JIMMA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF BIOLOGY

Evaluation of Earthworm Culture Using Locally Available Bio-wastes and its Impact as an Alternative Protein Source on the Growth Performance and Survival of Juvenile African Catfish (*Clarias gariepinus*) at Jimma University, Southwestern Ethiopia

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A Research Thesis Submitted to Department of Biology, College of Natural Sciences, Jimma University in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Sciences in Biology (Aquaculture and Fisheries Management)

June, 2024

Jimma, Ethiopia

Acknowledgements

Foremost my thanks go to my research advisers Dr. Tokuma Negisho and Dr. Mulugeta Wakjira for their advice, encouragement, assistance and unreserved support both during the preparation of the proposal and the write up of the thesis. No words are good enough to extend my heartfelt gratitude to them. Their unreserved sharing of their knowledge and experiences is greatly acknowledged.

Next, I would like to thank Jimma University for allowing me to join this MSc program and Jinka University for giving me to learn MSc program. I would like to express my deepest gratitude to my instructors of Jimma University and all staff members of Jimma university Biology Department for their kind cooperation and assistance throughout my study. Afterward, my thanks go to my father Gelsimo Bersha and to my mother Ayana Guyita for your vision and decision to devote in intellectual property for my study. Lastly but not the least, my gratitude goes to all those who contributed in one way or another to the successful realization of this study and who deserve deepest appreciation to me.

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Acronyms/Abbreviations

ANOVA Analysis of Variance

AOAC Association of Official Analytical Chemist

BM Bio-waste mixture

CP Crude Protein

EWM Earth Worm Meal

FAO Food and Agricultural Organization

FCR Feed Conversion Ratio

GHG Green House Gases

NFE Nitrogen-Free Extract

NRC National Research Council

PER Protein Efficiency Ratio

SGR Specific Growth Rate

SR Survival Rate

TSS Total Suspended Solid

Abstract

African catfish is widely distributed throughout Africa and is one of the continent's most important aquaculture species. It is known for its adaptability and resilience to adverse environmental conditions. However, a sustainable source of feed is one of the bottlenecks in expanding its farming. This study was aimed to evaluate earthworm (Eisienia fetida) culture with local available biowastes and its impact as alternative protein source on catfish Juvenile. Various local available biowastes (T1: fruit leftover, T2: biowaste mix, T3: vegetable leftover and T4: cow dung) were tested as they were Suitable substrate for vermiculture. For each treatment, 50 cocoons from on-site earthworm production were inoculated to determine hatching perormance. Subsequently, equal earthworm juveniles harvested from each biowaste were fed with the corresponding biowaste to determine growth performance and feed utilization parameters, and both testes were examined for 42 days each. The suitable substrate was further utilized for mass production of worm to utilize as feed for fish. Catfish juveniles were obtained from Boye River and acclimatized for 14 days during which they fed on basal diet. A total of 64 juveniles with uniform average size was randomly distributed in each diet treatment (D100:100%, D60:60%, D30:30% and D0:0% earthworm inclusion). Growth performance and feed utilization parameters of fish were collected every fortnight for 60 days. Earthworm meal and catfish juvenile's carcass were analyzed for their proximate composition .The vermiculture tests revealed that, There was statistically significant differences ($p<.05$) in number of hatchlings in T4 than all the other treatments and T2 had the highest biomass obtained, growth rate ,crude protein and lower feed conversion ratio. Average weight gain, feed conversion ratio, specific growth rate and protein efficiency ratio among all diets were shows statistically significant differences ($p<.05$). Crude protein level obtained from D30 was highest among diets. In general, T4 and T2 induced better hatching performance and growth performance parameters of E.fetida respectively and the inclusion of 30% earthworm meal was best supported juvenile catfish rearing.

***Key Words**, Bio-waste, Cattle blood, Clarias gariepinus, Earthworm meal, Eisenia fetida, Tank*

1. Introduction

1.1 Background of the Study

Aquaculture is the farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants. Globally, aquaculture production is the most immediate solution to declining capture fisheries, however, it faces a number of challenges that prevent it from reaching its full potential (Barbu *et al*., 2016). The major constraint to the emergence of aquaculture in developing countries is feed quality and its cost (Siddhuraju and Becker, 2003). So, responsible aquaculture sector should focus on reducing production costs, improving efficiency of the production systems and promoting environmental sustainability.

Fish feeds play a major role in aquaculture production; however, they pose a great challenge to fish farming as they contribute up to 70% of aquaculture total financial inputs (FAO, 2009). Fishmeal has for ages been credited as the main dietary protein source in formulating a nutritive diet for cultured fish due to its superior nutritional qualities and palatability properties. Nevertheless, the consistent use of fishmeal as the main source of protein in fish feeds has progressively made the commodity costly, unreliable and unsustainable due to increased demand, diminishing wild catches, ecological concerns and competition from humans and other animal feed manufacturers. Consequently, the continued use of fishmeal has affected the economic success of most farmers a phenomenon that has prompted expanded research on alternative protein sources. 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).

In recent years, animal protein sources for use as ingredients in fish feed have become increasingly scarce and therefore expensive (Davis, 2015) and yet plant protein sources used for replacing them are also expensive and have anti-nutritional factors in them that reduce their efficiency of utilization in fish (Hossain *et al.*, 2003) which brings about the necessity to develop new protein sources. Therefore, non-conventional protein sources, such as earthworm and maggot meal, have gained interests to provide an alternative protein source thanks to their nutritional values that are close to that of fish meal (Fadaee, 2012).

Agro-industrial wastes have been recognized as non-conventional feed resources because they are often cheaply available and do not suffer competition with livestock and human as it is with fish meal and most grains (Ogello *et al*., 2017). Kitchen waste and livestock manure are among the agricultural household residues commonly used in fish nutrition due to their abundance and low cost of obtaining them. However, their utilization in fish feeds production is often restricted by biosafety, processing and ethical issues (Cheng & Lo, 2016).

Vermicomposting biotechnology, which is a joint action of earthworm and micro-organisms on organic matter, has been used for ages as a natural and cheap technique to treat and bio-transform agro-industrial residues to safe and steady compounds (Musyoka *et al*., 2019). Consequently, the vermicomposting by-products (earthworm, vermicast and vermiliquid) have been optimized and utilized to supply nutrition in various intensities of aquaculture. The accrued biomass is tested as direct protein sources (Vodounnou *et al*., 2016), while vermicast and vermiliquid are examined as organic fertilizer to promote pond primary productivity (Kaur & Ansal, 2010).

Earthworm, *Eisenia fetida*, has been considered as a suitable vermicomposting candidate vis-a-vis fish feed production due to its superior nutritional attributes comparable to that of fishmeal, higher growth and reproduction rate and ability to tolerate wide range of climate conditions (Vodounnou *et al*., 2016). Earthworm meal has been tested in a broad range of fish diets from low level (10%) to high level (100% as a fish meal replacer). It has been generally shown to have positive effect on growth performance and feed utilization parameters, but not on all tested fish species (Mohanta *et al*., 2016).

African catfish is widely distributed throughout Africa and it is one of the most important commercial freshwater fish species in many parts of the continent. It is known for its adaptability and hardiness to adverse environmental conditions (Macharia *et al*., 2002). Goose and Richter (1996) identified *C. gariepinus* as the most important species for aquaculture in Africa due to its omnivorous eating habits, resistance to handling stress, high meat content, ability to handle low water levels by breathing atmospheric air and its ability to withstand high stocking densities. Therefore, this study was aimed to establish vermiculture to Jimma University and to evaluate earthworm culture with local available biowastes and its impact as alternative protein source on growth performance and survival of African Catfish (*Clarias gariepinus*) Juvenile.

1.2 Statement of the Problem

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). Further, Very limited research has been carried out on use earthworm in fish diet and as the culture environment (nutrient/medium) plays a great role in determining the nutrient composition of earthworm, there were no reports by earlier researchers on culture environment for earthworm and difficulties were faced to compare the nutrient composition of the earthworm found in our study and the earlier reports (Mohanta *et al*., 2016).

Key challenges for aquaculture development in Ethiopia were; lack of cheap and efficient locally available fish feeds, lack of locally selected and certified fish seeds, Seasonality, shortage and very high prices of feed ingredients for sustainable and affordable delivery of compound feeds (Seyoum *et al*., 2018).

Uncontrolled disposal of Organic wastes causes environmental pollution, landfill ,eutrophication, economic loss, health risks and climatic change by producing greenhouse gases(Degefe *et al.*,2016).These wastes have attracted research interests on away to recycle and reuse them as ingredients, supplement feed ,bio fertilizers and energy production in fish culture(Ghosh,2004).

African catfish is endemic throughout Africa and in the Middle East and remains an important aquaculture species for food security in developing countries (Ouma *et al*., 2022). Around 81% of catfish globally rely on some form of commercially formulated diet as a supplement to natural food produced in the culture systems to attain optimal fish performance (Naylor *et al*., 2021). However, in catfish farming, the formulation of quality and relatively cheaper feeds is one of the leading factors confronting sustainable production of the species in most developing countries (Mbokane *et al*., 2022)

It is against this backdrop the present study was aimed to evaluate earthworm culture with local available bio-wastes and its impact as alternative protein source on growth performance and survival of African Catfish (*C. gariepinus*) Juveniles in fish tank.

1.3 Research Questions

- Which culture substrate/medium will induce higher Hatchability rate, growth rate, biomass and better proximate composition of earthworm?
- What percentage of earthworm inclusion can show better growth performance, survival rate and fillet proximate composition of African catfish juvenile fed on it?

1.4 Objectives

1.4.1 General Objective

The general objective of this study was to evaluate earthworm culture with local available bio-wastes and the impact of utilizing EWM as alternative protein source on growth performance, survival rate and proximate composition of African Catfish (*C. gariepinus*) Juveniles.

1.4.2 Specific Objectives

- To evaluate and compare earthworm culture with locally available bio-wastes on hatching performance and number.
- To evaluate a bio-waste which produce earthworm with higher proximate composition in its meal
- To evaluate the percentage inclusion of EWM which shows better growth performance and survival rate on African catfish (*C. gariepinus*) juvenile.
- To evaluate the impact of earthworm meal inclusion on the proximate composition of African catfish juvenile fillet.

1.5 Significance of the Study

The results of this study will play a significant role in offering simple scientific information on inexpensive biotechnologies that can help to address challenges related to fish nutrition and processing agro-industrial wastes and the use of EWM in fish feeds. Additionally, it will provide solutions to environmental pollution and offer eco-friendly and cost-effective alternatives fish feed resources.

The study will also provide insights on recycling household, on-farm, and market-produced bio-wastes into valuable products. It will provide information on the appropriate inclusion of EWM in

the diet of African catfish juveniles, which promotes better growth and survival, as well as the bio-waste that best supports earthworm culture. Furthermore, the study's results will aid in formulating a nutritionally balanced feed for African catfish juveniles, and the impact of EWM on its fillet proximate composition and serve as a foundation for future research.

2. Review of Related Literature

2.1 Vermi-culture

Vermiculture is defined as artificial rearing or cultivation of worms (Earthworms) and the technology is the scientific process of using them for the betterment of human beings (Dominguez, 2004). Vermiculture was first introduced in the 1970s by a biology teacher, Mary Appelhif. She developed the idea of using red wiggler worms (*Eisenia fetida*) for composting in indoor and outdoor systems to convert kitchen waste to worm compost. In the pasts Large scale culture of earthworms (Vermiculture) is used as a vital tool for organic waste management to produce worm castings as fertilizer with potential market value and a novel protein source, the earthworm biomass which can be included in animal feeds including fish (Edwards, 1985).

2.1.1 Contribution of vermiculture to pisciculture

planktons have protein contents ranging between 40 and 60%, like most conventional artificial fish feed sources, which is optimal for culturing fish(Silva and Anderson,1995), in vermi-compost manured ponds, Kumar *et al*.(2012) noticed a correlation between improved phytoplankton biomass and zooplankton abundance and diversity, with 68.38, 19.77, and 11.38% occurrence of rotifers, cladocerans, and copepods, respectively ,and Further, the vermicompost provides food directly to zooplanktons and fish. Though the vermicompost alone might contain low protein value for feeding fish directly, the microbes adhering to organic manures improve their nutrition to contents suitable for the majority of aquatic organisms.

