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Original Research Article

Physicochemical Properties and Proximate Composition of Agro-Industrial Wastes Appropriate for Recycling and Culturing Earthworm, *Eisenia fetida* for Aquaculture Nutrition

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Abstract: This study analyzed the proximate composition and acceptability of four agro-industrial wastes i.e. coffee husks (CH), barley waste (BW), kitchen waste (KW), cattle manure (CM) and their mixture (M) by earthworms, *Eisenia fetida*. The acceptability was evaluated by placing *E. fetida* (buried in spent vermicompost) next to fresh and pre-composted test substrates (separated by 2mm mesh net), then counting the number of earthworms migrating from the old food to the new substrate after 1, 2, 12 and 24 hours. All the substrates had temperatures and pH within the optimum conditions of 20-30°C and 5-9, respectively as required by *E. fetida* except for BW, which had a low pH of 4.5±0.2. The moisture contents recorded in all substrates was below the optimum conditions of 80-90% required by *E. fetida.* In a dry matter basis, the BW had significantly high crude protein (17.87±1.63%) and lipid (8.43±0.93%) and was low in ash (7.23±1.07%) and fibre contents (18.73±1.42%). All the substrates had a C/N ratio below 20, an indication that they are suitable for producing mature vermicast with the potential to increase primary natural productivity in semi-intensive ponds. In the acceptability experiment, most earthworms moved towards M and CM substrates within the first and second hours, indicating high preference to M and CM compared to KW and CH. However, there was no significant difference ($p>0.05$) in the number of earthworms migrating towards all pre-composted substrates. The results showed that BW, KW, and CH has the greatest potential to promote vermiculture and improve aquaculture nutrition.

Key words: Agro-industrial wastes, barley waste, coffee husks, *Eisenia fetida*, fish feeds, kitchen waste, livestock manure

Introduction

The aquaculture industry is recognized in promoting food security, creating employment and curbing malnutrition. Nevertheless, the aquaculture sustainability is threatened by the limited, expensive, and at times low-quality fish feeds, which barely meet the nutritional requirements of cultured fish. Fishmeal, which is the main protein source in fish feeds, has become expensive and scarce due to competition from humans and other livestock feed manufacturers, dwindling wild catches and ecological concerns. The unsustainable fishmeal-dependent aquaculture industry has necessitated research for alternate non-conventional protein sources.

The path to finding fishmeal replacer has been challenging due to cost, inferior nutritional profile, low palatability, poor digestibility and sustainability concerns associated with the non-conventional protein sources. Agro-industrial wastes of plant origin (such as coffee husks, barley waste, kitchen waste and cattle manure) have been credited as cheap source of protein and basal ingredients in fish feeds. However, their utilization in the aquaculture sector has been limited by low protein content with an unbalanced amino acid profile, elevated ash/fibre contents and endogenous anti-nutritional factors. Other concerns include unpalatability and, indigestibility to fish and biosafety concerns (Goswami *et al.* 2014; Ogello *et al.*, 2016).

Kitchen waste (KW) and cattle manure (CM) are among the most abundant residues globally with the potential to provide fish nutrition. The KW contributes to over 70% of urban municipal waste with each household estimated to produce an average of 500g of waste daily (Kale, 1993; Emporer *et al.,* 2016). Likewise, barley waste (BW) and coffee husks (CH) are among the commonly produced agro-industrial wastes, which are not consumed by humans, and have shown the potential to provide nutrition to aquaculture. About 10% of barley grains delivered to breweries factories (from the farms) are considered unsuitable for the malting process, hence discarded as waste or used for animal feed processing (Viergever and Tipper, 2014). During the brewing process, the malting and milling processes release over 85% of BW, which have 30% and 9% crude protein level wet weight, respectively (Kieran *et al.*, 2016; Agu *et al.*, 2008). On the other hand, for every unit input in coffee processing, over 51% of solid biomass is generated as wastes, 25% being CH (Mbugua *et al.*, 2014). The CH has a relatively low protein level, it is fibrous (37% dry matter) hence considered to have lower animal feed value (Bouafou *et al.,* 2011). Moreover, the use of CH for fish feeds at an inclusion level of more than 4% has been limited by the caffeine, tannin, and alkaloids, which at large amounts, reduces palatability and digestibility (Mazzafera 2002; Didanna 2014). Therefore, it is normally land-filled near processing factories where they lose nutrients and pose health and environmental risks.

