PERFORMANCE OF AFRICAN CATFISH (*Clarias gariepinus*, Burchell, 1822) LARVAE FED ON SPIRULINA AND REDWORMS AS PROTEIN ALTERNATIVES IN FORMULATED DIETS.

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DECLARATION

I, Callen N. Onura declare that this thesis is my original work and has not been presented for a degree award in any other University.

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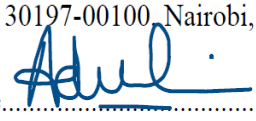
Signature.....  ..... Date: 14/08/2024

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This thesis is dedicated to my late father, William Onura Okenyoru, and my adorable trio Brian Sereti, Festus Orenge and Felix Omambia.

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LIST OF ABBREVIATIONS AND ACRONYMS

DDG	Dried Distiller Grains
DGR	Daily growth rate
DPH	Days post-hatching
ESP	Economic stimulus programme
FCR	Feed Conversion Ratio
FIFO	Fish in Fish Out
FL	Final length
FM	Fish meal
GM	Genetically modified
HUFA	Highly unsaturated fatty acid
IL	Initial length
IMs	Insect Meals
PER	Protein Efficiency Ratio
PUFA	Polyunsaturated Fatty Acids
SGR	Specific Growth Rate
UN	United Nations
UoN	University of Nairobi
USD	US dollar

APPENDICES

ABSTRACT

The contribution of aquaculture to food security and employment cannot be overemphasised. However, the limited availability of quality seed and feed has prevented it from realising its full potential. A vital component of fish feeds is aquatic animal protein sources like fishmeal and freshwater shrimp meal (*Caridina nilotica*). Continued use of aquatic animal proteins in fish feed further decreases capture fisheries catches. Alternatives to aquatic protein sources in the feed industry like soybean meal, are expensive and in competition with human food. Feed prices have skyrocketed and have made fish larvae' nutrition a hindrance to aquaculture development. This study investigated the growth, digestive capacity and stress tolerance of *Clarias gariepinus* larvae fed on *Spirulina platensis* or *Eisenia fetida* in formulated diets. The study was conducted at the University of Nairobi, Department of Biology under controlled conditions. Seven diets were formulated to be approximately iso-nitrogenous and iso-caloric. *Spirulina platensis* or *Eisenia fetida* partially replaced *Caridina nilotica* at 25%, 50%, and 75% and control of 100% *Caridina nilotica* to give the seven diets. In addition, a commercial diet (Gemma Micro, Skretting Co. Netherlands) with 100% fishmeal was used to compare the performance of formulated diets and what was available in the market. *Clarias gariepinus* larvae were artificially propagated and randomly stocked at 25 larvae per litre in 24 glass aquaria at 28 ℃. Larvae were fed at 20% of their body weight, decreasing to 10% at the end of week two at a frequency of five times a day. Fish in the aquarium were randomly assigned one of the eight experimental diets in triplicate. Larvae fed on these experimental diets were transitioned to fingerlings in the grow-out tanks at Makindi Fish Farm Muranga County, Kenya, to assess the effects of hatchery nutrition on growth. Fingerlings were fed on skretting and Raanan feeds at 5% body weight twice daily in grow-out tanks. Nutrient utilisation was determined by feed conversion ratio and protein efficiency ratio while weight gain, specific growth rate and survival determined larval growth performance. A partial cost analysis for all experimental diets was estimated. The spectrophotometric analysis determined the digestive capacity of the *Clarias gariepinus* larvae by specific enzyme activities (total protease, trypsin, alpha-amylase and lipase) at 0, 2, 7, 14-, 21- and 28 days post-hatching. Furthermore, stress tolerance by larvae fed on the experimental diets on four- and six-week-old larvae through exposure to ammonia solution for 24 hours was done to indicate larval robustness and fitness in culture water. Total mortalities, survival time in un-ionised ammonia and stress index indicated *Clarias gariepinus* larvae's stress tolerance at different ammonia levels in culture water and age. One-way analysis of variance compared means between treatments and t-test compared means between time points after passing normality and homogeneity tests ($p>0.05$). Replacing *Caridina nilotica* with 50% *Eisenia fetida* in the *Clarias gariepinus* larval diet posted the highest and significantly different ($p<0.05$) weight gain (0.43 g/larva), specific growth rate (9.57%/day), survival (81%), protein efficiency ratio (1.3) and feed conversion ratio (1.65)

compared to all other formulated diets. All experimental diets progressively activated and increased digestive enzyme activities in enzyme-specific patterns. Digestive enzyme activities were low and significantly different ($p<0.05$) in larvae fed on *spirulina platensis* diets. The highest and significantly different ($p<0.05$) specific enzyme activities (0.89 U/mg protein^{-1}min^{-1} total protease, 0.35 U/mg protein^{-1}min^{-1} alpha-amylase, 0.99 U/mg protein^{-1}min^{-1} trypsin and 60.23 U/mg protein^{-1}min^{-1}) was posted in larvae fed on 50%*Eisenia fetida* at 28 days post-hatching. Six weeks old larvae fed on a 50% *Eisenia fetida* diet were the most robust, with the lowest stress index of 342 and the longest survival time of 18.7 hours before the death of 50% of the exposed larvae in a 0.05 mg/litre unionised ammonia. A t-test revealed low and significantly different ($p<0.05$) stress indices in six-week-old larvae compared to those in week four regardless of dietary treatment. Fingerlings with hatchery nutrition of 75%*Eisenia fetida* and fed on Raanan or Skreeting in grow-out tanks had the highest and significantly different ($p<0.05$) weight gain of 13g and 12g/fingerling and the best feed conversion ratio of 1.19 and 1.39 respectively. A partial cost analysis revealed *Eisenia fetida* diets to be cheaper and had high returns on investment. *Caidina nilotica* can be replaced with reduced survival by 75% *Eisenia fetida* or 25% *Spirulina platensis* in the *Clarias gariepinus* larval diet. Larvae fed on 50% *Eisenia fetida* had the highest digestive competence and were the most stress tolerant. Hatchery nutrition of 75% *Eisenia fetida* positively influenced *Clarias gariepinus* fingerlings' performance in the grow-out. The study recommends *Eisenia fetida* protein use in fish larval feed production. Additionally, capacity building in *Eisenia fetida* production for fish feed industries is important. Further, a study on the formulation of diets combining 50% *spirulina platensis* and 50% *Eisenia fetida* is recommended.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Global aquaculture production has gradually increased to 126 million tonnes of live weight valued at USD 296.5 billion in 2021 (FAO, 2023). However, Africa experienced a growth rate decline from 17.9% in 2018 to 14.5% in 2020 (FAO, 2022). Additionally, Africa's share of the world's aquaculture production in 2021 was only 1.9% 2021 (Mair *et al*., 2023). Africa's reduced growth rate and low contribution to global aquaculture were attributed to COVID-19 effects and weak value chain linkages. On the other hand, Kenyan aquaculture production increased from 895 metric tonnes in 2009 to 19945 metric tonnes in 2020 (Opiyo *et al*., 2023). Sustaining this production rise requires aquaculture to have a cost-effective production system with a sustainable and robust supply chain. However, the industry faces many challenges, including a shortage of quantity and quality fish seed and feed (Griffin, *et al*., 2019). Seed quality is influenced by many factors including water and feed quality. However, supply shortages of aquatic animal protein and the skyrocketing prices of fishmeal continue to pose a significant challenge in ensuring the quality of fish feed. Cheaper protein alternatives that are less digestible and have lower nutritional value have been used leading to reduced survival and lower production at the hatchery level. As a result, fish species used in aquaculture have recorded decreased growth and survival rates across all life stages.