Vermiliquid has equally shown the prospects of directly improving plankton productivity, particularly zooplanktons such as Artemia, whose mass production is often limited by lack of nutritious feeds, costly and laborious tasks. Vahdat *et al*. (2018) demonstrated the potential of vermiliquid powder to promote the survival, growth, body composition, total carotenoids, and reproduction performance of *Artemia franciscana* in small-scale laboratory cultures. Therefore it is interesting to note that a symbiotic system is developed when vermiculture is integrated with aquaculture.

Although, earthworm is considered as a by-product of waste management process or vermiculture unit, it can be used as a potential non-conventional animal protein source for formulating the fish feed (Pereira & Gomes, 1995).

2.2 Bio-wastes

Bio-waste is referred to as biodegradable waste. In simple terms, it can be defined as any waste material that is organic or that is capable of decomposing under aerobic or anaerobic conditions they include: Forestry and agricultural residues, Animal waste, Manure Sewage sludge, Commercial food waste, Kitchen scraps, Garden waste Paper and cardboard and natural textiles. Humans, livestock, and crops produce approximately 38 billion metric tons of organic waste worldwide annually (Ghosh, 2004). Such a vast amount of solid waste can have significant impacts on the disposal and methane emission from the anaerobic fermentation process. The management and safe disposal of these wastes has become a global priority. Organic wastes are naturally transformed into plant nutrients by a variety of soil decomposers involving bacteria, fungi, and earthworms (Oyedele *et al*., 2006).

2.2.1 Fruit leftovers

Fruit Waste means solid waste deriving from fruits that is biodegradable and includes the peel, skin, pulp, seeds and leaves. Solid waste is complex in character and its volume is greatly increased due to increase of living standards and population density. Hence the importance of efficient solid waste management is increasingly recognized. Several fruits, such as the mango (30–50%), orange (30–50%), pineapple (40–50%), and banana (20%), a significant amount are often wasted. Common fruits, including the mango, banana, orange, watermelon, and lemon, account for between 25 and 57 million tons of waste annually (Leong and Chang, 2022).

These fruit wastes can pose major environmental challenges, such as water and soil pollution, the greenhouse effect, eutrophication, global warming, and other health problems if not effectively handled due to their high biodegradability and fermentability (de Medeiros *et al*., 2020). Although some fruit waste is used as animal feed (Caipang *et al*., 2019), landfill, incineration, and open burning are the most frequently used methods to dispose of fruit waste (Rifna *et al*., 2021). However, these methods or approaches could result in other problems, such as the generation of secondary waste.

2.2.2 Waste papers

Waste paper is paper that you throw away because it has been used or is not needed. Papers are usually discarded indiscriminately leading to environmental pollution by littering and burning. The

accumulation of excess volumes of solid waste such as the waste paper and the search for clean energy are topical global issues (Mokatse *et al*., 2016). Its recent discovery in industries has helped to curb the environmental pollution, as these papers can be recycled so that they can be re-used instead of being wasted (Kerr, 2007). Solid waste does not only occupy valuable land but also contributes largely towards environmental pollution (Meizah *et al*., 2015).waste papers include newspapers, office, toilet and foolscap papers.

2.2.3 Vegetable leftovers

Vegetable waste is the non-edible parts of vegetables that are discarded during collection, handling, transportation and processing. In the past, those wastes have been mixed in to municipal waste streams and sent to landfills or incinerators for final disposal. As landfills close at an alarming rate, and fewer incinerators are under development, the disposal of vegetable waste becomes a serious problem in many cities. A number of supermarkets and wholesales markets are anxious to find an alternative means of disposal. According to the estimation of the United Nations (FAO, 2014), every year, one-third of the food produced in the world (about 1.3 billion metric tons) is wasted. The waste generated by the fruit and vegetable sector (fruit and vegetable waste, FVW) is estimated to represent up to 60% of the food waste generated annually in the world (Gustavsson *et al*., 2011).

In this regard, roughly one-third of all fruits and vegetables produced worldwide are wasted before reaching the consumer (Kader, 2005), and this estimation was reported to be even higher in Italy (about 87%) (Segre and Falasconi,2011). FVW represents a significant economic issue for companies and poses environmental problems, due to its high moisture and biodegradability. Moreover, it involves a loss of valuable biomass and nutrients. Therefor the technologies that convert these wastes in to valuable products are needed.

2.2.4 Cow dung

Cow dung can be defined as the undigested residue of consumed food material being excreted by herbivorous bovine animal species. Being a mixture of faeces and urine in the ratio of 3:1, it mainly consists of lignin, cellulose and hemicelluloses. It also contains 24 different minerals like nitrogen, potassium, along with trace amount of sulphur, iron, magnesium, copper, cobalt and manganese. Cows can produce dung waste in large capacities every day. A cow weighing 454 kg can produce

up to 30 kg of faecal waste every day. If the farmer has 5 cows, the amount of dung that is produced will reach 150 kg per day.

Farmers tend to throw them on empty land or pile them up at the edge of the pen (Fathurrohman *et al*., 2015). The accumulation of cow dung waste in the environment results in decreased environmental quality. This is because the dirt that is disposed of directly is still hot, so it can inhibit plant growth. The application of environmentally appropriate technology to overcome these problems needs to be done gradually to maintain the balance of the production sector with the preservation of the surrounding environment (Damanik *et al*., 2014).

2.3 Earth worm Taxonomy, Morphology and life cycle

Earthworms are macroscopic clitellate oligochaete annelids that live in soil. They are classified in the kingdom Animalia, phylum Annelida or Annelids and class Citellata, Annelida in Latin meaning a little ring, scientific name *Lumbricus terrestris*. They are segmented, bilaterally symmetrical invertebrates, displaying indeterminate growth after sexual maturity and reaching up to 30 cm in length. They have a digestive tract with a mouth, a crop and a gizzard, intestine and anus. The circulatory system consists in blood vessels and several pairs of hearts (5 in Lombricids).

They have no lungs and respiratory exchanges are done through the skin. Earthworms are hermaphroditic: they reproduce by copulation between two individuals at the level of an external gland the clitellum producing the cocoon where the eggs are laid. The cocoons are tiny and lemon-shaped, usually deposited near ground surface, and deeper when dry conditions occur. The period of incubation before hatching is variable, depending upon species and environmental conditions. Recently hatched earthworm is not pigmented but they need only a few days to get their adult color. Under favorable conditions, they reach maturity in several weeks. The clitellum is an indicator of maturity (Dominguez *et al*., 2011).

2.3.1 Ecology of earthworm and candidate species for vermin culture and vermicomposting

Different species of earthworms have different life histories, occupy different ecological niches, and have been classified, on the basis of their feeding and burrowing strategies, into three ecological categories: epigeic, anecic, and endogeic (Bouché, 1977). Endogeic (soil feeders) and anecic species (burrowers) live in the soil and consume a mixture of soil and organic matter, and thus

excrete organomineral feces. Epigeic species of earthworms are litter dwellers and litter transformers; they live in organic soil horizons, in or near the surface litter, and feed primarily on coarse particulate organic matter. There are about 4000 species of earthworms. A handful of species has been used for vermicomposting as they display suitable characteristics like tolerance of a wide range of environmental and management conditions, short life-cycles, high reproductive rates, and good composting rate. *Eisenia andrei, Eisenia fetida* (also spelled *Eisenia foetida*), *Dendrobaena veneta*, *Perionyx excavatus* and *Eudrilus eugeniae* are the only extensively used species for vermicomposting (Dominguez *et al*., 2011)

2.3.2 Earth worm as alternative protein source in fish feed

In the last 35 years, some experiences were carried out with different fish species. Ghosh (2004) used fresh earthworms (*E. foetida*) as feed for catfish (*Clarias batrachus*) in India and observed a higher weight gain respect to the control fed with a traditional diet without EWM. Vodounnou *et al*. (2016) tested, for a 6-weeks period, EWM (from *E.foetida*) as substitute of fishmeal on *Parachanna obscura*: higher growth rate and FCR were obtained with the fish diet containing 50% of EWM. Mohanta *et al*. (2016) evaluated weight gain, growth rate and FCR in rohu (*Labeo rohita*) using earthworm (whole, custard and pellet from *E.foetida*) and highlighted how the pellet achieves the best performances for all the evaluated parameters.

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

2.3.3 Role of earthworm as food and feed in assuring food security and valuing food waste

Earthworms, like insects, could represent a valuable solution. They can bio-convert fruit and vegetable waste, which this is a sustainable, cost-effective, and ecological approach that contributes to the reduction of food waste (Tedesco *et al*., 2019). Using fruit and vegetable waste as a substrate

for the growth of earthworms produces a new high-protein nutrient that can in turn be valued, first for animal nutrition and then for human consumption. The need for new food products is dependent on two specific aspects.

(i) The human population is increasing, with more than 821 million people still lacking regular access to adequate food, and, at the same time,

(ii) The demand for new protein sources, in particular for animal proteins, which are the most limiting and expensive in terms of resources, is also increasing (Alexandratos and Bruinsma, 2012).

Today, the food sector is considering the use of insects in human nutrition (Halloran *et al*., 2018), and, in this context, terrestrial invertebrates such as earthworms used as an alternative source of protein could represent a valid solution, especially if they are fed without land use competition and by reintroducing fruit and vegetable waste into the food supply chain, hence turning waste into a resource (Tedesco *et al*., 2019). Several authors (Zhenjune *et al*., 1997 and Vielma *et al*., 2007) have underlined the nutritional value of earthworms based on their nutrient content: earthworms of *E. foetida* species are an excellent source of biologically valuable protein, micronutrients, minerals, and vitamins in the human diet. *E. foetida* meal has high protein content in the range of 54.6% to 71% dry matter (Zhenjune *et al*., 1997) and is rich in amino acids considered essential for humans.

Earthworms are also rich in fat, with content ranging from 7.3% to 10% of dry matter (Sun and Jiang, 2017). As reported in Reg. (EC) no. 2015/2283 on novel foods, traditional foods from third countries with a history of safe food use can be considered a valuable source of food nutrients in our food chain. In some parts of Asia, Africa, and South America (Medina *et al*., 2003), earthworms have been introduced into the everyday diet and have also been included in the Dictionary of Food Science and Technology (Kennedy and Jin, 2009). Finally, to ensure a high level of protection of human health, the production of edible terrestrial invertebrates as food should be safe and wholesome and reared and marketed as food following European safety rules for food.

2.3.4 Vermi-composting: an eco-friendly approach for waste management and nutrient enhancement

Solid waste management has been an integral part of the sustainable society; however, the increasing volume and complexity of waste associated with the modern economy has become an

issue of global concern as it is posing a serious risk to ecosystems and human health (Bhat *et al*., 2018 and UNEP 2021). The rate of accumulation of organic waste from industries, domestic household and agricultural sectors imposes excessive burdens which need a holistic approach to treat this waste without harming the environment. In recent times, with better collection system, waste segregation and advanced technologies, developed countries are more efficient in treatment and recycling of solid waste (Lim *et al*., 2015); of which segregation, recycling, and reuse are the most useful solid waste management system.

Vermicomposting, which is described as bio-oxidation and stabilization of organic material involving the joint action of earthworms and mesophilic micro-organisms, is one of the most feasible and environment friendly technique for the bioconversion of industrial wastes/sludges into a useful and high quality vermicompost (Bhat *et al*., 2018). Vermicomposting accelerates the rate of waste degradation into manure thus modifying the physiochemical properties of the waste (Domínguez 2004 and Devi *et al*., 2020).

While biochemical reactions during the degradation of organic waste are mostly done by the microorganism, earthworms are the crucial drivers of the process as they aerate condition and fragment the substrate, thereby drastically altering the microbial activity (Kiyasudeen *et al*., 2014). Microorganism present in the earthworm gut has also significant role in nutrient transformation and degradation of waste into useful manure (Sun *et al*., 2020). The capability to accumulate heavy metals from industrial wastes/sludges by earthworms is reported wherein; the gut microbes and the chloragocyte cells of earthworms detoxify heavy metals (Bhat *et al*., 2018).