Many studies have reported that most agro-industrial wastes do not provide the required nutritional contents for fish growth and reproduction and recommend the need to vaporize them (Bouafou *et al.,* 2011; Devi *et al.,* 2019). Vermicomposting has been accredited as one of the simple, safe and cheap biotechniques of upgrading agro-industrial wastes for aquaculture nutrition (Zhenjun *et al.*, 2010, Rameshguru and Govindarajan, 2011). The vermicomposting is a biological process, which utilizes the mutual action of earthworms, micro-organisms, and enzymes to bio-convert organic wastes to safe and stable elements. The earthworms stabilize the organic matter physically through grinding, aerating and mixing while the micro-organisms and enzymes biochemically degrade the plant nutrient and humic acids suitable for agronomy. In the process, they improve the biosafety of organic wastes by suppressing pathogens, removing toxic compounds and remediating heavy metal accumulation (Adhikary, 2012; Goswami *et al.,* 2014).

The earthworm, *E. fetida* has been acknowledged as an appropriate vermicomposting agent *vis-à-vis* fish feed production due to its comparable nutritional attributes (particularly protein and amino acid) with fish meal, fast growth, high reproduction rate, and voracious nature that give it the ability to consume up to half of its body weight daily (Tohidinejad *et al.*, 2011; Musyoka *et al.*, 2019). The growth, reproduction, and maturation, as well as the lipid and protein levels of *E. Fetida,* depends on the quantity and nutritional quality of the culture substrate (Rorat *et al.,* 2015; Li *et al.,* 2016). In return, nutrition (principally protein with balanced amino acid, lipids, ash, and fibre contents), cost and palatability are the key attributes considered when selecting a sustainable fish feed ingredient.

There are several studies on the proximate analysis of agro-industrial wastes to determine their suitability to promote fish production. However, currently, there is limited scientific information citing the proximate analysis and acceptability of organic wastes to both fish and earthworms production. Therefore, the present study analyzed the physicochemical properties and acceptability of the agro-industrial wastes, to determine their potential to grow earthworms and fish as well as producing stable and mature vermicompost suitable for improving the natural productivity of ponds. This is because the proximate composition and acceptability of feed material by earthworms are some of the key indicators for determining the suitability of culture substrates. The data obtained is expected to form the basis for recycling the agro-industrial wastes through vermicomposting to high-value by-products (vermicompost, earthworm biomass as well as bedding i.e. mixture of earthworm and vermicompost) suitable for improving aquaculture nutrition. This will simplify and significantly reduce fish feed cost, improve yields and promote the utilization of agro-industrial wastes, thus promoting food security and environmental integrity.

Materials and methods

Selection of agro-industrial wastes

Through market surveys and literature reviews, the study identified and selected agro-industrial wastes of plant origin in Kenya based on cost, quantity (volume and availability over time), their present use and biosafety (free from pathogens or potential pollutants). The study targeted agro-industrial wastes, which are not affected by seasons and those present in most homesteads and major processing industries. Kitchen wastes were selected due to their abundance in most homesteads and are known to contain high organic matter and have a faster decomposition rate (Sharma and Garg, 2017). The kitchen wastes were collected from nearby households mainly comprising of eggshells, fruit peelings and vegetables remains. The eggshells were highly preferred because they help in raising the pH of the earthworm substrate during vermicomposting (Nair *et al.,* 2006). Acidic fruits and vegetables such as lemons, oranges, and onions were sorted out because they might have lowered the pH content of the substrate to levels not optimal for the worms.

The cattle manure was considered because it is readily available in most agricultural homesteads, contains high organic matter, and is considered highly appropriate natural feed for earthworms (Lee, 1985; Vodounnou *et al.,* 2016; Sharma and Garg, 2017). Soil and urine free manure were carefully collected from nearby farms. The BW was procured from the East Africa Breweries Limited, Nairobi, Kenya. The BW consisted of barley grains (full grains that upon reaching the brewery from the farms are graded and considered unsuitable for the malting process), barley husks (the outer layer of the suitable barley grain milled out during the malting process) and some tiny straws and chaffs collected after sieving.