The African catfish (*Clarias gariepinus*) is a freshwater species with global aquaculture importance. The species tolerates variable environmental conditions, rapid growth, and a better feed conversion ratio (Ngugi *et al*., 2007). The species' larvae feed within the water column or on the surface. Its feeding physiology, digestive morphology, and nutritional requirements vary from those of adults. This variation is attributed to the larvae's drastic digestive shifts, small mouth gape, and selective feed preference skewed towards small particle sizes (Rathore *et al*., 2016). Furthermore, the species larvae are susceptible to the diets they are fed on within the first weeks of exogenous feeding. All these larval challenges have contributed to the failure to achieve this species' full aquaculture production potential.

Clarias gariepinus larvae, like many other fish species, rely on live feed for enhanced growth and survival in their initial life stages. The live feeds have high nutritional value for fish larvae and the advantage of exogenous enzymes for improved digestion. However, live feeds (like *Artemia* nauplii) require specialised equipment and technical skills for hatching, variable nutritional content and a potential source of microbial infection in the hatchery (El-Sebaie *et al*., 2014). *Artemia's* nutritional content depends on the species of phytoplankton it feeds on and habitat abiotic factors. *Artemia* microbial infection risk to cultured species depends on how efficiently it is rinsed during hatching processing and the storage temperature after hatching (Ali & Juancey, 2004).

Research on dry diets as alternatives to live feed has been tried with inconsistent results. The dry diets do not require hatching and allow manipulation of nutritional content to suit species-specific and age-specific nutrient requirements. Nevertheless, these dry diets heavily depend on expensive and scarce aquatic animal protein sources, which are also highly digestible and nutritionally balanced, and some (for example, fishmeal) contain unknown growth factors (Radhakrishnan *et al*., 2014). Therefore, aquaculture intensification is likely to push the prices of aquatic animal protein further up. The increased aquatic animal protein prices amplified fish production costs and the prices of fish products beyond the means of a sizable human population. Increased production costs will reduce aquaculture's contribution to global food security and economic resilience. Reliance on aquatic animal protein in fish feed threatens the viability and health of cultured species. Aquatic animal species destined for fishmeal accumulate microplastics in their muscles from the habitat and provide a route of microplastics to cultured species. Ingested fishmeal containing microplastics interferes with the normal body functioning of cultured species through bioaccumulation. A continued scientific and innovative search for economical and nutritionally complete protein alternatives to replace aquatic animal protein sources in starter diets is a priority. Therefore, non-conventional protein sources like microalgae, leaf protein concentrate, insects, and earthworms in fish feed have been explored (Han *et al*., 2018).

Microalgae like *Spirulina platensis*, Geitler,1925, present a primary protein source for aquatic organisms at some stage in life. Proteins, amino acids, polyunsaturated fatty acids, fatty acid profiles and bioactive characteristics of *S. platensis* are important in aquatic animal nutrition (Yarnold *et al*., 2019). Likewise, *S. platensis* has attractant properties that stimulate feed ingestion in catfish larvae (Dabrowski, 1984). However, the availability of *S. platensis* in some countries is limited. Also, its use in aquaculture competes with human diet supplementation. Thus, there is a need to evaluate the performance of *S. platensis* against other non-conventional primary proteins.

A range of earthworm species, such as *Eisenia fetida*, are used in fish feeds; however, full utilisation of these worms is limited because they are sensitive to handling stress and are not adaptable to many climatic conditions (Vodounnou *et al*., 2016). However, *E. fetida* is adaptable to different culture substrates and can balance its energy expenditure (Sharma & Garg, 2018). The species has high levels of limiting amino acids, vitamins, and minerals and has a nutritional content closer to fishmeal and chicken eggs (Antonova *et al*., 2021). Success stories of its use in poultry and pig feed nutrition have been reported (Castro-Bedriñana, *et al*., 2020).

Spirulina platensis and *E. fetida* are both low in the natural environmental food chains exploited as protein alternatives for aquafeed industries. The availability of their proximate composition in the literature is high. However, the effects of *S. platensis* or *E. fetida* as partial or complete protein alternatives in formulated

starter diets on fish larvae's digestive capacity and stress tolerance remain scanty. Linking the hatchery nutrition of *S. platensis* or *E. fetida* and grow-out performance is of economic value in aquaculture production.

1.2 Problem Statement

Demand for fish fingerlings exceeds supply because of the inadequate quality and quantity of larval fish feed and seed. The larval feed relies on the expensive and nutritionally variable live feed. Expensive fish feeds have resulted in aquaculture apathy due to economic setbacks associated with low larval growth and survival. Dry diets have replaced live feed with unpredictable results because of limited information on fish larvae nutrition and potential digestible protein alternative sources. Furthermore, dry diets rely on finite but highly digestible aquatic animal protein sources such as fishmeal and *C. nilotica*. The supply of these protein sources is declining due to climate change, which has increased global warming, and ocean acidification. This has led to a hike in fish feed prices and shortages in supply. The use of potential alternatives to aquatic animal protein sources like plants, invertebrates, stem cells or biotechnological proteins has been developing. However, the amino acid profile of plant proteins is incomplete, stem cell proteins are still in the experimental phase, and they are not mass-tested. Invertebrate proteins from the class Insecta, Mollusca, and Annelida are abundant and diverse, with huge potential as protein alternative sources in the feed industry. However, the performance of these alternatives has been characterised by poor growth and

high mortalities in fish larvae. Therefore, fish larvae remain a hindrance to aquaculture sustainability and environmental integrity.

Non-conventional protein alternative sources like microalgae (*S. platensis*) and earthworms (*Eisenia fetida)* have replaced aquatic animal protein sources in the recent past. However, the performance of such formulated feed is mainly evaluated based on fish biological characteristics, feed intake, nutrient utilisation, and survival. Nevertheless, the influence of *S. platensis* or *E. fetida* diets on digestive capacity is little known. Additionally, knowledge of the robustness and fitness of larvae fed on *S. platensis* or *E. fetida* to withstand variable culture conditions is mostly ignored. Studies have compared growth rates of fingerlings in grow-out ponds and have reported significant differences without links to hatchery nutrition. The use of *S. platensis* or *E. fetida* to replace *C. nilotica* in formulated larval nutrition remains scanty.

1.3 Justification of the Study/Rationale

1.3.1 African Catfish (*Clarias gariepinus*) Larvae

African catfish is suitable for both tropical and subtropical freshwater aquaculture. The species is among those that contribute 75% of global aquaculture production and 21% of aquaculture in Kenya (Nyoje *et al.,* 2018; Naylar *et al*., 2021). It grows faster than other catfishes with high-quality muscles and is tolerant to high stocking densities and diseases. However, the species has low larval survival and its larval stage demands nutritional care to guarantee a positive

influence on its later life stages. Understanding the species' digestive capacity is a guide to selecting protein ingredients for its larval stage and designing tailored feed for optimal growth and survival. Information on *C. gariepinus* larvae's environmental stress tolerance provides cues for its survival in different culture conditions during stocking and appraise experimental diets.

1.3.2 Choice of Protein Alternatives (*S. platensis* and *E. fetida*)

Spirulina platensis and *E. fetida* are natural feeds for fish with a high nutritional content that occupies a low position in the food chain and are highly digestible. It is possible to manipulate the culture media of *S. platensis* or *E. fetida* to match species and age-specific desired nutrients. Moreover, they have a short life span with high reproduction rates to ensure higher biomass for fish feed formulations with minimal water and land usage. Their use in the fish feed industry cushions aquaculture intensification against plant and animal protein production failure due to climate change and ever-declining aquatic protein sources. *Spirulina platensis* and *E. fetida* use in feed formulation contributes to the future production of quality and healthy larvae in aquaculture. Their increased use in aquafeeds is one of the roadmaps towards aquafeed resilience by narrowing the larval feed demand gap. Using *S. platensis* and *E. fetida* in larval feed will increase fish farmer returns by producing volumes of high-quality feed at a reasonable price.