During composting of organic wastes, nitrogen loss occurs through ammonia, nitrogen oxides,or other forms leading to the loss of fertilizing value of composts as well as promote GHG emissions (Joseph *et al*., 2020). In this context, vermicomposting, a reliable, efficient and environmentally friendly method is gaining great interest among many researchers across the globe. Though studies regard that vermicomposting also generates GHGs, it is reported that controlled vermicomposting process reduces GHG emissions (Joseph *et al*., 2020). During vermicomposting of sewage sludge, Lv *et al*. (2018) reported that vermicomposting enhanced the degradation of organics and accelerated the mineralization process of nitrogen, whereas the increase in the C/N ratio reduced GHG emission. Besides, product of vermin-technology (vermicast) is known to harbor higher

number of phosphate solubilizing bacteria and occurrence of such beneficial microorganism convert good amount of phosphorus from insoluble form in any organic waste material.

In vermicomposted manure, nutrients via available nitrogen, phosphorus, potassium and calcium are reported to be higher compared to surrounding soil (Rajkhowa *et al*., 2015). The physical properties of soil such as resistance to corrosion, aeration, drainage, infiltration and porosity, are also reported to improve due to addition of vermicompost (Singh *et al*., 2020). The beneficial effects of vermicompost based substrates in agriculture accelerates growth, increases crop yields, creates favorable environment for beneficial micro-organisms, permanently improves soil structure, increases plant secretion and is very ideal for use in organic farming and in artificial environments (Olle, 2019). Therefore, with large scale production facility vermicompost can be used as an effective replacement for inorganic bio-fertilizer.

An innovative discipline of vermiculture biotechnology i.e., the breeding and propagation of earthworms and the use of its castings has become an important tool of waste recycling all over the world (Aalok *et al*., 2008). Subsequently, vermitechnology is widely used for the conversion of solid waste into manure from the municipal wastes, paper mills and food industries (Bhat *et al*., 2018). Vermicomposting facility and its practices have developed at a rapid rate and vermicasts produced by earthworms are presently used as a nutrient supplement for organic food production and in agricultural sector (Mahmud *et al*., 2020).

2.4 Biochemical composition of *Eisinia 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 (Stankovic *et al*., 2011). They are responsible for growth, tissue development, energy source and are 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 and Metian, 2008).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. 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 (Liti *et al*., 2005).

Essential amino acids are often unbalanced in the common fish feeds. *E.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 (Vodounnou *et al*., 2016).lipid contents on dry matter of *E. fetida* 18% (Mohanta *et al*., 2016). Likewise, the worm has a high and a recommendable ratio of poly- unsaturated fatty acids (linolenic ω-3 and ω-6) that are critical for formulating diets of many fish species (Mukti *et al*., 2012). *E.fetida* contains about 45.8% saturated, 22.2% monounsaturated, 31% polyunsaturated, 23.5 of n-6 and 8.3of n-3 fatty acids (Gunya *et al*., 2016). Furthermore, the worm has tolerable crude fiber levels (10.9%) that are not too high to affect digestibility in fish. Consequently, the presences of cellulose in the worm promote high-protein assimilation efficiency to cultured fish (De Chaves *et al*., 2015).

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). 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) that are within the recommended dietary requirement of most fish (NRC, 1993).

2.5 African catfish

2.5.1 Taxonomic position and habitat of African catfish (*C. gariepinus*)

The African catfish belongs to the kingdom Animalia, phylum Chordata, sub-phylum Vertebrata, class Actinopterygii (ray-finned fishes), order Siluriformes (catfish) and family Clariidae (Nelson, 2006). The family Clariidae consists of two notable genera; namely Clarias and Heterobranchus. *Clarias gariepinus*, commonly named as African sharp tooth catfish, is scaleless and "air breathing". The genus *Clarias* was reviewed in the 1980s and some species were synonymized with *C. gariepinus*. These were *Clarias lazera* of West and North Africa, *Clarias capensis* of Southern Africa, Clarias mossambicus of Central Africa, in the name *C. gariepinus* (Teugels, 1986).

African catfish (*C. gariepinus*) lives in a variety of freshwater environments including lentic waters like lakes, ponds and pools. It is also very prominent in flowing rivers and around dams. It is very

adaptive to extreme environmental conditions and can live in pH range of 6.5-8.0. They are able to live in very turbid waters and can tolerate temperatures of 8-35°c. Their optimal temperature for growth is 27-30°c (Verreth, 1993). They are able to move across land to another water source during damp conditions (Skelton, 2001) and thus can breathe atmospheric air by means of a supra-bronchial or accessory respiratory organ. They simply extend their strong pectoral fins and spines and begin crawling through shallow pathways. This species can live in very poorly oxygenated waters and is one of the most recent species of fish to live in such uninhabitable place by other fish species (Eyo and Mgbenka, 1992). They are also able to secrete mucus to prevent drying and are able to burrow in the muddy substrate of a drying body of water (Skelton, 2001).

2.5.2 Geographical distribution and aquaculture potential of African catfish

African catfish (*C. gariepinus*) is considered as an indigenous and most important species for aquaculture in the African continent (Akankali *et al*., 2011). It has wide distribution in Africa, ranging from the Nile to West Africa and from Algeria to Southern Africa (De Graaf and Janssen, 1996). It is the most sought species by fish farmers and consumers and as commands it has very good commercial value in Nigerian markets (Gaffar, 1996). In 2009 investment in catfish farming was documented by catfish farmers association of Nigeria (CAFAN) and reported over 24 billion naira profits annually (Akankali *et al*., 2011).

African catfish is an endemic species having a ubiquitous distribution in rivers, streams, dams and lakes in Kenya. All the Clarias species reported in Kenya inhabit wetlands or wetland open interface. These groups of fish (Siluriformes) are widely consumed. Successful culture/captive breeding of this species and thus fingerling rising has been done in the country. It can be raised in high densities resulting in high yields and fetches a higher price than tilapia as it can be sold live at the market (Macharia *et al*., 2002).

Clarias gariepinus has been considered as an ideal species for the development of aquaculture in Africa (Gadissa and Devi, 2013). It is very important to the sustainability of the Aquaculture industry in the continent. In recent past, fish fingerlings were captured from the wild however, due to problems associated with wild fish seed, seasonality in availability, uncertainty of species of fish seed, diseases and limited quantity of harvestable fish seed; it has become an unreliable source for commercial fish farming (Shepherd and Bromage, 1992). *C. gariepinus* is relatively insensitive to

disease and does not have high water quality requirements. It tolerates high concentrations in the water of ammonia (NH3) and nitrite (NO2) in water. Low oxygen concentrations are tolerated because the fish utilizes atmospheric air as well as dissolved oxygen since it has well developed air breathing organs (De Graaf and Janssen 1996). It can be raised at high densities, resulting in high yields and fetches a higher price than other fish species as it can be sold live at the market. Moreover, *C. gariepinus* can be fed with large variety of agricultural by products and make this species an excellent candidate fish for aquaculture (Macharia *et al*., 2002).

2.5.3 African catfish aquaculture status in Ethiopia

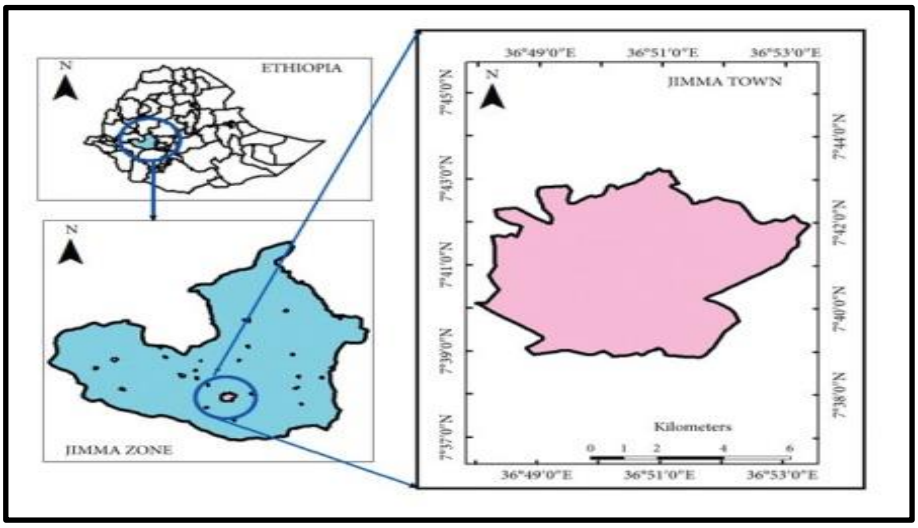
In Ethiopia aquaculture has been proposed by the government of Ethiopia as an option to boost fish production and as an alternative means to food security and poverty alleviation. Nowadays it is considered as an integral part of rural and agricultural development policies and strategies. It was started in the form of extensive aquaculture (Lakew *et al*., 2016).In Ethiopia, it is widespread in almost all water bodies (lotic and lentic systems) such as in the rift valley, Abay, Awash, Baro-Akobo, Omo-Gibe, Tekeze, and Wabishebele-Genale basins (Awoke, 2015, Golubtsov and Mina, 2003).

C. gariepinus is both an ecologically and economically important fish in Ethiopia. Hence, knowledge of its biology could have significant importance for the development of catfish aquaculture in the country (Dadebo *et al*., 2014). Even though there were different types of fish species in Ethiopia mainly dependence on Nile tilapia was seen. That is Nile tilapia is benchmark of the country fish farm production hence, narrowing Ethiopian production diversity of aquaculture. Even though, African catfish has great economic importance next to Nile tilapia in the country fisheries product, yet its farming is not well practiced. Fortunately, African catfish farming has witnessed an increased production of aquaculture in Ethiopia (Natea *et al*., 2017).

3. Materials and Methods

3.1 Description of experimental site

The experiment took place at the aquaculture experimental site within main campus of Jimma University. Jimma University is situated at an elevation of 1700 meters above sea level, about 352 kilometers southwest of Addis Ababa. It is located at coordinates 7.68°N and 36.854°E in the Jimma Zone of the Oromia region, southwest Ethiopia. The study area experiences an average daily temperature of 20.71°C and an average annual precipitation of 123.01 mm, based on data from Jimma metrological station in 2012. The Experimental site covers an area of approximately 506.25 square meters.



(Source: (Desye *et al*., 2022)

Figure 1 : Study area map

3.2 Source of earth worm and pre- test of the bio wastes

Earthworms (*E. fetidia)* were sourced from Jimma Agricultural Research Center in, southwestern Ethiopia, specifically from vermiculture/vermcomposting research site focused on natural resource management. Upon acquisition, the worms underwent a 14-day acclimatization period during which they were fed with cow dung. Subsequently, a range of locally available biowastes was evaluated to determine their suitability as substrates for vermiculture. These biowastes included cow dung, fruit leftovers (banana, avocado and papaya (1:1:1)) student meal leftovers, waste paper and vegetable leftovers (cabbage, potato peel and lettuce (1:1:1)). Each waste type, 50 grams in

quantity, was moistened with water and placed in five separate boxes. Four adult earthworms were then inoculated into each box, and the setup was left for 24 hours. The initial assessment revealed that the earthworms survived in all biowaste except for the student meal, indicating that this waste was unsuitable as a substrate for vermiculture. Consequently, the remaining four biowastes were individually and in combination subjected to further testing to ascertain their efficacy as vermiculture substrates.

A) Jimma agricultural research center

B) Student left over meal

c) Fruit waste

d) Cow dung

e) Vegetable waste f) Waste papers

Figure 2: Jimma agricultural research center and bio wastes utilized

3.2.1 Collection, preparation of Bio- wastes and plastic boxes

Fruit leftovers were gathered from juice cafes and the fruit market in Jimma town, while cow dung was sourced from a nearby dairy farm. Vegetable leftovers were collected from various sources including nearby hotels, markets and restaurants in Jimma town. Waste paper was obtained from the discarded materials in Jimma University offices.