The CH is the discarded outer layer of coffee beans extracted after the harvesting and drying processes. It is different from the coffee pulp, which is also a discarded outer layer of coffee beans, but that is extracted through a wet process. Therefore, the CH was picked free of charge, from coffee factories within Kiambu and Machakos Counties in Kenya. The CH was sundried for one day to disinfect, reduce foul smell and reduce the caffeine and tannin content (Degefe *et al.,* 2016). In addition, all four potential substrates were mixed (25% of each) and then analyzed separately.

Earthworms, *E. fetida* acceptability test

Two kilograms of *E. fetida* were procured from Kamuthanga fish farm in Machakos County, Kenya and acclimatized for two weeks by feeding them with a mixture of the four substrates. The acceptability test was done to determine the potential of the agro-industrial waste for culturing earthworms. To test the acceptability, the current study modified the bait harvesting method demonstrated by Kostecka and Garg (2015). Their method first starves the worm for about a week then places new food at the surface prompting the hungry earthworms to migrate towards the feed. However, the current study, in triplicates, placed 100 *E. fetida* (buried in the exhausted feed) in one corner of 80 L bucket then positioned fresh substrate next to the earthworms, but separated by 2mm mesh net (as shown in Fig. 1). The number of earthworms migrating

Fig. 1. Photographs of experimental setup of earthworm culture

from the old to the new substrate was counted after 1, 2, 12 and 24 hours. This procedure was repeated after pre-composting the substrates for two weeks. The pre-composting was done by sprinkling the substrates with water then turning them after every 48 hours.

Proximate analysis

The proximate biochemical analysis of the test samples was done in triplicates. The pH and temperature were tested by mixing each substrate sample with water in the ratio of 5:1 according to APHA (1998). The moisture, organic matter and ash content were determined using single samples to reduce the chances of the dry sample absorbing moisture from the air according to the AOAC (1995) standards. The samples were oven-dried at 70°C until they attained a constant weight, which gave the moisture content (expressed as a percentage of the initial sample weight). The same sample was ignited immediately on a muffle furnace at 550°C to burn off the organic matter (OM) and remain with the ash content. The Kjeldahl digestion, distillation and titration method described by AOAC (1995) standards was used to determine the nitrogen content, which was further converted into crude protein using the conversion factor of 6.25, which is known to be equivalent to 0.16g nitrogen per gram of protein. Crude fibre and crude lipids were determined using the acid-base hydrolysis, and solvent extraction method, respectively. The C content was analyzed using the method of partial oxidation described by Dynoodt and Sharifudin (1981). The K and Ca were determined using the atomic absorption method of ignition described by Loh *et al.* 2005, while P was estimated using Tandon (1993) calorimetric method of using molybdenum and sulphuric acid. The C/N ratio was calculated by dividing C and N obtained.

Data analysis

The data were analyzed by SPSS (Statistical software package) version 17.0 using univariate analysis to determine statistical differences between means. Tukey's post hoc test (at 5% probability level) compared differences among the means in cases where significant differences ($p<0.05$) were observed. The data was then presented as mean ± standard deviation (SD).

Results

Physicochemical characteristics and proximate analysis of the substrates

Apart from temperatures there was a significant difference ($p<0.05$) in other physicochemical properties among all treatments tested. There was a significant difference ($p<0.05$) in pH and moisture with the highest being in CM (8.27±0.25% dry matter) and KW (72.10±0.1% dry matter) while the least was in BW (4.5±0.2% dry matter) and CM (20.4±0.62% dry matter), respectively. Equally, there was a significant difference ($p<0.05$) in ash and fibre (which are usually the inverse of organic matter) with CM recording the highest contents (14.63±0.47 and 27.17±0.9% dry matter) in both but KW and M having the least levels of 5.47±0.15 and 18.43±0.35% dry matter, respectively. The crude protein and N contents were significantly high ($p<0.05$) in BW (17.87±1.63 and 2.86±0.26% dry matter) and least in CM (5.65±0.6 and 0.9±0.1% dry matter), while lipids levels had no significant difference in KW, CM, and M as well as between CH and BW.