1.3.3 Formulated Diets

Formulated diets for *C. gariepinus* larvae eliminate variable supply and nutritional inconsistency in live feed used in larviculture. The formulated diets ensure the availability of larval feed to satisfy hatchery managers' demands for quality and quantity production. They variably influence larval performance, digestive capacity, and later fish developmental stages. Therefore, this study intends to contribute to the criteria of selecting protein ingredients for optimal larval nutrition and transition to juveniles through sustainable feed and larvae production.

1.3.4 National and International Links of the Study

The provision of formulated dry diets spurs aquaculture through enhanced fish growth and survival at low feed costs. The study addresses Kenya’s Vision 2030, the Big Four agenda, and the African Vision 2063 on food security, social welfare, and economic growth for their citizens. This can be made possible through enhanced aquaculture production of seafood, employment creation and income generation.

Internationally, *S. platensis* and *E. fetida* protein alternatives to *C. nilotica* are essential in minimising pressure on aquatic animal protein sources. The study also addresses Sustainable Development Goals (SDGs) 2 and 14, which aim to ensure no hunger among the global population while conserving and using aquatic

resources sustainably for sustainable development. Also, S. *platensis* or *E. fetida* use contributes to the blue economy and climate-smart aquaculture.

1.4 Research Questions

i. What is the growth performance, nutrient utilisation, and survival of *Clarias gariepinus* larvae fed on *Spirulina platensis* and *Eisenia fetida* as partial protein alternatives in formulated diets?

ii. What is the digestive enzymatic activity in *Clarias gariepinus* larvae fed on *Spirulina platensis* and *Eisenia fetida* as partial protein alternatives in formulated diets?

iii. What is the stress tolerance of *Clarias gariepinus* larvae fed on *Spirulina platensis* and *Eisenia fetida* as partial protein alternatives *nilotica* in formulated diets?

iv. What are the effects of hatchery nutrition on grow-out growth performance, nutrient utilisation and survival of *Clarias gariepinus* fingerlings in the grow-out?

1.5 Research Objectives

1.5.1 General Objective

To assess the growth performance, digestive capacity and stress tolerance of African catfish (*Clarias gariepinus*) larvae fed on *Spirulina platensis* and *Eisenia fetida* as partial protein alternatives in formulated diets

1.5.2 Specific Objectives

i. To determine the growth performance, nutrient utilisation and survival of *Clarias gariepinus* larvae fed on *Spirulina platensis* and *Eisenia fetida* as partial protein alternatives in formulated diets

ii. To determine the digestive capacity of *Clarias gariepinus* larvae fed *Spirulina platensis* and *Eisenia fetida* as partial protein alternatives in formulated diets

iii. To determine the stress tolerance of *Clarias gariepinus* larvae fed on *Spirulina platensis* and *Eisenia fetida* as partial protein alternatives in formulated diets

iv. To determine the effects of hatchery nutrition on growth performance, nutrient utilisation and survival of *Clarias gariepinus* fingerlings in the grow-out

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aquaculture Industry

Demand for animal protein is steadily increasing as the global human population increases along with increased income, awareness of healthy foods, expanding global fish trade, improved distribution channels, competitive fish prices and urbanisation (Naylor *et al*., 2021). The demand gap is worsening with the stagnation of capture fisheries, sustained climate change impacts, shrinking arable land and water resources and food-feed-fuel competition. Aquaculture provides an alternative to narrow the protein demand gap.

Governments have made deliberate efforts to support the prioritisation of species diversification and aquaculture intensification as a way of r increasing fish availability for human consumption (Osmond & Colombo, 2019). Results have seen a steady rise in global aquaculture production from 114.5 in 2018 to 126 million tonnes of live weight in 2021 in Figure 2.1 (FAO, 2023). Kenyan aquaculture had an upward trend to 19945 metric tonnes, valued at KSh 6303 million in 2020 (Opiyo *et al*., 2023). However, sustained growth may be short-lived due to overreliance on scarce fishmeal in fish feed production. Fishmeal supply shortages have been occasioned by many factors including overexploitation of natural resources and low implementation rate of aquaculture technologies. Resulting in a short supply of reasonably priced and high-quality fish feed (Béné *et al*., 2015; Jonnathulla *et al*., 2019).

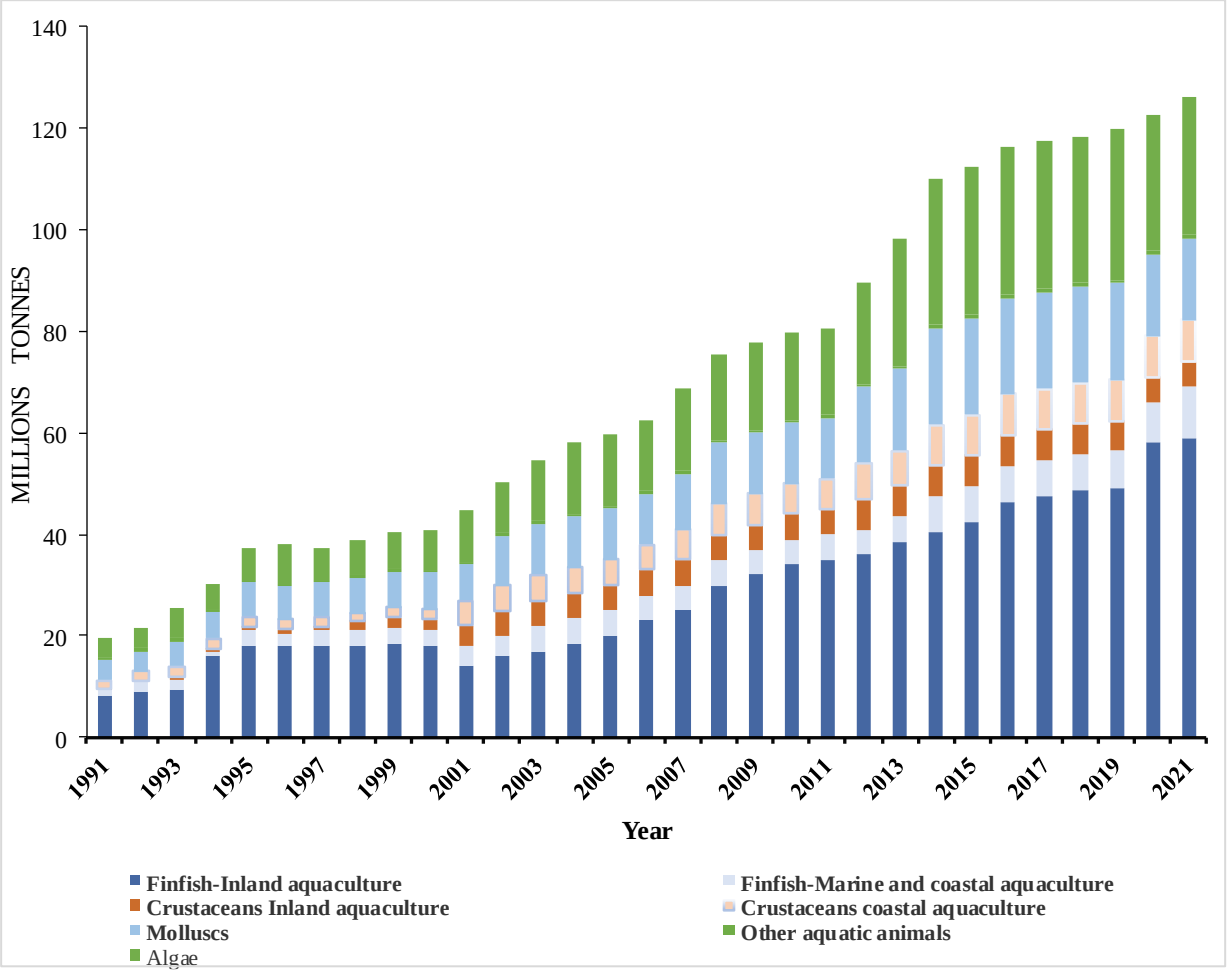

Figure 2.1: Global aquaculture production since 1991-2021 (adopted and modified FAO, 2023).