The pre-decomposition process involved specific steps for each waste type. Cow dung was pre-decomposed by adding water and turning it every 48 hours. Fruit and vegetable leftovers were cut into small pieces (approximately 1–2 cm in size) and placed in a designated area for pre-decomposition. Waste paper was soaked in water in a 24 liter container for 24 hours, and then thoroughly mixed, air dried and manually crushed to achieve a powdery form. This pre-decomposition phase lasted for 10 days.

Subsequently, eight culture plastic boxes were manufactured on-site using locally available plastics. These boxes had dimensions of 0.0624 m^2 in area and a height of 24 cm, providing the necessary environment for the vermiculture experiments (Photo. C).

a) Fruit leftover

b) Vegetable leftover collection

c) Grinding the bio-wastes

d)locally made plastic boxes

Figure 3: Bio wastes collection, preparation and plastic boxes locally made

3.2.2 Bio wastes nutrient analysis, earthworm bedding preparation and experimental diet formulation

All the bio wastes underwent proximate composition analysis at Jimma University College of Agricultural and Veterinary Medicine Laboratory following the AOAC (1990) Procedure.

For the bedding material, a mixture of soil, cardboard and dried leaves was prepared in a ratio of 2:1:1, respectively. The soil was sifted to remove impurities, cardboard was cut into small pieces and dry leaves were crushed to ensure a suitable particle size for worm movement. This mixture was then added to plastic boxes, filling them to a height of 5 cm in 8 boxes of the equal area. Subsequently, 500ml of water was added and left for 3 days to initiate microbial activity. In terms

of diet formulation, the biowaste mix (BM) was created by blending waste paper, cow dung, and fruit leftovers in a 2:1:1 ratio, while other bio-wastes were used individually.

A) Bedding ingredients

Figure 4: Bedding material ingredients

3.2.3 Earth worm culture experimental design and its feeding

The culture experiment involved four treatments utilizing different types of bio-wastes: T1 (fruit leftovers), T2 (Biowaste mix BM), T3 (vegetable leftovers) and T4 (cow dung). After initiating microbial activity in the bedding material, a total of 400 earthworm cocoons were manually harvested from on-site earthworm production. Subsequently, 50 cocoons were inoculated into each treatment replicate. Each inoculated cocoon was covered with 62.5 grams of the treatment substrate specific to its respective treatment. Then, 250ml of water was sprinkled onto the substrate, and the treatments were placed on a wooden shelf in a small green house constructed from wood and plastic sheeting.

Feeding the worm was carried out by adding 62.5 gram of selected substrate to respective treatment every week. The amount of feed given was determined based on the conversion rate of earthworms where 1kg of adult earthworms can convert up to 5 kg of waste per day, and approximately10 kg of adults earthworms can convert one ton waste per month (Mahmoud, 2011).

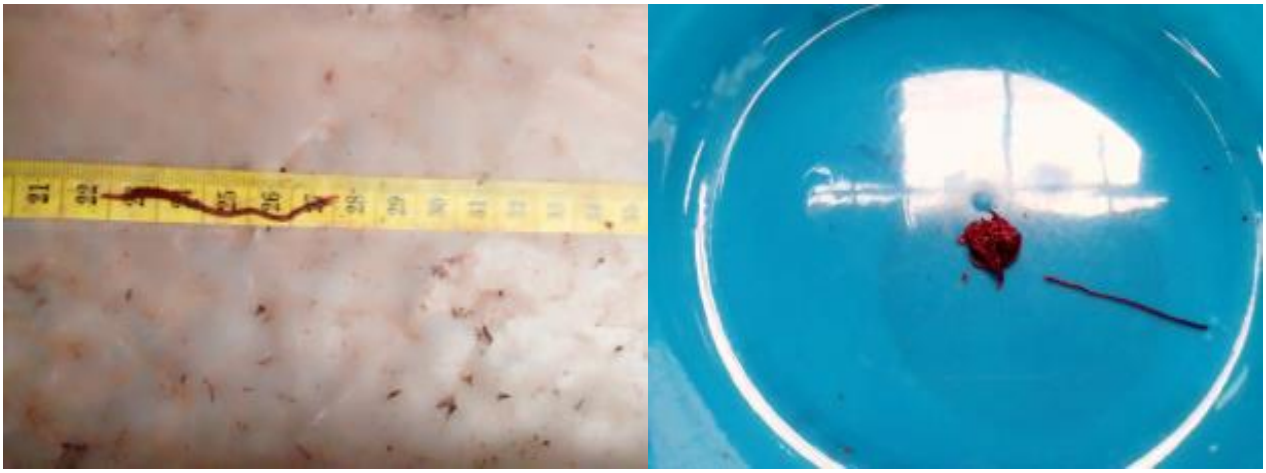
To determine hatching performance, earthworms were manually harvested according to the method outlined by Misra *et al*. (2003). , The number of individual worms found in each the respective treatment was counted after 42 days of experiment.

a) *E.fetida* cocoons b)experimental setup

Figure 5:*E.fetida* cocoons and experimental setup

3.2.4 Stoking density and feeding juvenile earthworms

Juvenile earthworms with a consistent initial weight and length of 0.022 ±0.01grams and 4 ±1.03centmetrs, respectively, were harvested from their respective treatments. A total of 760 juveniles were inoculated into each treatment, with 95 juveniles per replicate, and fed 10% of their body weight of bio-wastes. The amount of feed provided to the worms was adjusted every week based on their weight and length, which were assessed by sampling 10 individual earthworms from each plastic box. After a 6-weeks period, the final mean weight, length, and biomass of the harvested were determined. Growth performance and feed utilization experiments were conducted during this time frame. Additionally, the final earthworms harvested from each treatment were analyzed for their proximate composition.



a) Length measurement of *E.fetida* b) weight measurement

Figure 6: Length and weight measurement of *E.fetida*

3.2.4 Physic- chemical parameters in culture units

Moisture control was maintained by applying 250 ml of water to each treatment daily. Furthermore, to prevent worms from escape and protect against predators, mosquito nets were used to cover the boxes. Temperature control during the culture experiment was ensured by situating it under a shed.

For pH measurement of the vermi-compost, a representative sample was taken and mixed with distilled water in a ratio of 1:2.5 (10g compost and 25ml distilled water). This mixture was then covered and left undisturbed for 2 hours, with occasional shaking. Afterward, the pH was measured using a pH meter following the method outlined by Suthar(2007).

Daily temperature measurements were conducted using a thermometer, while moisture content was assessed every 5 days, following the procedure specified by AOAC (1990).

a)pH. Measuring b) culture units covered with mosquito net

Figure 7: pH measuring and experimental setup cover

3.2.5 Collection of biological data of earthworm culture

Data were collected on the hatching performances, growth rate, and feed utilization parameters of earthworms from each culture treatments. Earthworm growth rate was calculated using the formula:

$$\text{Earthworm growth rate (mg/worm/day)} = \frac{\text{Maximum biomass (mg)-Initial biomass (mg)}}{\text{Total Number of day} \times \text{Number of earthworms inoculated}}$$

$$\text{Feed conversion ratio (FCR \%)} = \frac{\text{Total feed intake (dry weight)}}{\text{Total live weight gain}} \times 100$$

$$\text{Protein efficiency ratio} = \frac{\text{Total live weight gain}}{\text{Total protein intake}}$$

Weight gain (WG, gm.)=final mean weight –initial mean weight

3.3 Vermi-culture

The suitable substrate was further utilized for vermiculture to facilitate mass production of worms. After 10 weeks of vermiculture, the worms were harvested every 3 weeks. Harvesting process was conducted in alternate weeks using different boxes and plastic containers, following the procedure described by Misra *et al*. (2003).

To harvest the worms, the compost was spread in a thin layer on the ground, prompting the worms burrow down to avoid light. The top layer was repeatedly removed until reaching the lowest layer where most of the worms were concentrated and could be harvested effectively.

The harvested worms were then processed to create Earthworm meal (EWM), following the procedure detailed by Mohanta *et al*. (2016). This process involved fasting the worms for 48 hours, boiling them in water with a pinch of common salt, thorough washing, and oven-drying for 24 hours, and finally grinding them into a powder using a kitchen mortar. The powdered EWM was packed into plastic bags until it was for use.

A) Earthworm mass cultivation

Figure 8:*E.fetida* mass culture unit

A)Compost+worm

B) Compost removing

C)Removing again

D) little compost left

E) Pure worms

Figure 9: Earthworm harvesting procedure from compost

A) Washed worms B)Fasting

c)Cooking D) Chopping

E) Chopped EWM F) Oven drying

Figure 10: Earthworm meal processing procedure

3.3.1 Processing of experimental diet ingredients and determining their biochemical composition

The basal feed ingredients, including rumen liquor, cattle blood, and poultry manure were sourced from the Jimma slaughterhouse and poultry farm, respectively. These ingredients were then transported to Jimma University main campus. A sample was taken from each ingredient and analyzed for proximate composition following the AOAC (1990) procedure.

During processing, cattle blood was collected in a 20L container and transported to the university. It was then poured onto a polythene tent sun drier, mixed with a pinch of common salt and dried for 14 days. Once completely dry, it was collected, ground into powder form, packed in plastic bags and stored in a moisture-free area until needed.

Similarly, rumen liquor and poultry manure were collected and air-dried for 14 days. Once completely dry, it was sieved with a 0.6 mm sieve, packed in plastic bags, and stored in a moisture-free area until used. This standardized procedure ensured the quality and preservation of these ingredients for use in the experiments.

a) Cattle blood collection and drying

Figure 11: Cattle blood collected and solar tent drier

3.3.2 Experimental diet formulation

After preparing all the experimental diet ingredients, four experimental diets were formulated to have the same nitrogen content. This was achieved by adjusting the crude protein content to 35% based on the major ingredients content using Pearson's square method. In this methods, the ingredient with a higher protein content than required was placed in the top left corner of the square, while those with lower protein content were placed in the bottom left corner. The difference between the two was placed in the opposite position in the top right corner. This value was then divided by the sum of the differences to determine the quantity required.

In Ethiopia, blood meal is commonly used as a protein source in animal diets. However, due to concerns such as unpalatability, poor amino acid balance, and potential health and safety issues associated with blood-based products, consumers are avoiding them. Therefore, in this study, cattle blood was replaced with different percentages of dried EWM in the experimental diets 100%, 60%, 30%, and 0%. The diet without EWM (D0) served as the control.

All the ingredients were thoroughly mixed; water was added to form dough. The dough was then pelletized using a pelletizing machine with a 3mm die. The pellets were air-dried, packed in plastic bags, and stored in a moisture-free environment until they were used in the experiments.

Table 1: Proportions (%) of feed ingredients used in experimental diets

	Diet treatments				
Ingredients	Diet 0	Diet 30	Diet 60	Diet 100	CP (wet weight %)
Cattle blood	22	18	12	0	80%
Earthworm meal	0	8	18	39.4	58%
Rumen liquor	43	42	39	33.9	19.9%
Poultry manure	35	32	31	26.7	24.5%
crude protein content	35%	35%	35%	35%	
Total	100	100	100	100	

a) Experimental diet formulation and pellet formation

Figure 12: Experimental diet ingredients and pellet formed

3.3.3. Fish culture

African catfish juveniles were captured from Boye River using gill net and transported to the experimental site in plastic containers. Upon arrival, the juveniles underwent a 14 day acclimatization period in a wooden tank equipped with continuous aeration provided by an electrically operated air-blower. During this time, they were fed on basal diet.

The experimental setup consisted of 8 rectangular tanks, each measuring 1.33 cubic meters. Each tank was randomly stocked with 8 pre-acclimatized juveniles, with an initial average weight of 31±0.34 grams and length of 14.9±0.199 centimeters. In total, 64 African catfish juveniles were used for the experiment.