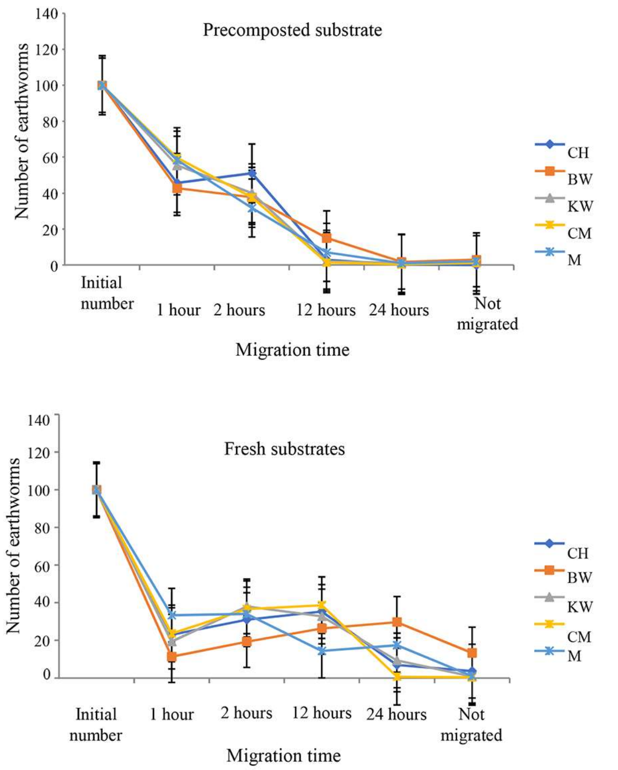

Fig. 2. Migration of *E. fetida* (Mean±SE) towards fresh and pre-composted substrates

The organic matter was significantly high ($p<0.05$) in CM (40.47±0.64% dry matter) and least in BW (24.87±1.25% dry matter). The C/N ratio was a reflection of C and N contents and was significantly low in BW (6.87±0.59% dry matter) and high in CH (20.68±4.88% dry matter), while C and Ca was high in CH (20.67±1.33% dry matter) and KW (3.86±0.29% dry matter), respectively. The CM had a significantly high ($p<0.05$) P and K contents of 0.42±0.03 and 3.77±0.12% dry matter, respectively.

Acceptability of substrates by earthworms, *E. fetida*

There was a significant difference ($p<0.05$) in the number of earthworms migrating towards the five substrates (as shown in Fig. 2). Within the first and second hours of the experiment, most earthworms had migrated towards the M and CM substrates, closely followed by KW and CH. The CH and CM had most of the earthworms migrating before 12 hours while BW attracted the least earthworms with more than 10% not moving at all even after the third day. There was no significant difference ($p>0.05$) in all earthworms migrations after pre-composting with the majority of worms migrating within the first day.

Discussion

The quality and quantity of earthworm depend on the substrate (nutrition, texture, and acceptability), environmental

Table 1. Physicochemical characteristics (Mean ±SD) of agro-industrial wastes (% dry matter)
Note. Different alphabets (a<b<c<d) in the same rows symbolize non-homogenous means ($p<0.05$)

Variables	Coffee husks (CH)	Barley waste (BW)	Kitchen waste (KW)	Cattle manure (CM)	Mixture (M)
Temperature °C	29.67±0.21^{a}	25.87±0.15^{a}	27.17±4.06^{a}	28.13±0.47^{a}	27.80±1.35^{a}
pH	5.47±0.15^{b}	4.5±0.2^{a}	6.53±0.21^{c}	8.27±0.25^{d}	6.07±0.38b^{c}
Moisture	44.47±0.47^{b}	20.5±0.17^{a}	72.10±0.1^{c}	20.4±0.62^{a}	44.37±0.40^{b}
Ash	11.37±0.31^{c}	7.23±1.07^{b}	5.47±0.15^{a}	14.63±0.47^{d}	10.4±0.53^{c}
Crude fibre	24.97±1.62^{b}	18.73±1.42^{a}	24.87±0.51^{b}	27.17±0.9^{b}	18.43±0.35^{a}
Crude protein	6.48±1.57^{a}	17.87±1.63^{c}	5.7±0.26^{a}	5.65±0.60^{a}	10.56±0.76^{b}
Crude lipids	7.83±0.95b^{c}	8.43±0.93^{c}	6.03±0.45^{a}	5.47±0.15^{a}	6.3±0.26^{ab}
Organic matter	29.73±0.21^{b}	24.87±1.25^{a}	35.7±2.85^{c}	40.47±0.64^{d}	35.87±0.21^{c}
Carbon	20.67±1.33^{c}	19.53±0.21^{c}	13.87±0.31^{a}	16.7±0.1^{b}	14.3±0.26^{a}
Nitrogen	1.04±0.25^{a}	2.86±0.26^{c}	0.91±0.04^{a}	0.9±0.1^{a}	1.69±0.12^{b}
C/N	20.68±4.88^{b}	6.87±0.59^{a}	15.22±0.37^{b}	18.64±2.16^{b}	8.5±0.76^{a}
P	0.2±0.01^{a}	0.15±0.04^{a}	0.31±0.02^{b}	0.42±0.03^{c}	0.37±0.02^{bc}
K	0.36±0.03^{a}	0.45±0.05^{a}	0.58±0.03^{b}	0.84±0.04^{c}	0.62±0.07^{b}
Ca	3.33±0.56^{bc}	1.46±0.31^{a}	3.86±0.29^{c}	3.77±0.12^{bc}	2.96±0.15^{b}