Aquaculture has many trade-offs including economics, sustainability, environmental integrity and biosecurity. However, aquaculture contributed 16% of total animal protein consumed globally (Kim *et al.*, 2019; Jones *et al.*, 2020). In Kenya, fish constituted only 5.7% of animal protein consumed (Opiyo *et al.*, 2018). Therefore, the contributions of fish to the fight against poverty, elimination of hunger and malnutrition globally can not be underestimated.

Africa has great aquaculture potential, with a surface area of 37% suitable for fish farming (FAO, 2022). African governments have prioritised aquaculture technology, genetic advancement, and innovations to increase production. to increase production. They have also encouraged private-sector participation in this endeavour. New Partnership for Africa Development (NEPAD) and the implementation of a Special Program for Aquaculture Development in Africa (SPADA) are key players driving these African aquaculture priorities (Adeleke *et al*., 2020). However, Africa only contributed 1.9% to global aquaculture despite a 20-fold production increase in 2021 (FAO, 2023). The low contribution was due to weak value chain linkages and minimal enforcement of enabling policies and technologies in Africa (Jonnathulla *et al*., 2019). Notwithstanding, Kenyan aquaculture has maintained an upward trend from 895 in 2009 to 19945 metric tonnes in 2020 (Opiyo *et al*., 2023). This has led to the rise in demand for quality fish feed and seed. One of the costly operational expenses in aquaculture is feed due to its limited availability and price shocks on quality protein sources for feed formulations (Munguti *et al*., 2021; Naylor *et al*., 2021).

2.2 Status of the Fish Feed Industry

By 2050, the projected increase in fish consumption per person is expected to range between 60 - 70 % (Kim *et al*., 2019; Etowa *et al.*, 2022). Thus, there is an expected demand rise for aquaculture products and the elusive fish feed in most developing countries. The main challenge in fish feed production is the over-reliance of feed industries on fishmeal protein and fish oil from finite natural

resources. Aquaculture has surpassed capture fisheries (Figure 2.2) in production and this trend is predicted to remain until 2050 (Jones *et al*., 2020). Consequently, it is imperative to support aquaculture by identifying and producing fish feed ingredients with high nutritional content. Preferred protein ingredients should be those whose nutritional content can be manipulated using culture techniques to meet species- and age-specific nutrients.

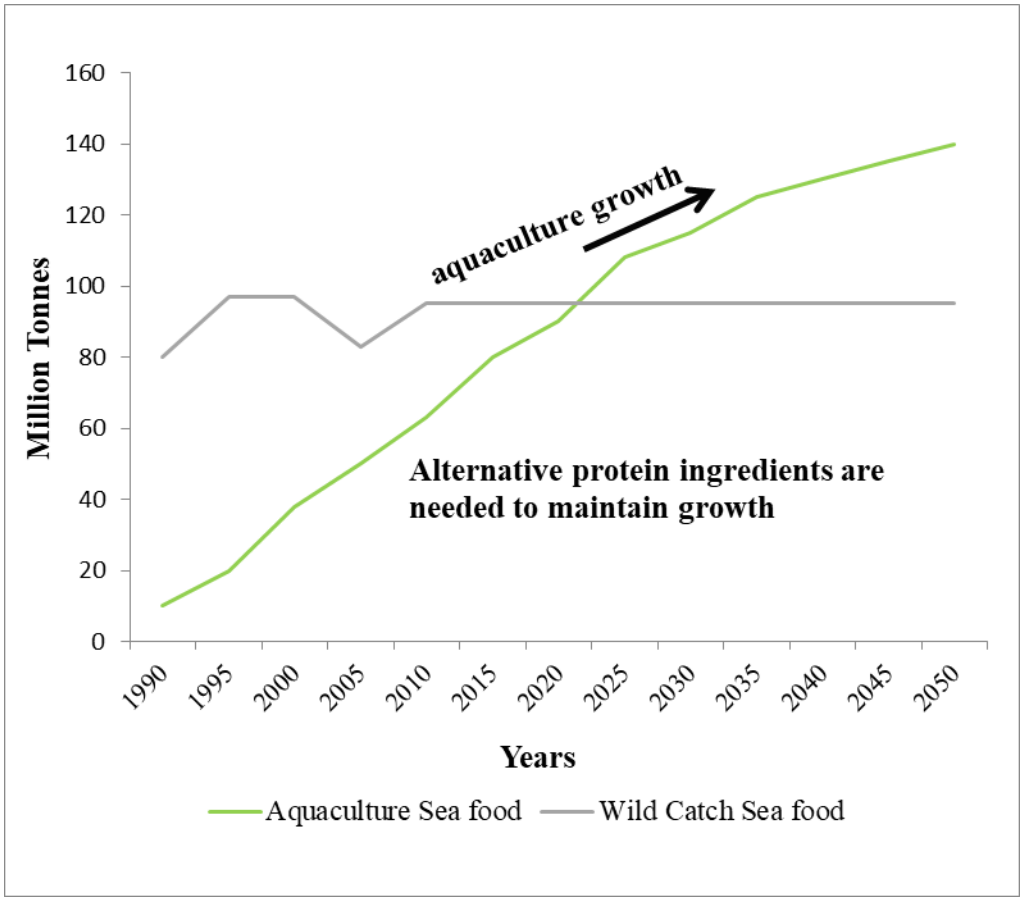

Figure 2.2: Projected capture and aquaculture seafood production by 2050 (Adopted and modified, Jones *et al*., 2020).

To sustain aquaculture production Salin *et al*. (2018), projected a global fish feed of 101.3 million tonnes by 2025. Internationally, fish feed production stood at

52.914 million metric tonnes valued at US$ 72.5 billion in 2022 with a decrease of 2.38% in Africa over the same period (Yildiz & yilmaz, 2024).

In Kenya, the introduction of the Economic Stimulus Programme (ESP) in 2009/2010 increased fish production nevertheless stretched the demand for fish feed (Nyonje *et al*., 2018). The main protein ingredients are forage fish like silver cyprinid (*Rastrineobola argentea*), freshwater shrimp (*Caridina nilotica)* wheat (*Triticum aestivum),* rice bran (*Oryza sativa*), sunflower cakes (*Helianthus annuus*) and cassava (*Manihot esculenta*) (Munguti *et al*., 2021). In addition, Kenya like many developing countries, uses by-catch fish oval, insects, worms, algae and stem cell proteins as protein sources in aquaculture.

Volumes of fishmeal used in global aquaculture have reduced though its use in fish farming has steadily increased due to intentional intensification in fish production (Kim *et al*., 2022). Deliberate intensification coupled with limited knowledge of feed formulations and processing, proper handling and market accessibility have resulted in the underdevelopment of feeds in all fish developmental stages (Munguti *et al*., 2021).

2.3 Catfish Species in Aquaculture and Their Production Status

Globally, aquaculture production relies on carp, tilapia, salmon, trout, and catfish as the main species cultured (Dauda *et al*., 2018). Catfishes are characterised by a cylindrical body that is mostly scale-less, ventrally flattened and has barbel(s) around the mouth (Armbruster, 2011). The catfish comprises 40 families and

3407 species with a global distribution except in the Antarctic (Jokar *et al*., 2023). They constitute 12% of the teleosts (Sullivan *et al*., 2006). Catfishes are among the model organisms in many fields including toxicology, evolutionary biology and fisheries for they inhabit marine and freshwater ecosystems (Thirukanthan *et al*., 2023).