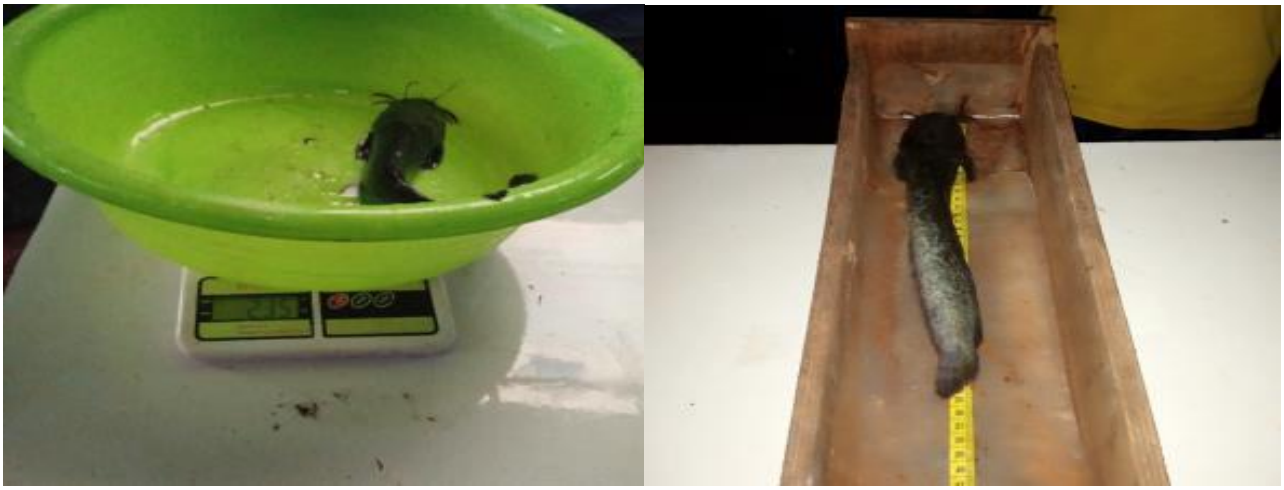
The fish were hand-fed twice a day by broadcasting feed at the periphery of each tank for a period of 60 days. Continuous aeration was provided in the tanks using an electrically operated air-blower, and the photoperiod followed a natural cycle of 12 hours of light and 12 hours of darkness.

Figure 13: African catfish raring tanks

3.4 Collection of biological data of African catfish juvenile

The total length and average weight of African catfish juveniles were measured every two weeks using a measuring board and electronic balance, respectively, in order to determine growth performance parameters. Additionally, the experimental tanks were observed daily to record any mortality, and any dead fish were removed and not replaced to gather survival rate data. Finally, at

the end of the experiment, the final weight and length of all remaining fish in each tank were measured. Further, three fish from each tank were sacrificed for proximate composition determination



A) Measuring length and weight of African catfish

Figure 14: Length and weight measuring of African catfish juvenile

3.4.1 Growth and feed utilization parameters of African catfish juvenile

Data on growth performance and feed utilization parameters were evaluated by the following:

1. Specific growth rate (SGR %) $= \frac{\ln \text{final weight} - \ln \text{initial weight}}{\text{Experimental duration (days)}} \times 100$

2. Feed conversion ratio (FCR %) $= \frac{\text{Total feed intake (dry weight)}}{\text{Total live weight gain}} \times 100$

3. Protein efficiency ratio $= \frac{\text{Total live weight gain}}{\text{Total protein intake}}$

4. Weight gain (WG, gm.)=final mean weight –initial mean weight

5. SR (%) $= \frac{\text{final number of fish}}{\text{Initial number of fish}} \times 100$

3.4.2 Water quality parameters in fish tanks

Tank water was partially renewed every 3 day and completely changed per week a time when the fish were kept in separately in holding buckets as the tanks get cleaned. Water temperature, dissolved oxygen, salinity, electrical conductivity, TDS were monitored daily twice using multi physic parameter Palin test kit (model MICRO 800), pH was measured using Adwa(model AD 8000), while nitrogen compounds were monitored every three days using Palin test kit (model photometer 7500).

A) photometermicro8000 b)PH meter

b) Palin test kit micro 7500 d) Colors observed

Figure 15: Water quality parameters measurements

3.5 Proximate composition analysis of ingredients, EWM and African catfish fillet

In the present study after all the samples required for proximate composition analysis was prepared, the samples were the subjected for analysis following the procedure of AOAC (1990). Accordingly,

3.5.1 Protein content determination

The total nitrogen (crude protein) was determined by the Kjeldahl method. A known weight (0.5 g) of the prepared sample was taken and dropped at the bottom of the Kjeldahl digestion flask together with 6-8 glass beads, 4-5 spatula full of a granular mixture of CuSO4 and K2SO4 as the catalyst. 20ml of concentrated H2SO4 was then added carefully. The flask was gently heated on a Gerhardt heating mantle in an inclined position in a fume cupboard until full digestion (when the liquid changed from brown colour to colourless. The contents of the flask were transferred to a clean 100ml volumetric flask and made up to volume 25ml, an aliquot was used for distillation. Then the formula for Percentage Nitrogen is given as:

$$\% \text{ Nitrogen} = \frac{\text{ml acid titrated} \times \text{Normality of acid titrated} \times 0.014}{\text{Weight of the sample}} * 100$$

$$\text{Crude protein} = \% \text{ Nitrogen} * 6.25$$

Where, 6.25=animal conversion factor for nitrogen to protein

3.5.2 Ether extract content determination

Ether extract content was determined by the Soxhlet extraction method, accordingly, rinse all glassware with petroleum spirit, drain, and dry in an oven at 102 º C for 30 min. and cool in a desiccator. Place a piece of cotton wool in the bottom of a 100 mL beaker. Put a plug of cotton wool in the bottom of an extraction thimble and stand the thimble in the beaker. Accurately weigh 5 g of sample into the thimble. Add 1 - 1.5 g of sand and mix the sand and sample with a glass rod. Wipe the glass rod with a piece of cotton wool and place cotton wool in the top of the thimble. (Addition of sand is not required for analysis of meat meal). Dry the sample in an oven at 102ºC for 5 hours. The drying step may be omitted in the analysis of meat meal.

Allow the sample to cool in a desiccator. Take the piece of cotton wool from the bottom of the beaker and place it in the top of the thimble. Insert the thimble in a Soxhlet liquid/solid extractor. Accurately weigh a clean, dry 150 mL round bottom flash and put about 90 mL of petroleum spirit

into the flask. Assemble the extraction unit over either an electric heating mantle or a water bath. Heat the solvent in the flask until it boils. Adjust the heat source so that solvent drips from the condenser into the sample chamber at the rate of about 6 drops per second. Continue the extraction for 6 hours.

Remove the extraction unit from the heat source and detach the extractor and condenser. Replace the flask on the heat source and evaporate off the solvent. (The solvent may be distilled and recovered).Place the flask in an oven at 102ºC and dry the contents until a constant weight are reached (1-2 hours). Cool the flask in a desiccator and weigh the flask and contents.

$$\% \text{ Ether extract (Crude fat)} = (W2 - W1) \times \frac{100}{S}$$

Weight of empty flask (g) = W1

Weight of flask and extracted fat (g) = W2

Weight of sample = S

3.5.3 Ash content determination

The ash content was determined by incineration in a carbonite Sheffield LMF3 mufflers furnace at 500°C (AOAC, 1990). The difference in weight of the samples before and after heating was taken as the ash content.

$$\% \text{ Ash content} = \frac{w2 - w_0}{W1 - w_0} * 100$$

Where, w =Empty crucible

w1 =Ash sample weight before heating

w2 = Dry sample weight after heating

3.5.4 Moisture content determination

Dry the empty dish and lid in the oven at 105°C for 3 h and transfer to desiccator to cool. Weigh the empty the dish and lid. Weigh about 3 g of sample to the dish. Spread the sample with spatula. Place the dish with sample in the oven. Dry for 3 h. at 105°C.After drying, transfer the dish with partially covered lid to the desiccators to cool. Reweigh the dish and its dried sample.

$$\text{Moisture (\%)} = \frac{(W1 - W2) * 100}{W1}$$

Where, w1= weight (g) of the sample before drying

W2= weight (g) of the sample after drying

3.6 Data Analysis Techniques

The data collected were subjected to the one-way analysis of variance (ANOVA) using the SPSS (version 20.0).The data without significant differences were presented as mean and standard deviation(SD).The difference between means were tested by Duncan's multiple range test at p (<0.05) .

4. Results

4.1 Physicochemical parameters in vermin-culture treatments

The physicochemical parameters of the earthworm culture are detailed in Table 2. Although slight differences were observed between treatments, there were no statistically significant differences ($p > 0.05$) in the tested parameters (temperature, pH, and moisture content) across all culture treatments.

Table 2: Physic-chemical parameters of earth worm culture (mean ± SD).

Variables	№ sample	Treatments				
		T1	T2	T3	T4	P -value
Temperature(°c)	6	21.9±0.1	22.1±0.07	22.2±0.79	22.4±0.32	0.56
pH	6	7.6±0.84	7.7±0.42	7.3±0.42	7.7±0.98	0.278
Moisture	6	75.5±1.12	74±0.24	76.5±1.94	74.5±1.36	0.622

Note: Values represent mean ± standard deviation of duplicate tests.

4.2 Number of hatchlings and hatching performance of *E.fetida* cocoon

Number of hatchling, mean number of hatchling and hatching success per *E.fetida* cocoon were presented in table 3. The cocoons incubated in T4 had the highest hatchling numbers, mean number hatchling and hatching success per cocoon. However T3 had lowest number of hatchling, mean number of hatchling and hatching success per cocoon. There were statistically significant differences in mean number of hatchlings across treatments, ($p < 0.05$) with T4 recording the highest.

Table 3: Number of hatchlings and hatching performance by *E.fetida* cocoons

Variables	№ sample	Treatments				
		T1	T2	T3	T4	P -value
Number of hatchlings from one cocoon	2	1-3	2-4	1-2	3-4	-
Mean number of hatchlings per cocoon	2	2 ± 0.14^{a}	2.85 ± 1.03^{b}	1.59 ± 0.55^{a}	3.94 ± 1.04^{c}	0.021
Mean number of hatchlings emerged	2	100 ± 2.82^{a}	142.5 ± 2.9^{b}	79.5 ± 1.5^{a}	197 ± 1.2^{c}	.004
Hatching success		50%	71.25%	39.75%	98.5%	-

Note: values represent mean ± standard deviation of duplicate tests. Different alphabets (a<b<c) in the same rows symbolize non-homogenous means (p<.05).

4.3 Growth performance and feed utilization parameters *E.fetida* juvenile

The growth performance, feed utilization and total biomass of *E.fetida* juvenile are presented in table 4. The obtained biomass showed significant differences ($p < 0.05$) across treatments, with T2 recording the highest followed by T4, T1 and T3. The growth rate of juvenile earthworms showed statistically significance differences($p<0.05$) across the treatments, with T2 exhibiting the highest growth, followed by T4, T3 and then T1.Statistically significant difference ($p < 0.05$) were also observed in feed conversion ratio and length gain among all culture treatments. The feed conversion ratio was lowest in T2, however juveniles incubated T4, T1 and T3 shows increase in feed conversion ratio. Length gain was highest in T2, followed by T4, T3, and then T1 as presented in table 4.