conditions, stocking density, handling, harvesting, processing and test procedures (Rorat *et al.* 2015; Vodounnou *et al.* 2016). Despite the observed temperatures being within the optimal range (20-30°C) required by earthworms, it is advisable to continuously sprinkle the substrates with cold water during vermicomposting (Edwards, 1985). This is because temperature tends to rise during vermicomposting due to environmental factors and fermentation of substrates such as barley (López *et al.,* 2008; Bravo *et al.*, 2018). The average pH of 4.5±0.2 in barley waste is below the optimal pH value of 5-9 required by *E. fetida* (Edwards, 1985). This can be adjusted upward by sprinkling the substrate with lime water. Nevertheless, continuous monitoring of all substrates is crucial because pH contents can either increase or decrease during vermicomposting. The decrease in pH is due to the accumulation of humic acid from earthworm-related gut micro-organisms and also as a result of the mineralization of N and P (Hassain and Abbasi, 2018). On the other hand, the increase in pH was caused by the microbial mineralization of proteins into ammonia (Fernández-Gómez *et al.*, 2010). All the substrates had moisture content below the optimum (80-90%) required by earthworms, thus the continuous sprinkling of water was necessary. However, the sprinkling of water should be controlled because excess moisture during vermicomposting can suffocate the earthworms, attract mites and dissolve more CO_2, which is known to increase acidity by creating carbonic acid (Loh *et al.*, 2005). Also draining excess water would mean losing nutrients that could be valuable for fish diet.

The nutritional composition of a substrate is crucial because, apart from determining the growth performance and nutritional content of the earthworm, it influences the earthworm meal utilization in fish (Sakthika *et al.,* 2014). High nutritious substrates translate to quality and quantity earthworms an attribute required in fish feed production. Protein structure, phospholipids, ash and fibre contents of an ingredient are the key attributes considered when selecting fish feed ingredients. The protein levels are inversely proportional to lipid content in animal tissue and their energy trade-off during metabolism promotes the utilization of low-cost feeds (of high lipids but fewer proteins) in fish (Mohanta *et al.*, 2016). The lipids observed in all substrates are within the 4.4% dry matter recommended requirement of Nile tilapia (*Oreochromis niloticus*) production (Winfree and Stickney, 1981). Lipids are crucial in fish farming because they are more metabolizable, contain higher energy per unit and are a cheaper component compared to protein (Doreau and Chilliard, 1997). Besides, the lipids block entry of water to pelleted fish diet, hence maintaining water stability, reducing nutrient leaching and wastage, subsequently improving feed utilization efficiency (Lim and Cuzon, 1994). Nonetheless, the crude protein observed in all treatments is below the 30% dry matter optimum required for *Oreochromis niloticus* production (FAO 2019). All the substrates had fibre contents higher than the recommended requirements of 8-10% dry matter for fish (FAO 2019). Nonetheless, with continuous vermicomposting, fibre contents in substrates decrease due to uptake by earthworm and microorganisms.

There is a positive correlation between earthworm biomass and organic matter content (Fonte *et al.*, 2009). Metabolizable organic matter is crucial during vermicomposting because, apart from providing food to the earthworms, it encourages the growth of fungal biomass and microbes, which help in mineralization, stabilization, mobilization, and solubilization of micronutrients (Aira *et al.,* 2006; Suthar and Gairola, 2014). Besides, their presence and richness are indicators of good quality, stable and mature vermicast.