Global catfish aquaculture production stood at 6.13 million tonnes valued at USD 9.98 billion in 2021 (FAO, 2023). In 2018, Asia was the most important region in catfish production, accounting for 92.3% of the global catfish production. Africa followed at 4.3% valued at US$731,432 while, 3.4% was shared among the remaining regions (Gisbert *et al.,* 2022). Notwithstanding the regional catfish production differences, the species of aquaculture importance includes Amur catfish (*Silurus asotus*), Channel catfish *(Ictalurus punctatus*), Stripped catfish (*Pangasius hypophthalmus*), African catfish or sharp tooth catfish (*C. gariepinus*), stinging catfish (*Heteropneustes fossils),* butter catfish (*Ompok bimaculatus),* Mekong catfish (*Pangasius bocourti*), silver catfish (*Rhamdia quelen),* pacamã (*Lophiosilurus alexandri), C. macrocephalus*, *C. fuscus*, *C. batrachus*, *Pangasius bocourti,* and a hybrid of *C. macrocephalus Pseudoplatystoma* species (Dauda *et al*., 2018; Karanja *et al*., 2021).

2.4 African Catfish (*Clarias gariepinus*, Burchell, 1822)

2.4.1 General Description and Taxonomy

The African catfish (*Clarias gariepinus*, Burchell, 1822), is a species in the genus of *Clarias*, family Clariidae, order Siluriformes and Actonopterygii class. *Clarias gariepinus* (Figure 2.3) is a pan-African species. The species has barbells with taste buds around the mouth for food detection and navigating turbid waters (Teugels, 1990). It thrives well in turbid and fast-flowing rivers, streams, lakes, or swamps. *Clarias gariepinus* can walk through land using its spinny pectoral fins in search of prey or a suitable habitat (Bruton, 1979). In addition, the species breathes air using the dendrite in the supra-bronchial/accessory air-breathing organ associated with the gills (Gisbert *et al*., 2022). The species tolerates low dissolved oxygen levels and irregular desiccation in different habitats (Bruton, 1979). Furthermore, the species uses a self-generated electric discharge to defend itself against intraspecific aggression (Teugels, 1990).

Clarias gariepinus attains a large weight of up to 60 kg and a total length of 1.7m in its natural habitat (Weyl *et al*., 2016). Morphologically, it has a cylindrical dark-pigmented scaleless body with long anal and dorsal fins. The species is highly ossified and has a dorsal-ventrally flattened head with a terminal mouth (Gisbert *et al*., 2022). *Clarias gariepinus* is a food fish, bait in Nile perch fisheries and has also been used to control the Tilapia population in ponds (Musa *et al*., 2013).

Figure 2.3: African catfish, *Clarias gariepinus* (Burchell, 1822).

2.4.2 Natural Habitat and Feeding Habits

Clarias gariepinus is an omnivorous freshwater species that prefers shallow and swampy areas (Teugels, 1990). However, Tesfahun (2018) considered the adult stage of *C. gariepinus* to be an opportunistic feeder due to dietary shifts based on prey abundance, season and habitat differences. A possible explanation for *C. gariepinus'* diverse feeding strategies is filter feeding, planktivore, piscivorous, detritivore, and predatory (Weyl *et al*., 2016). *Clarias gariepinus'* opportunistic feeding ability has been linked to high levels of digestive enzymes including pancreatic amylase, gastric lysozyme which digests high-fibre plant proteins and gastric and pancreatic protease for effective digestion and utilisation of various diets (Uys & Hecht, 1987). Despite its omnivorous feeding behaviour, *C. gariepinus* has an intestinal morphology close to that of carnivore species.

2.4.3 Social Organisation and Feeding Behaviour of African Catfish Larvae

African catfish larvae are secretive voracious feeders, feeding at the water surface or within the water column (Bruton, 1979; Weyl *et al*., 2016). These larvae successfully utilise live feed but are limited in utilising dry feed at first feeding. The larvae mostly swim away from light and have an unconscious resting behaviour (Tesfahun, 2018). Their feeding behaviour is reported to be synchronised by light and darkness (Hossain *et al*., 1998). Nevertheless, this was challenged by the study's conclusion that species feeding is more chemo-sensory than visually dependent (Mukai & Leong, 2011). Their high activities during the dark come with the economic benefit of increased survival and reduced cannibalism when larvae are very active.

Clarias gariepinus larvae prey size is restricted by head size, yolk size, and the structural differentiation of the feeding apparatus (Verreth *et al*., 1992). Nonetheless, species cannibalism is speculated to start on the fifth day after hatching due to the heterogeneous sizes of the larvae (Makai & Leong, 2011). This continues into the adult stage and necessitates grading to avoid economic loss. Cannibalism is highly associated with hunger, feeding, and feeding strategies other than a direct consequence of social hierarchy on growth (Verreth *et al*., 1992; Adriaens *et al*., 2003).

2.4.4 Production Status of African Catfish and Challenges

African catfish was first domesticated in the 1950s, though its initiation into aquaculture goes back to the 1970s (Weyl *et al*., 2016). The species' importance in aquaculture was due to its faster growth rate, early maturity, efficiency in feed utilisation, higher fecundity and disease tolerance. The ability of African catfish to stand a wide range of environmental conditions like a temperature of 8–35 °C and a salinity of 7–15 mg/l was also of importance in aquaculture (Hecht & Appelbaum, 1987; Hecht *et al*., 1996). African catfish culture rose in the 1980s because of the development of artificial propagation and mass-rearing techniques to enhance fish seed supply (Hecht, 2009). The species-enhanced production followed its demand rise due to consumer acceptance (Dauda *et al*., 2018). Nigeria leads sub-Saharan Africa in the production of African catfish. Further, *C. gariepinus's* upward trend is expected to rise to 93 million tonnes by 2030 (Etowa *et al*., 2022). This species is successfully cultured in cages, tanks, raceways, ponds and recirculation systems (RAS). However, high mortalities at its larval stage remain the greatest bottleneck in achieving the projected production statistics (Nyonje *et al*., 2018).

2.4.5 Fish Larval Nutrition and Challenges

Fish larval nutrition is important in later life development as it stimulates cellular development like growth (Clarkson *et al*., 2017). The larval feed should be palatable, digestible, and nutritionally balanced for enhanced growth, health, and

well-being (Gisbert *et al*., 2022). Despite the qualities of larval feed, fish larvae remain quite vulnerable and require special nutritional requirements for efficient growth, development, and survival (Ngugi *et al*., 2007; Hamre *et al*., 2013). This is probably because of their rapid physiological and morphological development. Differences in digestive morphology and physiology between catfish larvae and adults are responsible for variable nutritional requirements (Grosell, *et al.*, 2010). Feeding physiology, ontogeny, and nutritional requirements within and between species vary greatly, making it difficult to generalise the nutrient needs of different life stages and species (Hamre *et al*., 2013). The larvae survival depends on hatchery personnel experiences, and abiotic and biotic factors in the culture system (Verreth *et al*., 1994; Gisbert *et al*., 2022). However, the success and expansion of aquaculture rely on larval nutrition, which is faced with numerous challenges that threaten the industry. For instance, larval attributes like drastic ontogenetic shifts before the maturation of digestive morphology and physiology, and a small mouth gape are among the constraints to aquaculture expansion (Verreth *et al*., 1994; Hamre *et al*., 2013). In addition, there is limited information on the larval nutritional requirements for different fish species (Jobling, 2012; El-Sebaie *et al*., 2014; Rathole *et al*., 2016). *Clarias gariepinus* larvae are sensitive and have record mortality of up to 80% within the first two weeks of exogenous feeding (Hecht & Appelbaum, 1987). This problem is attributed to the slow development of its digestive system for efficient nutrient absorption and utilisation (Verreth *et al*., 1992; Ngugi *et al*., 2007). As such, *C. gariepinus* larvae

depend on the expensive and laborious live feed, *Artemia* nauplii. However, there are reports on the successful weaning of the fish larvae on dry feed with an initial meal of *Artemia* nauplii (Uys & Hecht, 1985) and mixed feeding (Chepkirui-Boit *et al*., 2011).