Table 4: Growth performance and feed utilization parameters of *E.fetida* (Mean ± SD)

Variables	№ sample	Treatments				P-value
		T1	T2	T3	T4	
Initial weight(g)		0.025 ± 0.01^{a}	0.023 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.1^{a}	0.329
Final weight(g)		0.55 ± 0.02^{a}	1.04 ± 0.01^{c}	0.8 ± 0.02^{a}	0.92 ± 0.03^{b}	0.035
Biomass gained(mg)	6	53.9 ± 1.3^{a}	92.4 ± 1.64^{c}	39 ± 2.89^{a}	66 ± 0.9^{b}	0.002
Specific growth rate (%)	2	1.45 ± 0.001^{a}	2.37 ± 0.01^{b}	1.62 ± 0.001^{a}	1.36 ± 0.02^{a}	0.047
FCR (%)	2	2.21 ± 0.02^{a}	1.1 ± 0.0^{a}	1.57 ± 0.01^{b}	1.54 ± 0.01^{a}	0.031
Initial length(cm)	1	3.9 ± 0.01^{a}	4.1 ± 0.12^{a}	4.18 ± 0.02^{a}	4.05 ± 0.03^{a}	0.65
Final length(cm)	1	8.4 ± 0.21^{a}	10 ± 0.15^{c}	8.8 ± 0.024^{a}	8.95 ± 0.08^{b}	0.021
Length gain(cm)	2	3.4 ± 0.28^{a}	5.9 ± 0.14^{c}	4.1 ± 0.42^{a}	4.9 ± 0.14^{b}	0.01
Growth rate(mg/worm/day)	2	1.8 ± 0.33^{a}	7.8 ± 1.43^{c}	1.5 ± 0.5^{a}	4.7 ± 1.36^{b}	0.009

Note: values represent mean ± standard deviation of duplicate tests. Different alphabets (a<b<c) in the same rows symbolize non-homogenous means ($p<.05$).

4.4 Weight gain of *E.fetida* in different substrates /week

Figure: 1 displays the weight gain of *E. fetida* cultured in various biowastes. Noticeable differences in weight gain were observed across all treatments. Specifically, the biowaste mix (T2) exhibited the highest weight gain, while fruit leftovers (T1) showed the lowest. Each treatment group displayed a distinct exponential growth curve, with none of the curves overlapping throughout the 42-day experiment period.

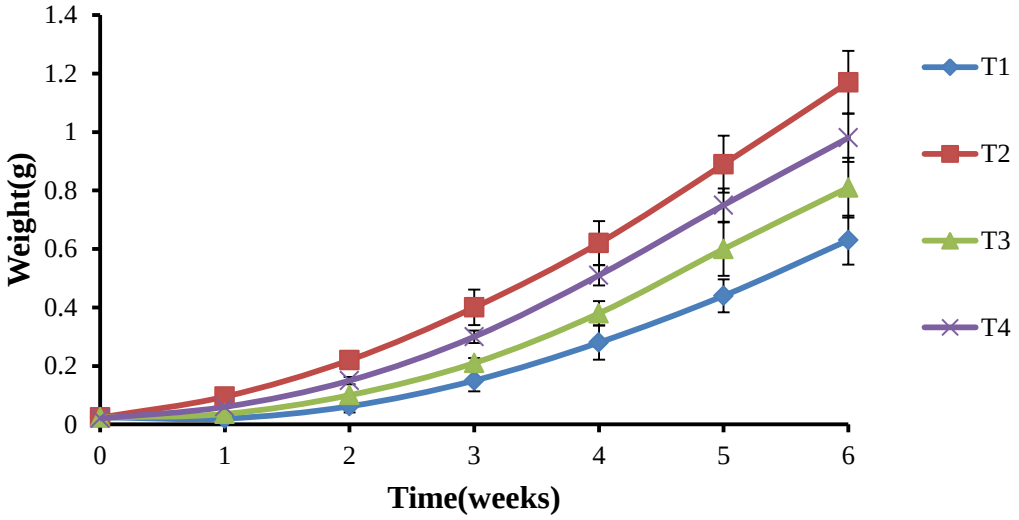


Figure 1: Mean weights (±SE) (g) changes of *E.fetida* fed on different biowastes

4.5 Proximate composition of EWM

The crude protein content obtained from EWM fed on different bio-wastes showed statistically significance differences ($p<.05$) across the treatments. With T2 had the highest crude protein level, followed by T4, T1 and T3.

In terms of ether extract, T1 had the highest content, followed by T2, T3, and T4, although the difference was not statistically significant ($p>.05$). There were statistically significant differences ($p< 0.05$) in ash content between treatments. With T3 exhibited the highest ash content, followed by T1, T4 and the lowest in T2.

Table 5: Proximate composition of EWM (wet weight %) (Means ±SD)

EWM	№ sample	Treatments T1(FW)	T2(BM)	T3(VW)	T4(CD)	P –value
Crude protein (%)	3	41.9±1.28^{a}	58.8 ±1.05^{b}	37.4±1.85^{a}	49.1±2.21^{ab}	0.031
Ether extract (%)	3	7.7±2.22^{a}	6.7±0.9^{a}	5.9±2.28^{a}	4. 7±0.97^{a}	0.456
Ash (%)	3	7.8±1.32^{bc}	3.7±0.84^{a}	8.9±1.42^{c}	5.3±0.58^{ab}	0.029

Note: values represent mean ± standard deviation of duplicate tests .different alphabets (a<b<c) in the same rows symbolize non-homogenous means ($p<.05$).

4.6 Water quality parameters in catfish raring tank

The water quality parameters in the catfish raring tanks are detailed in Table 6. Although slight differences were observed between treatments, there were no statistically significant differences ($p > 0.05$) in the tested parameters (temperature, dissolved oxygen, salinity, pH, and ammonia nitrogen content) across all culture treatments.

Table 6: Water quality parameters in fish tank (Mean ±SD)

Variables	№ sample	Diet treatments				
		D100	D60	D30	D0	P – value
Temperature(°C)	8	22.38 ± 0.78	22.6 ± 0.7	22.08 ± 0.35	22.77 ± 0.6	0.426
Dissolved oxygen(Mg/L)	8	4.18 ± 0.24	4.62 ± 0.17	5.20 ± 0.08	5.06 ± 0.6	0.426
Salinity(PSU)	8	0.14 ± 0.01	0.11 ± 0.02	0.13± 0.02	0.13 ± 0.02	0.141
pH		7.56 ± 0.01	7.58± 0.01	7.55 ± 0.02	7.56±0.02	0.456
NH_4(Mg/L)	8	0.75 ± 0.08	0.76 ± 0.13	0.52 ± 0.11	0.59 ± 0.1	0.191

Note: values represent means ± standard deviation.

4.7 Growth performance and feed utilization African catfish juvenile

Fish fed D30 exhibited the highest biomass of 191,950 grams, followed by D60, D100 and D0 with biomass of 151,215, 120,088 and 88,120 grams, respectively. There were no statistically significance differences ($p>0.05$) in mortality among all experimental fish groups. However, D100, D60 and D30 showed the highest survival rates, while D0 had the lowest.

Significant differences ($p < .05$) were observed in average weight gain. With The highest mean weight gain were recorded in fish fed D30, followed by D60, D100 and then D0 showing the lowest weight gain. Furthermore, significant differences ($p < 0.05$) were noted in feed conversion ratio among all treatments. With Fish fed D30 had the lowest feed conversion ratio, however it was increasing in those fed D60, D100 and the highest D0. Specific growth rate and protein efficiency ratio shows statistically significantly differences ($p<0.05$), across the diets. With D30 exhibited the highest followed by D60, D100 and D0 had the lowest values, as detailed in table 7.

Table 7: Growth performance and feed utilization parameters of *C. gariepinus* juvenile fed on different diets (Mean ±SD)

Variables		Diet treatments				
	№ sample	D100	D60	D30	D0	P- value
Initial weight(g)		31.13±0.02^{a}	31.55±0.0^{a}	30.89±0.05^{a}	30.78±0.01^{a}	0.23
Weight gain(g)	4	60±2.49^{ab}	75.6±1.0^{b}	95.97±2.8^{c}	44.06±0.36^{a}	0.014
Survival rate (%)	2	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	96.9 ± 2.41^{a}	0.479
Specific growth rate	2	1.8±0.1^{b}	2±0.13^{bc}	2.26±0.28^{c}	1.47±0.09^{a}	0.027
Feed conversion ratio	2	1.4±0.06^{b}	1.23±0.04^{ab}	1.03±0.02^{a}	1.7±0.01^{c}	0.03
Protein efficiency ratio	2	0.66±0.05^{ab}	0.85±0.1^{bc}	1.04±0.09^{c}	0.54±0.12^{a}	0.007

Note: Values represent Means± standard deviation of duplicate tests .different alphabets (a<b<c) in the same rows symbolized non-homogenous means (p<.05).

4.8 Weight gain of catfish juvenile fed on different diets

Figure 2: displays the weight gain of African catfish juvenile fed on different experimental diets. Noticeable differences in weight gain were observed across all treatments. Specifically, the 30% inclusion of earthworm meal (D30) exhibited the highest weight gain, while 0% inclusion of earthworm meal (D0) showed the lowest weight gain. Each treatment group displayed a distinct exponential growth curve, with none of the curves overlapping throughout the 60-day experiment period.

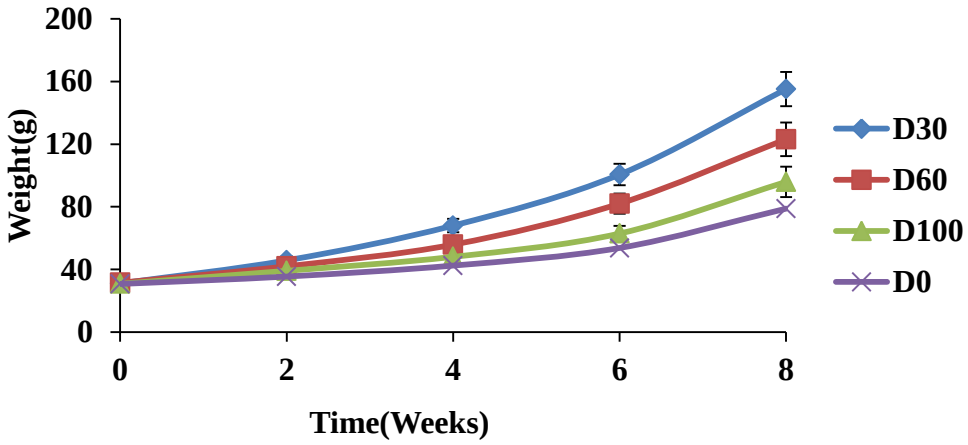


Figure 2: Mean weight (±SD) (g) change of *C. gariepinus* juvenile fed on different diets

4.8 Proximate composition of *C. gariepinus* fillets

Table 8: presents the proximate compositions of *C. gariepinus* fed on four different diets. Crude protein content obtained from fish fed on different diets did not showed statistically significance differences ($p>.05$) across the diet treatments. With D30 had the highest crude protein level, followed by D60, D100 and D0 showing the lowest levels.

There were no statistically significance differences ($p>.05$) in ether extract among all diet treatments. However, there were statistically significance differences ($p<.05$) in ash content across the diets treatments. With D100 had the highest ash content followed by D60,D0 and D30.

Table 8: Proximate composition of *C.gariepinus* fillets fed on four different diets %)(wet weight (Means ±SD)

Variables	№ sample	Diet treatments				
		D100	D60	D30	D0	P- value
Moisture content (%)	3	70.1 ± 0.3^{bc}	61.01 ± 1.24^{a}	74.8 ± 1.51^{c}	65.7 ± 0.8^{b}	0.014
Crude protein (%)	3	18.1 ± 2.82^{a}	19.9 ± 1.27^{a}	20 ± 2.82^{a}	16.9 ± 2.8^{a}	0.34
Ether extract (%)	3	3.8 ± 0.44^{a}	3.9 ± 1.13^{a}	2.8 ± 1.00^{a}	3.8 ± 1.2^{a}	0.612
Ash (%)	3	4.8 ± 1.2^{b}	1.1 ± 1.38^{a}	0.7 ± 1.67^{a}	3 ± 0.51^{a}	0.045

Note: Values represent Means± standard deviation of duplicate tests .different alphabets (a<b<c) in the same rows symbolized non-homogenous means (p<.05).

5. Discussion

In order to understand the population dynamics, productivity and energy flow in earthworms, studying their life cycle pattern is highly essential. Particularly, for effective vermiculture, knowledge on life cycle of composting worms on various wastes is crucial (Bhattacharjee and Chaudhuri, 2002). It is well known that food sources influence not only size of an earthworm population but also their growth and reproduction rates (Dominguez, 2004).Moreover the present study evaluated the suitable biowaste for vermiculture and revealed that cow dung is biowaste best supported higher mean number of hatchling and biowaste mix was best supported growth performance, feed utilization parameters and produced earthworm with higher crude protein content.

