ingredient in feeds for Nile tilapia (Oreochromis niloticus). Aquaculture Research, 46, 346–357.

Mukti, P. B., Mahapatra, S. C., Rao, P. S., Chakrabarty, D., & Pal, H. (2012). Nutritive potential of earthworm, Eisenia fetida meal in the diet for Nile tilapia (Oreochromis niloticus) fingerlings. International Research Journal of Pharmaceutical and Applied Science, 2(5), 117–123.

Müller, J. L., Pucher, J., Tran, T. T., Focken, U., & Kreuzer, M. (2012). The productive potential of earthworm Eisenia fetida. Cell Tissue Research, 302, 263–270. https://doi.org/10.1007/s004410000284

Olvera-Novoa, M., Olivera-Castillo, L., & Martinez-Palacios, C. A. (2002). Sunflower seed meal as a protein source in diets for Tilapia rendalli (Boulanger 1896. Fingerlings). Aquaculture Research, 33, 223–229. https://doi.org/10.1046/j.1365-2109.2002.00666.x

Patil, R., & Biradar, M. (2017). Earthworm's coelomic fluid: Extraction and importance. International Journal of Advanced Scientific Research, 2(2), 1–4.

Pérez-Godínez, E. A., Juan, C., & Martha, B. (2017). Growth and reproductive potential of Eisenia fetida (Sav.) on various zoo animal dung after two methods of pre-composting followed by vermicomposting. Waste Management, 64, 67–78. https://doi.org/10.1016/j.wasman.2017.03.036

Pigatin, L. F., Atoloye, I. O., Obikoya, O. A., Borsato, A. V., & Rezende, M. O. (2016). Chemical study of vermicomposted agro-industrial wastes. International Journal of Recycling Organic Waste Agriculture, 6, 55–63. https://doi.org/10.1007/s40093-016-0117-7

Popek, W., Łuszczek, E., & Rościszewska, M. (1996). Influence of supplementary feeding of earthworm (Eisenia fetida) (Annelida, Lumbricidae) on growth, gonad maturation and spawning in goldfish (Carassius auratus). Agriculture Academy, 310, 45–53.

Pramanik, P., & Chung, Y. R. (2011). Changes in fungal population of fly ash and vinasce mixture during vermicomposting by Eudrilus eugeniae and Eisenia fetida, documentation of cellulase isozymes in vermicompost. Waste Management, 31, 1169–1175. https://doi.org/10.1016/j.wasman.2010.12.017

Pramanik, P., Ghosh, G., Ghosal, P., & Banik, P. (2007). Changes in organic-C, N, P and K and enzyme activities in vermicompost of biodegradable organic wastes with liming and microbial inoculants. Bioresource Technology, 98(13), 2485–2494. https://doi.org/10.1016/j.biortech.2006.09.017

Prasanthraj, J., & Kannan, J. (2011). Assessing the growth of earthworms with respect to environmental factor and feeding materials. Crop Resources, 41, 253–254.

Pucher, J., Ngoc, T., ThiHanYen, T., Mayrhofer, R., El-Matbouli, M., & Focken, U. (2014). Earthworm meal as fishmeal replacement in plant based feeds for common carp in semi-intensive aquaculture in rural Northern Vietnam. Turkish Journal of Fisheries and Aquatic Sciences, 14, 557–565. https://doi.org/10.4194/1303-2712-v14_2_27

Pucher, J., Steinbronn, S., Mayrhofer, R., Schad, I., El-Matbouli, M., & Focken, U. (2013). Improved sustainable aquaculture systems for small-scale farmers in northern Vietnam (Chapter 8). In H. L. Fröhlich, P. Schreinemachers, G. Clemens, & K. Stahr (Eds.), Sustainable land use and rural development in southeast Asia: Innovations and
policies for mountainous areas (pp. 281–317). Berlin, Germany: Springer Environmental Science and Engineering.

Ramesh Guru, G., & Govindarajan, B. (2011). Study on growth of Oreochromis mossambicus fed with Vermiwash (Earthworm Coelomic fluid) diet. *Journal of Bioscience Resources*, 24(4), 232–238.