2.4.6 Diets and Their Nutritional Values for African Catfish Larvae

Clarias gariepinus Larvae, like any fish larvae, undergo anatomical and physiological transitions in different structures and systems, necessitating a full understanding of larvae nutrition, digestive capacity and metabolic systems (Gisbert *et al.*, 2008). To ensure smooth changes in larval physiology, the timing of first feeding and the type of starter feed should be of key interest to avoid starvation and water pollution.

Different starter feeds have been used, with live feeds being the most preferred. Live feeds like *Artemia* nauplii and Daphinia, are rich in nutritional content and provide the high protein necessary for all fish larvae growth and survival (Beg *et a*l., 2016).

Generally, the use of *Artemia* nauplii outperforms other starter feeds. The *Artemia* nauplii swims within the water column and in the process, it stimulates larvae feeding for digestion and utilisation. However, Syukri *et al.* (2022), reported a low survival rate in freshwater fish larvae fed on *Artemia nauplii* due to starvation. *Artemia* nauplii's success shortcomings in larviculture are attributed to its fast death rate in freshwater and decreased nutritional value along its

developmental stages (Léger *et al.*, 1986). Therefore, using *Artemia* nauplii in larval nutrition does not guarantee larval success.

Dry diets have been used in larviculture though, the results are inconsistent and with high larval mortalities. To improve fish larval performance, dry diets have been mixed (co-feeding) with live feed in equal proportions. This has enhanced growth and survival rates in fish larvae like the African catfish (Awaïss & Kestemont, 1998). The improved performance was attributed to the advantage of exogenous neuropeptides from the live feed that stimulate larvae digestive enzyme activities for effective diet utilisation and the synergy of combining proteins (Kolkoviski, 2001).

2.4.7 Nutritional Requirement for African Catfish Larvae

Fish require a nutritionally balanced diet for proper growth, functioning and development. The gross composition of feed ingredients, processing and finished feed storage determines feed quality (Munguti *et al.,* 2014). Fish nutritional requirements vary with the age and size of the fish. However, there is limited information on fish larvae nutrient requirements and formulation (Verreth *et al*., 1994; Kpogue *et al.,* 2013). This results in challenges in developing a larval feed with all the nutritional requirements for effective physiological and metabolic functions.

Generally, fish larvae require carbohydrates, lipids, proteins, vitamins and minerals in different proportions depending on species and feeding habits. Earlier

studies have reported requirement levels for *C. gariepinus* larvae of 50–59% crude protein, 9–11% lipids, 20–21% carbohydrate, and digestible energy of 3042 kcal/kg–3824 kcal/kg (Machiels & Henken, 1985; Uys & Hecht, 1985; Uys, 1989; Yilmaz & Mutlu, 2006; Vital *et al*., 2016). Except for methionine at 2.5% dietary protein dry matter (Uys & Hecht, 1985; Uys, 1989), information on amino acid and mineral requirements for *C. gariepinus* larvae is scanty.

High-quality protein ingredients are expensive and in short supply. Protein availability for larval utilisation depends on its structure, which may be altered. Therefore, there is a need to evaluate the performance of different protein sources. Protein level requirements need a good understanding for optimal growth and minimal environmental pollution (Kpogue *et al*., 2013). Protein quality and feed management determine the protein requirements, larval weight, feed allowable and protein energy sources based on economic and environmental conditions.

Amino acids (AA) are important energy sources and attractants in the diet. Amino acids also stimulate digestive hormones like gastrin, cholecystokinin, and insulin for enhanced digestion and assimilation (Hamre *et al.*, 2013). In *C. gariepinus* larvae, except for methionine, the quantities for all other amino acids (AA) included in the larval feed formulation are not specified (Jobling, 2012). This may lead to low retention efficiency and increased oxidation of AA due to their imbalances in dry diets (Pinto *et al.*, 2009). Carbohydrates are the most important and cheapest source of larval energy. *Clarias gariepinus* requires 21% of the carbohydrates and effectively utilises it using pancreatic amylase at start feeding

(Uys & Hecht, 1985). Dietary lipids are an important source of energy and essential fatty acids to aid in soluble fat vitamin absorption. *Clarias gariepinus* requires 9% of crude lipids for proper functioning (García-Ortega *et al*., 2000). However, high lipid levels in a formulation make feed processing difficult and increase fat deposits in fish. Minerals and vitamins are required at negligible levels and may be provided through premixes (Nabulime *et al*., 2015).

2.5 Challenges with Fishmeal

Fishmeal is a standard and nutrient-rich feed ingredient for terrestrial and aquatic animals, though it may be a quality organic fertiliser (Tacon & Metian, 2008; 2015; Jonnathulla *et al*., 2019). This is because of its good attributes, including high protein content, chemoattractant ability, palatability, and digestibility, with high proportions of glutamic acids, minerals, vitamins, and growth promoters for enhanced fish performance (Mugo-Bundi *et al.*, 2015; Naylor *et al*., 2021). The availability of fishmeal depends on the fast-growing pelagic species with short life cycles and a low market value (Bandara, 2018; Jonnathulla *et al*., 2019). According to Jonnathulla *et al*. (2019), 4.5 million metric tonnes of fishmeal, valued at USD 1,600 per metric tonne were produced in 2018 (Figure 2.4), with an expected increase of over 80% by 2025 (Kim *et al*., 2019). Global production of fishmeal is led by Peru with 20.73%, followed by 10.26% from Vietnam (Jannathulla *et al*., 2019). The price of fishmeal has steadily risen from USD 425/tonne in 2000 to USD 1596/tonne in 2018 (Jonnathulla *et al*., 2019).

However, fish feed represents insignificantly 4% of global animal feed produced, though it consumes 70–73% of the fishmeal produced per year (Shepherd &Jackson, 2013). Therefore, the continued use of fishmeal in aquaculture for enhanced fish production further threatens the sustainability of capture fisheries (Naylor *et al*., 2009; Alfiko *et al*., 2022). Overexploitation of forage fish species for feed industry use and their low recruitment as a response to climate change resulted in a decrease in fishmeal production by 26.5% between 2000 and 2018, though consumption increased steadily from 53% to 69% over the same period (Parolini *et al*., 2020; Naylor *et al*., 2021; Kim *et al*., 2022). This has pushed fishmeal prices upwards without an equal rise in fish and fish products.

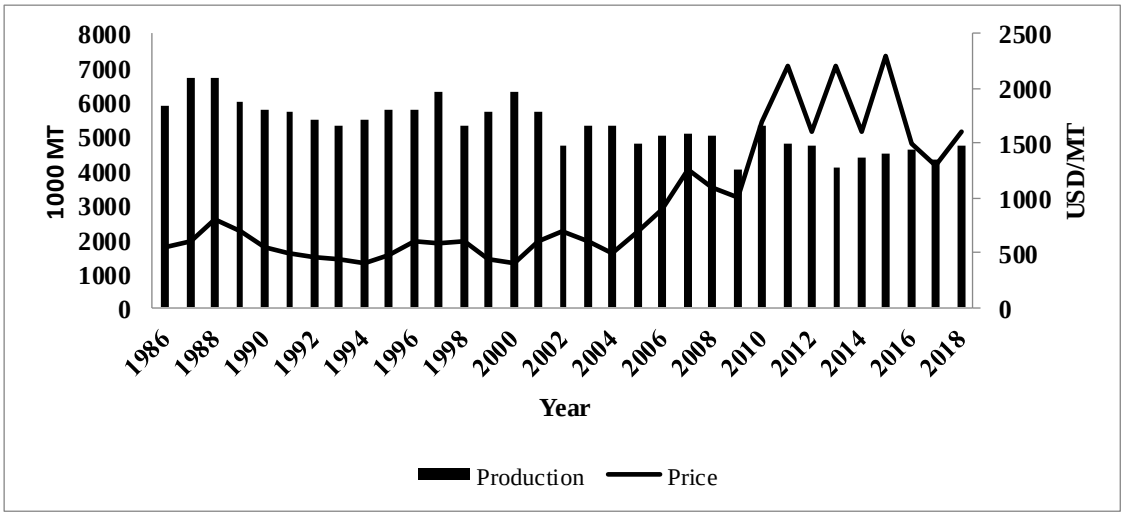

Figure 2.4: Fishmeal production and price between 1986/ 2018 (Adopted and modified, Jannathulla *et al*., 2019).