Aquaculture sustainability is highly threatened by among others the expensive and unreliable fish feeds. Cost, quality and quantity are the crucial elements that determine the sustainability of fish feeds in aquaculture. A sustainable fish feed should be nutritionally complete, less costly, palatable, easily digestible, as well as capable of maintaining water quality and improving disease resistance, and not causing mortality(Mosyoka *et al*., 2020). Moreover the present study evaluated the impact of utilizing the interesting nonconventional alternative protein source obtained from *E.fetida* as alternative protein source in the diets of African catfish aquaculture and revealed that 30 % inclusion of earthworm meal induces better growth performance, feed utilization parameters and increased protein content of African catfish juvenile.

5.1 Physic-chemical parameters in vermiculture units

Cocoon production, rate of development and growth of earthworms are all critically influenced by environmental conditions. Epigeic earthworms are relatively tolerant of the environmental conditions of organic waste, so fairly simple windrow or bed systems with little management effort have been used to process these wastes on a large scale (Edwards *et al*., 2022). In the present study, the environmental parameters such as, temperature, pH and moisture were maintained. These parameters were show no statistically significant differences among all treatments ($p>0.05$). The measured values were in the optimal range for *E.fetida*.

The moisture content obtained the present study was in agreement with Bhattacharjee and Chaudhuri (2002), who reported that bed humidity of 70–80% is required for reproduction of *E*.

eugeniae and *E. fetida* and also results of Haukka (2017), who reported that that the humidity of the media required for *E. fetida* earthworm is 60-90%.

pH obtained in the present study was in agreement with(Edwards and Lofty, 2013) who reported that, most epigeal earthworm species prefer a pH in the acidic range of neutral when exposed to a pH gradient of 5–9.

The average temperature obtained in the present study was in agreement with (Dominguez and Edwards, 2011), who reported that, optimal temperature range for breading of *E. fetida* and *E. andrei* in organic waste was (15–20 (limits: 4–30) °C). Therefore, all parameters were within the optimal range for *E. fetida* , that means the different biowasts have no adverse effect on different culture treatments physic-chemical parameters.

5.2 Number of hatchlings and hatching performance by *E.fetida* cocoon

Significantly higher mean number of hatchlings was obtained from cow dung in the present study .This results was higher than mean number of hatchling per cocoon reported by Edward (1988) who reported that mean number of hatchling per cocoon of *E.fetida* was 3.3±0.57. The increase in mean number of hatchling in the present study than the other studies is may be because cultures units were maintained at controlled environmental condition and also cow dung alone may contain high nitrogen content. This is also supported by Garg *et al*.(2005) who reported that , it is also may be the dung, which is an important and a unique factor for the earthworm maturation and for the production of cocoons and hatchling from the cocoons.

Hatching success in the present study was higher than that obtained by Ali and Kashem (2018) who reported that The cocoons produced generally demonstrated good hatching performance in *Eisenia fetida* (86.6%) than *Eudrilus eugeniae* (75.3%).Giraddi *et al*. (2010) who reported that better hatching success of cocoons that indicate the cocoon viability.

Since, there are no or few published reports in literature regarding the effect of substrate quality on cocoon hatchling success and number of hatchling that emerge from cocoon, it is difficult to relate totally the direct impact of feeding material on such kind of biological activities. However, Suthar (2007) reported that N-content in substrate might be the primary determinant of cocoon hatching success. The nitrogen content of the culture media affects the cocoon production rate and their

further development by influencing the nutritional need of the protein for earthworms. It could be hypothesized that earthworm produce more viable cocoons in nitrogen-rich culture media due to efficient protein supply in their diets.

5.3 Growth performance and feed utilization parameters of *E.fetida* juvenile

Statistically significantly highest biomass was obtained in treatment which contains biowaste mix in the present study. This may perhaps due to the in increased number of biowaste ingredients, therefor the lack of one nutrient in other bioweste will be present in other biowaste so this means this biowate was provided complete nutritional requirement for *E.fetida*. The best biomass production recorded in present study is in agreement with the report of Omoyinmi *et al*. (2005), who used sawdust and sand culture media for raising earthworms.

Another possible explanation for best growth and production in T2 is the production of bacteria involved in the breaking down of cellulose and possible biochemical by-products activated in presence of cow dung that are naturally more numerous in T2, when compared with other substrates (Aston 1984). As BM also contains waste papers which were rich in cellules. These bacteria are the protein source for worms and needed in decomposition of organic matter. Suzuki & Kurihara (1981) demonstrated that aquatic oligochaete, *Aelosoma hemprichi* could feed exclusively on bacteria, showing remarkable rapid population growth on this diet.

According to Meharaj and Manivannan (2015), the growth rate (mg/earthworm/day) is an excellent and acceptable comparative index to compare the growth of earthworms in different organic wastes. Significantly higher growth rate of *E.fetida* was obtained from earthworm fed on BM. This is may be due to increased bio-wastes ingredients, quality and nutritional contents of the bio wastes. The growth rate of the earthworm is affected by the type and quality of feeding material (Jayakumar *et al.,* 2018). The results of the present study was more or less similar to the results of Chauhan and Singh(2013) who reported that significant growth rate occurred which was 7.18 ± 0.42 mg/worm/ day in CWGr((goat dung + wheat straw + gram bran) combination.

Growth pattern of *E.fetida* over experimental period of 6 weeks shows, weight of earthworms in all treatments increased gradually till the end of experiment. However the highest weight gain was observed in treatment containing BM. The increased mean weight gain is may be due to nutritional

quality of the culture media, the maintenance of culture units in controlled condition and feeding practice.

Feed conversion ratio (FCR) is an important indicator of the excellence of feed. A lower FCR indicates better utilization of the feed by the fish (Mugo-Bundietal,2013).The lowest Feed conversion ratio and highest specific growth rate obtained in treatment 2 .This indicates that this substrate is accepted by the worms and efficiently converted in to the earthworm weight gain and also The low FCR might be due to the high proportion of protein derived from ingredients which are easily digestible.

5.4 Proximate composition of EWM

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 *et al*., 2011). They are responsible for growth, tissue development, energy source and are 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 and Metian, 2008).The result of the present study revealed that the highest crude protein level obtained the treatment containing BM.

The crude protein obtained in the present study is higher than that obtained by Mohanta *et al*. (2016) who evacuated the gut contents of *E. fetida* before biochemical analysis and got crude protein contents of 52% dry weight however slightly lower than that obtained by Rondón *et al*. (2003) who obtained 62.28% dry weight. The lowest Crude protein level obtained in control diet is lower than that obtained by Zhenjun *et al*. (1997), who obtained 39.9% dry weight. This may be due to the reason that the 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 and also the retention of amino acid in to earthworm body when used vegetable leftover as feed source for the worms is may be lower.

Nevertheless, all the crude protein levels in the present study were higher than the minimum required for growth and reproduction by the most cultured fish species. This is in agreement with (Liti *et al*., 2005).Accordingly, the minimum required crude protein levels for growth and

reproduction by the most cultured fish species 30%dry matter. Therefore all the selected bio wastes were produced EWM with crude protein which was in recommendable level.

Ether extract was significantly higher in treatment one. The highest ether extract obtained in the present study was more or less in an optimum range to those obtained by(Mohanta *et al*., 2016), who reported earthworm contain 6-11% fat. The increase in the amount of ether extract in present study is may be due to the reason that some fruits contain high fat content.

The highest ash content obtained in this study was similar with that reported by Sogbesan and Ugwumba (2008) who reported that earthworms contain (on dry mass basis) 8.9% ash. The increase in ash in the present study in treatment three is may be due to the reason that vegetables were rich in cellulose. This is in agreement with (Erdogan *et al*., 2019) who reported that, Vegetable fibers contain high amount of cellulose, which can be extracted and reused as a raw material in various industry.

5.5 Growth performance and feed utilization parameters of African catfish juvenile

The exponential growth curve observed in the current study shows that all experimental diets were adequate, palatable and essential nutrients (particularly protein) to support fish growth. The non-overlapping of the growth curves during experimental period means there is significant difference compared to control diet. Studies have shown *E. fetida* meal to contain similar protein, amino acids, lipids, minerals and vitamin contents with FM (Dedeke *et al*., 2010, Vodounnou *et al*., 2016 and Zhenjun *et al*., 2010).Growth performance parameters (weight gain, specific growth rate, length gain and FCR) were statistically significantly differences ($p<.05$) among the different diet treatments.

The highest specific growth rate was obtained in diet containing 30 % inclusion of earthworm meal is similar to that reported (2.55 ± 0.04) by Kpogue (2013) in *P. obscura* fingerlings. This value also agreed with the results that Monebi and Ogwumba(2012), obtained while studying the utilization of the earthworm *Eudrilus eugeniae* in the diet of Heteroclarias fingerlings where the optimum inclusion level of the earthworm meal in diet was 25%.

Feed conversion ratio (FCR) is an important indicator of the excellence of fish feed. A lower FCR indicates better utilization of the feed by the fish (Mugo-Bundi *et al.*, 2015). FCR obtained in the

present study in fish fed diets D30 is similar or even better to the earlier researchers who used different non-conventional dietary protein sources such as fisheries by-catch and processing waste for tilapia (1.34–1.55, Goddard *et al*., 2008), garden snail meal in *C. gariepinus* (1.21–1.44, Sogbesan *et al*., 2006).The results of the present study were also more or less similar with (Ogunji *et al*., 2008) who reported that FCR values of 1.2–1.5 have been reported as normal range for fish reared with properly compounded diet. The results of the present study was also higher than that obtained by Tadese *et al*.(2021),who reported FCR values ranged from 3.07 to 4.44 in utilizing *E.fetida* as fish meal replacement in diets of *C. gariepinus fingerlings.*

The protein efficiency ratio (PER) obtained in D30 is comparable to the PER reported by different authors using various non-conventional protein sources in the diet of fish (0.75–1.25, Mondal *et al*., 2011 for *Labeo bata* with fermented fish offal meal);0.99–1.05, Thompson *et al*., 2007 for sunshine).

The increase (weight gain, length gain, specific growth rate, survival, PER and lower FCR) in 30% inclusion level was in agreement Velasquez *et al*. (1991) Worm meal obtained from the common earthworm (*E. foetida*), when used to replace 25 and 50% of the fish meal component in diets for rainbow trout, gave higher growth rates in fish fed these diets compared to fish fed the control diet without any worm meal.

Fish growth, reproduction, immunity and behavioral activities are highly reliant on the essential amino acid profile in the protein component of a diet, owing to the fish inability to synthesize the 10-indispensable amino (Andersen *et al*., 2016 and Dedeke *et al*., 2010). Improper amino acid profile and balance with vitamins disrupt their patterns in the body, consequently impairing growth, appetite, food intake, absorption and assimilation (Andersen *et al*., 2016 and Zhenjun *et al*., 2010).

The lowest weight gain, SGR, PER and the highest FCR in diet containing 0% (control) were attributed to nutritional composition, unpalatability and unbalanced essential amino acids of the blood meal. As the level of blood meal inclusion increased after 10%, the response of the fish to the diet became poor. This finding indicate that fishmeal in the diet of *C. gariepinus* can only be efficiently replaced with 10% Blood Meal and this observation agree with the result of Otubusin (2001) who reported that the replacement of fish meal with 10% blood meal in pelleted feeds was adequate for tilapia, *Oreochromis niloticus* production in floating bamboo net-cages.

Total replacement of cattle blood protein (D100) with earthworm meal protein resulted in decreased weight gain and feed utilization than other treatments, however, still better than the control treatment D0. The decreasing growth performances in treatment D100 are perhaps due to the presence of the high amount of coelomic fluid, which is known to be toxic to fish and reduces feed palatability (Ngoc *et al*., 2016 and Musyoka *et al*., 2019). Kobayashi *et al*. (2001) reported toxicity of coelomic fluid of the earthworm (*E. fsoetida*) to vertebrates. Moreover, the tendency of decreased growth in treatment D100 may be due to the fact that the hard cuticle in the earthworm contains the chitin. Chitin is a polymer of glucosamine and is insoluble in common solvents. Our study revealed inclusion level which induces better growth performance, feed utilization and survival rate were 30 and 60% inclusion level of earthworm meal.