The organic matter (particularly C/N ratio P, K and Ca contents) in a substrate determines the quality of vermicast (*vis-à-vis* organic fertilizer production) produced after vermicomposting (Adi and Noor, 2009; Suthar and Gairola, 2014). Mature vermicompost is considered suitable and acceptable if it has a C/N ratio of below 15 and not above 20, respectively (Morais and Queda, 2003). Given all the substrates tested in the current study had a C/N ratio below 20; this is an indication that they are suitable for producing stable and mature vermicompost. The vermicompost has shown the potential (when applied as organic fertilizer in semi-intensive ponds) to increase primary natural productivity, promote water retention and stabilizing the bottom sediment (Ghosh, 2004).

Nevertheless, the C/N ratio increases during vermicomposting due to the decrease in C and the accumulation of N contents. The N increases as a result of enzymatic and microbial secretion, mineralization of non-nitrogenous matter, mucus and casts accumulation from earthworms as well as from nitrogen-fixing bacteria (Suthar, 2006; Suthar and Gairola, 2014). On the other hand, C decreases due to microbial respiration of carbon dioxide, which gets released to the environment. Kaushik and Garg (2003) reported a massive decrease of C/N ratio from 131 to 26.4 during vermicomposting of solid textile mill sludge mixed with cow dung (30:70) using *E. fetida* worms.

The P, K, and Ca contents are the other components, which indicate the quality and maturity of vermicompost. They increase during vermicomposting due to solubilization as the substrates pass through earthworm gut. Besides, the vermicomposting improve their bioavailability to vegetation when compared to their parent compound.

Substrate acceptability is a crucial attribute in vermicomposting. The pre-composting of the substrates before vermicomposting not only increases substrate acceptability but also reduces anaerobic conditions, salinity, toxic/acidic compounds that might kill or impair earthworms growth, raises pH, stimulate biodegradation and fastens stabilization period (Gunadi and Edwards, 2003; Adi and Noor, 2009). Therefore, the low pH and unacceptability recorded in substrates such as in BW can be overcome by pre-composting the substrates before vermicomposting. In addition, it is recommended to include at least 10% of cattle manure during pre-composting to help increase pH and stimulate biodegradation (Bhat *et al.*, 2016; Vodounnou *et al.*, 2016). This is because the manure was found to contain the highest pH and highly acceptable due to the recommendable organic matter (Loh *et al.*, 2005; Li *et al.*, 2016).

Culture substrates determine the quality of the *E. fetida* produced and consequently the fish feed formulated from the earthworm. This is because, hard textured substrates produce earthworm with elevated chitin, which is the exoskeleton that helps the worms burrow and move around (Musyoka *et al.* 2019). Chitin is an indigestible protein, which if not treated, lowers digestibility in fish if inclusion levels of *E. fetida* meal exceed 25% in fish feed formulation (Tacon *et al.* 19 83). Additionally, substrate quality is crucial in culturing earthworm destined to feed fish production because, harvested worms are not devoid of the culture material (especially through the conventional hand-harvesting technique), which if not thoroughly cleaned affects the water quality of the fish pond if the worms are to be fed alive. Consequently, the culture substrate particles in harvested worm might affect the final proximate composition of the formulated fish diet. Besides, the presence of a substrate in the earthworm gut reduces protein content by up to 30% during analysis (Zhenjun *et al.*, 2010). The slimy, moist and wiggling nature of earthworms complicates its handling and makes harvesting laborious and time-consuming.

Conclusion

Substrate selection is fundamental for successful vermicomposting. The substrate constituents determine the quality and quantity of earthworms produced, the period of vermicomposting and the maturity and stability of vermicompost. The present study shows the substrates tested are acceptable (after pre-composting) and have physical and nutritional attributes required to produce quality and quantity earthworms as well as mature and stable vermicompost. However, it is only the substrates of BW, KW, and CH, which have shown the potential to culture fish in a semi-intensive system, whereby the limiting nutrients can be supplemented by pond natural productivity. Therefore, this study recommends for further study to bioconvert BW, KW, and CH using *E. fetida* then amalgamate the vermicomposting by-products (i.e. vermicompost, vermiliquid and earthworm biomass) to complement limiting nutrients and simply the complications associated with earthworm handling and harvesting indented for fish production.

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