Intensive efforts to reduce the use of fishmeal in fish feed from 11-23% in 2014 to a projected low of 6% in 2025 are in place (Salin *et al*., 2018; Ghamkhar & Hick,

2020). Nevertheless, there remains a steady increase in the use of fish meal in aquafeed due to the expansion of global aquaculture production (Ghamkhar & Hick, 2020; Sandström *et al*., 2022). To cushion aquaculture from high fishmeal prices and production costs a search for alternative protein sources in aquafeed with similar or close nutritional values as fishmeal is inevitable (Naylor *et al*., 2021; Alfiko *et al*., 2022). Alternative proteins to fishmeal should be of consistent nutritional quality, palatable, highly digestible, and widely available at a low cost with a low quantity of anti-nutritional factors and crude fibre (Naylor *et al*., 2009; Bandara, 2018; Kim *et al*., 2019; Munguti *et al*., 2021).

2.6 Freshwater Shrimp (*Caridina nilotica*) as a Protein Source in Aquaculture

Caridina nilotica (Roux, 1833) is a forage-protandrous hermaphrodite freshwater shrimp that greatly contributes to the sustainability of the many Lakes and rivers fishery in Africa (De Necker *et al*., 2024). This species is a *Rastrineobola argentea* by-catch and is actively involved in the energy transfer within the food webs of lakes. It has been identified in the stomachs of Nile perch juveniles, catfish, and *Bogrus dogmac.* However, data on capture quantities of *C. nilotica* are limited despite the species' increased abundance lately (Musere, 2017). In Lake Victoria alone the species accounted for 23% (509424 tonnes) of the total Lake Victoria capture fisheries in 2022 (Basooma *et al*., 2023). The increase in *C. nilotica* biomass was attributed to the favourable development of anoxic

conditions and the decline of Haplochromine species in the lake. *Caridina nilotica* is used as bait in haplochromine hand-line fisheries and as a protein ingredient in livestock, poultry, and aquaculture diets (Radhakrishnan *et al*., 2014; Cashion *et al*., 2017; Han *et al*., 2018). Earlier studies have successfully used and recommended *C. nilotica* as a protein ingredient in grow-out tilapia farming (Liti *et al*., 2006) and African catfish larvae (Chepkirui-Boit *et al*., 2011). High palatability, crude protein of 66%., 10% crude lipid and 6% carbohydrate are the main characteristics supporting its use in feed industries (Mugo-Bundi *et al*., 2015). However, the sustainable exploitation of *C. nilotica* is doubtful. The quantities of this species may decline over time, especially with the intentional aquaculture intensification to fulfil the UN sustainable development goal of ensuring food and nutritional security for all. Further, climate change effects threaten the abundance and population structure of *C. nilotica* thus, its use as a protein ingredient in aquaculture may be limited. Alternative proteins to cushion against climate change are required for aquaculture sustainability (Han *et al*., 2018). Fishmeal has been partially or completely replaced by plants, animals, plant and animal by-products, algae, worms and genetically modified protein ingredients (Alfiko *et al*., 2022).

2.7 Conventional Protein Alternatives to Fishmeal

2.7.1 Plants and Their By-Products

Plant protein sources like soybean, corn, rice, sunflower, cottonseed, lupine, canola, corn gluten, barley, and wheat have been used with variable success because they are readily available and relatively cheaper compared to fishmeal (Naylor *et al*., 2021). However, plant proteins are on international markets and are therefore subject to price shocks, and their use in fish feed competes with other animal feed industries and human consumption. Further, the performance of plant proteins in aquaculture is suboptimal because of their low protein and limiting amino acids (lysine and methionine) content. Antinutritional factors (like lectin, trypsin inhibitors and other non-starch polysaccharides) also reduce feed intake, digestibility and utilisation in plant-based diets (Nagappan *et al*., 2021). Further, the use of plant proteins decreases water quality due to the presence of indigestible fibres like cellulose, hemicellulose and lignin (Hardy *et al*., 2009; Ytrestøy *et al*., 2015; Montoya-Camacho *et al*., 2019). Additionally, suboptimal performance is due to phytochemicals that disrupt fish health (Bandara, 2018; Daniel *et al*., 2018). Researchers have developed technologies to reduce antinutritional factors in plant proteins to increase their digestibility. The technologies include purification to produce soy protein concentrates, incorporating enzymes (cellulases, hemicellulases) in feeds and bacterial or yeast fermentation (Ray *et al*., 2022). However, soy protein concentrate's prohibitive

market price limits its utilisation in aquaculture. To reduce competition from human consumption, plant by-products such as Dried Distiller Grains (DDGs) from the alcohol industry have been used with some success. The DDGs' variable nutritional content depends on grain type, soluble quantity included during fermentation and production technology and methods (Khalila *et al*., 2018). Therefore, the use of DDGs in aquafeed remains doubtful as they are less digestible and have low palatability, a protein level of 29.4% and high crude fibre of 9.2% (Naylor *et al*., 2009; Ray *et al*., 2022).

2.7.2 Animal Alternatives and Their By-Products

Animal protein alternatives to fishmeal present challenges of increased microbial contamination, zoonotic infections, fatty acid rancidity, and prohibitive cultural beliefs in most developing countries (Ayadi *et al.,* 2012; Kobayashi *et al.,* 2015). Except for low lysine and methionine in feather meal, animal by-products like bonemeal, feather meal, blood meal, and poultry waste positively affect fish performance due to their balanced amino acid profiles, (Naylor *et al*., 2009). However, their digestibility varies according to processing methods and the presence of antimicrobial residues (Bandara, 2018). Inconsistency in protein quality due to different processing methods and hygiene concerns remains high in larval nutrition. Animal proteins destined for the feed industry should be handled with utmost care to minimise microbial loads that may threaten fish and human health.

2.7.3 Genetically Modified Proteins

The use of genetically modified (GM) feed ingredients in fish feeds and their application in aquaculture nutrition are at the infant stage (Osmond &Colombo, 2019). Genetically modified feed ingredients are important sources of protein, lecithin, long-chain poly-unsaturated (DHA and EPA) fatty acids., and macro- and micronutrients (Sissener *et al*., 2011). An analysis of feed formulated using genetically modified ingredients has been found to have a longer shelf life, lack anti-nutritional factors, have high levels of essential amino acids and PUFAs and, minimal inherent toxicants (Alfiko *et al*., 2022). Genetically modified proteins in aquafeed have faced many challenges including minimal consumer acceptance of GM-fed fish and strict regulation of GM use in many nations (Osmond & Colombo, 2019). Effects of GM protein alternatives on fish performances vary depending on the species and its age, GM inclusion level and type and, duration of feeding trials (Sessener *et al*., 2011). In aquaculture, GM utilisation is intended to reduce pressure on wild stocks and preserve natural aquatic ecosystems though this is largely debatable.

2.7.4 Non-conventional Protein Alternatives

Arable land and water resources have shrunk and sustaining the production of livestock and plant protein alternatives to fishmeal for feed industries is not guaranteed. Natural resource reduction has been linked to increased deforestation and non-sustainable use of natural resources (Liland *et al*., 2021). This presents an

opportunity to explore non-conventional protein alternatives like insects, microalgae, and worms in aquafeed. Many of these non-conventional protein alternatives have high production rates even on small, non-arable land with minimal water use to achieve the biomasses required to attain fish feed production.