5.6 Water quality parameters in fish tanks

After nutrition, water quality is the second key fundamental aspects of any fish production (Mosyoka *et al*., 2020). The lack of significant difference in the current study on water quality parameters (temperature, pH, DO, salinity and ammonia) between the culture units shows the experimental diets did not have a negative influence on the fish growth performance. Nonetheless, the water quality parameters obtained were within the optimal requirements for *C. garipnus* (Sogbesan *et al*., 2006; Amisah *et al*., 2009 and Eyo *et al*., 2004). Nitrogen-ammonia is also in optimum rage (Roye *et al*., 2019).Given that most of the mortalities were experienced a day after samplings, it is henceforth believed the fish deaths were caused by handling stress, but not by water quality or the experimental diets.

5.7 Proximate composition of catfish juvenile fillet

Proximate analysis of the fish fillet indicated that, crude protein obtained in D30 was higher, however that of D60 and D100 were consistent with those of (Nubia, 2014) who found that, the protein content of wild catfish was 19.33±0.25%. The increase in crude protein level in the current study is may be due to the diet quality that it contained balanced amino acid profile which induces good amino acid retention in to the fish muscle. This is because essential amino acids are requisites for nitrogen retention in fish muscles and growth (Peres & Oliva-Teles, 2009).

Ash obtained from D100 was statistically significance differences ($p<.05$) from other diets. The results of the present study were higher than those stated by (Deng, 2018) who found that the ash

content in *C. gariepinus* was 1.7±0.06%. On the other hand, (Nubia *et al*., 2014) found that the ash content in wild catfish was (1.40%). This was in agreement with (Mohanta *et al*., 2016) who reported that he growth and dietary performance of fish fed whole frozen earthworm (D-1) and earthworm-based custard (D-2) was not encouraging; it may be due to the presence of more chitin that is derived from cuticle when earthworm was incorporated at higher levels (100% in D-1 and 60% in D-2) in the diets. Therefore an increase in ash content of *C. gariepinus* might be attributed to be due to the presence of more chitin that is derived from cuticle when earthworm was incorporated at higher levels in the diets.

6. Conclusion and recommendations

6.1 Conclusion

It is generally concluded that cow dung is identified as the appropriate biowaste that optimally supports the hatching of *E.fetida* cocoon, while BM enhances its growth performance and feed utilization parameters, leading to the production of earthworms with higher levels of crude protein. The inclusion of 30% earthworm meal in the diets of African catfish juveniles results in improved growth performance, feed utilization parameters, survival rate, and yields African catfish juveniles with elevated crude protein levels in their fillets. Consequently, earthworm meal has the potential to partially or fully substitute cattle blood in the diets of African catfish (*C. gariepinus*) juveniles.

6.2 Recommendations

- Further research should be required on incorporating the byproducts of vermiculture in to aquaculture productions (use as pond bio fertilizer, fish feed ingredient) and in Urban agricultural production.
- EWM production should be scaled up and awareness should be given to all fish farmers of Ethiopia
- Determining the impact of nitrogen content of biowaste ingredients on hatchability rate of earth worm cocoon needs a research interest
- Instead of using cattle blood as alternative protein source utilizing EWM is better.

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8 Appendices

I. Appendix A:physic chemical parameters in vermiculture units

Temperature								
Week	T1.1	T1.2	T2.1	T2.2	T3.1	T3.2	T4.1	T4.2
0	21.6	21.6	21.6	22.3	21.3	21.7	21.5	22.23
1	25.7	25.8	25.4	25.3	24.8	24.8	23.6	24.5
2	19.1	19.2	19.4	19.5	19.6	19.7	20	20.2
3	24.2	24.7	24.8	24.7	24.9	25	25	24.7
4	23.1	23.2	23.5	23.6	23.8	23.8	24	24
5	19.7	19.6	19.5	19.6	19.7	19.7	19.8	19.9
6	21.3	21.2	21.7	21.8	22	22.4	22.7	22.6
pH								
1	7.7125	7.5575	7.2625	7.24	7.535	7.435	7.56	7.4225
2	7.6925	7.6975	7.7375	7.5625	7.57	7.265	7.74	7.73
3	7.5775	7.6875	7.56	7.695	7.6975	7.6975	7.675	7.5725
4	7.4075	7.5475	7.625	7.705	7.455	7.5475	7.5975	7.6175
5	7.585	7.24	7.15	7.5275	7.49	7.4725	7.4225	7.4225
6	7.6325	7.505	7.12	7.49	7.585	7.5025	7.4225	7.5475
Moisture								
1	71.02	73.5	71.23	72.12	76.25	76.025	73.2	75.006
2	75.14	75.6	74.56	74.02	78.1	74.36	75.64	73.046
3	78.16	74.2	76.5	72.1	75.36	77.214	76.09	72.034
4	77.2	71.2	75.02	73.03	77.35	76.21	74.047	74.023
5	73.4	76.25	74.2	74.04	76.51	77.1	73.64	75.094
6	75.23	73.13	75.23	73.325	76.25	77.012	72.35	76.01

II. Appendix B: Growth performance and feed utilization parameters earthworm

Treatments	Specific growth rate	Feed conversion ratio	Length gain	biomass gained	growth rate
T1.1	1.47	2.37	3.2	55.86	1.963
T1.2	1.36	2.035	3.6	51.05	1.8726
T2.1	1.8	1.13	5.8	89.3	7.686
T2.2	2.93	1.07	6.0	95.08	6.809

T3.1	1.68	1.061	4.4	35.31	1.621
T3.2	1.56	2.0746	3.8	43.48	1.511
T4.1	1.21	2.045	4.8	69.803	4.661
T4.2	1.51	1.05	5.0	63.75	4.7321

III. Appendix C:weight gain of earthworm per week

Week	T1.1	T1.2	T2.1	T2.2	T3.1	T3.2	T4.1	T4.2
0	0.023	0.026	0.023	0.024	0.023	0.024	0.02	0.021
1	0.03	0.04	0.095	0.055	0.028	0.03	0.051	0.071
2	0.09	0.06	0.19	0.17	0.12	0.04	0.18	0.12
3	0.11	0.19	0.45	0.35	0.3	0.12	0.23	0.43
4	0.27	0.23	0.52	0.74	0.5	0.3	0.48	0.6
5	0.44	0.34	0.71	0.99	0.7	0.5	0.64	0.82
6	0.8	0.3	0.99	1.09	0.95	0.65	0.89	0.95

IV. Appendix D: water quality parameters in fish tanks

				WATER QUALITY PARAMETERS			
	Week	Temp	DO	Sal	EC	TDS	PH
T1	1	20.55	1.195	0.3	722.25	490	7.845
	2	19.6	4.1625	0.085	269.5	176	7.6775
	3	18.35	4.6175	0.085	265.75	171.75	7.3525
	4	17.575	4.6375	0.12	372.75	240.75	7.59
	5	19.63	4.715	0.16	461	300	7.7325
	6	18.625	4.3375	0.215	544	354.25	7.7075
	7	17.325	3.615	0.1175	361.5	234.25	7.4725
T2	1	20.075	2.4	0.3075	650.25	419.5	7.775
	2	18.95	2.0825	0.1625	427.75	278.75	7.6175
	3	18.075	3.2375	0.0975	311.5	201	7.45
	4	17.325	3.615	0.1175	361.5	234.25	7.4725
	5	18.55	3.5025	0.14	438.75	284.25	7.6275
	6	18.775	3.6075	0.205	501	325.5	7.555
	7	18.1	4.57	0.145	410	266	7.695
T3	1	19.85	1.6225	0.25	588.25	371	7.69
	2	19.425	3.4975	0.17	426.75	277.5	7.6175

	3	18.325	4.54	0.095	292.5	189.5	7.5425
	4	17.325	4.9825	0.1075	339	218.5	7.4175
	5	18.15	4.5975	0.15	428.25	276.75	7.7
	6	18.475	4.365	0.16	446.75	290.5	7.6525
	7	18.025	4.415	0.145	415.25	268.25	7.6925
T4	1	20.05	1.015	0.13	397	258.25	7.4175
	2	19.525	3.8825	0.085	258.25	168	7.5575
	3	18.325	5.09	0.0725	223.5	145.75	7.565
	4	17.325	5.3525	0.07	234.25	151.25	7.555
	5	18.1	4.985	0.0875	284.25	183.75	7.6475
	6	18.525	3.86	0.1775	512	330	7.695
	7	18.075	4.28	0.1175	373.5	242.75	7.6175

V. Appendix E: growth performance and feed utilization parameters of Catfish juvenile

Diet	WG	SR	SGR	FCR	PER	LG
D0	42.1	100.00	0.54	1.65	0.17	7.53
D0	45.9	93.75	0.99	2.55	0.44	4.40
D30	98.661	100.00	1.67	0.10	2.76	18.41
D30	93.279	100.00	1.84	0.09	2.89	22.23
D60	73.52	100.00	1.48	0.76	1.87	16.22
D60	77.66	100.00	1.27	0.53	0.96	12.51
D100	58.3	100.00	1.02	0.95	0.68	9.07
D100	61.7	100.00	1.16	1.64	0.85	12.00

VI. Appendix F: weight gain of fish /weeks

Weeks	D0.1	D0.2	D30.1	D30.2	D60.1	D60.2	D100.1	D100.2
0	30.56	31	31	31.25	32.05	31.05	31	30.78
2	35	36.035	47.72	43.924	43.04	41.872	42	38.328
4	41.25	43.758	64.042	57.968	53.524	52.223	50.538	45.63
6	51.36	56.14	88.08	81.13	73.5	68.563	65.441	58.469
8	71.534	78.146	122.2	132	113.315	101	94.931	86.937

VII. Appendix G:proximate composition of Earthworm meal and African catfish fillet

		Crud Protein				
Lab.No	Sample.Code	SW(g)	Vb	V	N%	CP%
1	0% EW	0.5001	0.2	11	2.7024907	16.90567
2	30% EW	0.5007	0.2	11.3	3.2505207	20.40754
3	60% EW	0.5009	0.2	11.4	3.131931	19.57457
4	100% EW	0.5002	0.2	11.7	2.89322	18.12701
5	T1 EWM	0.5008	0.2	25.9	6.688097	41.92561
6	T2 EWM	0.5009	0.2	26.1	9.242589	58.26618
7	T3 EWM	0.5009	0.2	26.2	5.870553	37.4.44096
8	T4 EWM	0.5007	0.2	26	7.8507	49.10942
		Ash				
Lab.No	Sample.Code	CW(g)	SW(g)	(C+ Ash)W(g)	Ash%	
1	0% EW	32.6207	1.0016	32.659	3.023882	
2	30% EW	36.7587	1.0009	36.7981	0.736457	
3	60% EW	35.4046	1.0011	35.4443	1.1965638	
4	100% EW	32.5545	1.0001	32.5944	4.89601	
5	T1 EWM	35.8651	1.0004	35.9535	8.836465	
6	T2 EWM	31.924	1.0006	31.9998	3.75455	
7	T3 EWM	31.8828	1.0004	31.9717	8.886445	
8	T4 EWM	34.1944	1.0005	33.2776	5.315842	
		Ether Extract				
Lab.No	Sample.Code	CW(g)	SW(g)	A.Extracted(g)	EE%	
1	0% EW	29.8143	1.0001	29.8525	3.819618	
2	30% EW	30.9185	1.0004	30.9466	2.808876	
3	60% EW	30.0668	1.0006	30.1061	3.927643	
4	100% EW	29.5017	1.0001	29.5401	3.839616	
5	T1 EWM	30.0466	1.0009	30.1236	7.693076	
6	T2 EWM	29.8125	1.0002	29.8795	6.69866	
7	T3 EWM	30.9164	1.0004	30.9751	5.867653	
8	T4 EWM	29.8142	1.0003	29.8612	4.69859	