Refstie, S., Korsoen, O. J., Storebakken, T., Baeveftjord, G., Lein, I., & Roem, A. J. (2000). Differing nutritional responses to dietary soybean meal in rainbow trout (Onchorynchus mykiss) and Atlantic salmon (Salmo salar). *Aquaculture*, 190, 49–63. https://doi.org/10.1016/S0044-8486(00)00382-3

Reinecke, A. J., Viljpen, S. A., & Saayman, R. J. (1992). The suitability of *Eudrilus eugeniae*, *Perionyx excavatus* and *Eisenia fetida* (Oligochaeta) for vermicomposting in southern Africa in terms of their temperature requirements. *Soil Biology & Biochemistry*, 24(12), 1295–1307. https://doi.org/10.1016/0038-0717(92)90109-B

Ringa, E., Zhigang, Z., Rolf, O., & Seong, S. (2012). Use of chitin and krill in aquaculture – Effect on gut microbiota and the immune system: A review. *Aquaculture Nutrition*, 18, 117–131. https://doi.org/10.1111/j.1165-2095.2011.00919.x

Rondón, V., Ovalles-Durán, J., & León-Leal, M. (2003). Nutritional value and amino acid requirements of earthworm flour (*Eisenia fetida*). *Agriculture and Environmental Science*, 44(1), 43–58.

Sarkheda, T., Ronald, J., Siva Kumar, V., & Felicicita, J. (2014). Growth of Mystus montanus fed with two different earthworm meal. *International Journal of Environmental Science*, 4(4), 551–557.

Tripathi, P., & Bhardwaj, B. (2004). Earthworms in waste and environmental management. *Bioresource Technology*, 24, 725–283. https://doi.org/10.1016/j.biortech.2003.09.005

Singh, M. K., & Singh, P. (2014). *Handbook on vermicomposting: Requirements, methods, advantages and applications*. Hamburg: Anchor Academic Publishing.

Singha, R., Herat, S., Valani, B., & Krunalkumar, C. (2009). Earthworms: The ‘unheralded soldiers of mankind’ and ‘farmer’s friend’ working day and night under the soil: Reviving the dreams of Sir Charles Darwin for promoting sustainable agriculture. *American-Eurasian Journal of Agriculture and Environmental Science*, 5, 01–55.

Visvanathan, C., Trankler, J., Jospeh, K., & Nagendran, R. (2005). Vermicomposting as an eco-tool in sustainable solid waste management. *Asian Institute of Technology, Anna University, India.*

Vodounnou, D. S., Juste, V., Kpoug, D. N. S., Apollinaire, M. G., & Didier, F. E. (2016). Culture of earthworm (*Eisenia fetida*), production, nutritive value and utilization of its meal in diet for rainbow trout. *Aquaculture Research*, 57, 01–05.

Vodounnou, D. S., Kpoug, D., Tossavi, N. S., Mensah, G. A., & Fiooge, E. D. (2016). Effect of animal waste and vegetable compost on production and growth of earthworm (*Eisenia fetida*) during vermiculture.
International Journal Recycling Organic Waste Agriculture, 5, 87–92. https://doi.org/10.1007/s40093-016-0119-5

Wang, K. W., Takeuchi, T., & Watanabe, T. (1985). Effect of dietary protein levels on growth of Tilapia nilotica. Bulletin of the Japanese Society of Scientific Fisheries, 51, 133–140. https://doi.org/10.2331/suisan.51.133

Wu, G. (2009). Amino acids, review, research support, non-U.S. Gov't, research support. College Station, TX: N.I.H. Texas University.

Zakaria, Z., Rahayu, A., MohdSalleh, N., & Siti, N. M. (2013). Total nitrogen content from earthworm (Eisenia fetida) using TheKjeldahl method. IIUM Engineering Journal, 14, 1.

Zakaria, Z., Salleh, N. M., Mohamed, A. R., Anas, N. A., & Idris, S. A. (2012). Optimization of protein content in earthworm-based fish feed formulation for catfish (Clarius gariepinus). SainsMalaysiana, 41, 1071–1077.

Zhenjun, S., Xianchun, L., Lihui, S., & Chunyang, S. (1997). Earthworm as a potential protein resource. Ecology of Food and Nutrition, 36(2–4), 221-236. https://doi.org/10.1080/03670244.1997.9991517

How to cite this article: Musyoka SN, Liti DM, Ogello E, Waidbacher H. Utilization of the earthworm, Eisenia fetida (Savigny, 1826) as an alternative protein source in fish feeds processing: A review. Aquac Res. 2019;00:1-15. https://doi.org/10.1111/are.14091