2.7.4.1 Insects

Insects are traditionally important food sources for humans and other animals because of their high levels of amino acids, fatty acids and flavour. However, research on using insects like black soldier flies, house crickets, housefly maggots and silk meal worms in fish feed has surged. The ability of insects to convert organic wastes into a high biomass of quality proteins that are palatable, sustainable, and rich in valuable amino acids is an incentive for their use in the animal feed industry (Zlaugotne *et al*., 2022). Generally, insects contain high crude protein comparable to fishmeal, high saturated and mono-saturated fatty acids, and oleic acids with negligible levels of PUFAs but enhanced levels of lysine and methionine depending on the developmental stage of the insect and species (Bandara, 2018; Liland *et al*., 2021). However, nutrient utilisation and growth of fish fed on insect-based feed vary depending on the insect life stage, tolerance of fish species to insect meals, and insect protein quality and composition (Quang *et al*., 2022). There is limited use of insect meal in aquafeed due to low digestibility beyond 25–30% inclusion levels, variable quality and composition (Hua *et al*., 2019; Maulu *et al*., 2022; Quang *et al*., 2022)

2.7.4.2 Microalgae

Microalgae, including *Chlorella vulgaris, S. platensis and Synochococs,* are primary food sources for human and aquatic organisms. The microalgae have a balanced amino acid profile, high levels of lipids and high levels of polyunsaturated fatty acids., all of which are important for fish growth. Microalgae are also important in fish feed because of their enhanced metabolites (alkaloids, steroids, phenols, and saponins) and pigments (like carotene and C-phycocyanin) levels for improved fish health and muscle colouration, respectively.

Spirulina platensis is a species in the *Spirulina* genus, family Microcoleaceae, order Oscillatoriales and class Cyanophyceae (Figure 2.5). *Spirulina platensis* is a spiral-long thread-like blue-green algae found in many African and Asian lakes with high pH and salinity (Ragaza *et al.,* 2020). The species is produced in shallow ponds and bioreactors with low water and land use. The species has simple harvesting and preservation techniques that require no treatment (Velasquez *et al*., 2016; Rosenau *et al*., 2022). However, the production of *S. platensis* is expensive and translates to increased feed prices. This leaves algae production to a few monopolistic suppliers whose interest is profit and not product quality. Based on the culture medium, the species has a crude protein of 43–70% with balanced amino acids except for methionine and cysteine, 15–25% carbohydrate, 6-8% fat content, and a total fatty acid content of 81.2 mg/g dry weight. It is also an excellent source of vitamins, minerals, carotenoids, and

antioxidant pigment, making it a better protein compared to other vegetable protein sources and bioactive properties (Promya & Chitmanat, 2011; Radhakrisnan *et al*., 2014; Guedes *et al*., 2015; Abd-El Alim *et al*., 2018; Liestianty *et al.,* 2019; Ragaza *et al*., 2020; Yarnold *et al*., 2019). In addition to the high digestibility of 85–90%, possibly because of the fragile murein envelope (no cell wall) comparable to casein (Salmeán *et al*., 2015). This presents *S. platensis* as a complete nutritional ingredient in the aquaculture feed industry. Fish may be fed on live, off-shelf paste (flocculated or cryopreserved) pellets, green water or as a supplement protein in formulated diets (Velasquez *et al.*, 2016; Rosas *et al*., 2019; Nagappan *et al.*, 2021). Nevertheless, culture medium and temperature control protein quality and quantity in *S. platensis* (Ragaza *et al.*, 2020).

Spirulina platensis-fed larvae have reported increased growth, and high mortalities but enhanced overall health in Tilapia (Olvera-Novoa *et al*., 1998), Mekong catfish (Tongsiri *et al*., 2010), and *C. gariepinus* (Proyma & Chitmant, 2011). Further, improved nutrient utilisation and lipid metabolism in stinging catfish fed *S. platensis* have been reported. (Güroy *et al.,* 2012). This has been attributed to *S. platensiss'* adequate amino acid profile, attractants and crude protein content to stimulate feed ingestion in catfish larvae (Dabrowski, 1984). Earlier research reported the possibility of replacing fishmeal with 100% *Spirulina* though with reduced growth in *Carassius auratus (*Cao *et al*., 2018*),* *Labeo rohita* (Zhang *et al*., 2020), and *O. nilotica* (Velasquez *et al*., 2016).

Spirulina platensis is a promising alternative protein source for aquafeed; however, the accrued economic benefits from its use remain debatable. The greatest bottleneck to S. *platensis* uses in aquafeed is the inherent difficulties in maintaining its quality and purity besides the high cost of production (Sonani *et al*., 2015). Also, the presence of trypsin and amylase inhibitors and complex polysaccharides decreases the digestibility of *S. platensis* at high inclusion in a diet (Nagappan *et al.*, 2021; Liu *et al*., 2022). Nevertheless, this could be addressed by strict isolation and identification measures in the algae inoculation aliquot. However, *S. platensis* has a high protein production efficiency of 4-6 times a day, giving the highest biomass compared to any plant or animal protein alternative produced (Zhang *et al*., 2020). Using *S. platensis* as an alternative in the aquafeed industry is possible.

A  B

Figure 2.5: *Spirulina platensis* (A: Filament observed under a dissecting; microscope, B: powder) Source: Google.

2.7.4.3 Earthworm

For farmers, the most important family of epigeic worms are the Lumbricidae family. Epigeic worms feed on decomposing organic matter and live on the surface. The worms provide environmental cleaning services by reducing pollution through vermicomposting. They decompose organic matter produce organic mature and utilise the organic substrates to increase their biomass for animal feeds (Parolini *et al*., 2020). According to Sarkar, *et al*. (2006), common genera like *Allolophora, Aporrectodea, Bimastos, Dendrobaena, Eisenia, Tubifex,* and *Lumbricu*have a higher nutritional value than fishmeal or are similar to it. Although these genera have been used in aquaculture for the last 80 years, their use has recently increased due to the severe market prices for fishmeal and soybeans (FAO, 2023).

Worms offer naturally occurring alternatives to aquatic animal proteins, amino acids, fats, fatty acids, and vitamins (Herawati *et al*., 2016; Parolina *et al*., 2020). The performance of earthworms like *Eisenia fetida*, *Allolobophra longa*, *Tubifex tubifex* and *Lumbricus terrestis* in aquaculture feeds varies within and between species (Tacon, 1983; Nhi *et al*., 2000; Zakaria *et al*., 2013; Dedeke *et al*., 2013; Pucher *et al*., 2014; Vodounnou *et al*., 2016). the worms have a nutritional value similar to fishmeal however, most earthworms including blue worms (*Perionyx excavates*) and African crawlers (*Eudrilus eugeniae*) are not readily available for use in aquafeed. A state brought on by these worms' high-stress sensitivity and inability to adapt to different climatic conditions (Rehman *et al*., 2017).

Other earthworms that have been used in aquaculture include *Lumbricus terrestris* in trout feed, *Ailolobophora fetida* in carps and African catfish, *Dendrodrius subrubicusndus* in salmonids, and *Perionyx excavatus* in gubby and their nutritional value is not comparable to fishmeal. The aquafeed industry has also examined and utilised *Lumbricus rubellus*, *Libyodrilus violaceus* (Dedeke *et al*., 2013), *Hyperiodrillus africanus* (Dedeke *et al.,* 2010), and *E. fetida* (Tacon *et al*., 1983; Pucher *et al.*, 2014).

Eisenia fetida (Savigny, 1826) is a photophobic worm (Figure 2.6) whose body is covered with the indigestible nitrogenous polysaccharide chitin. Chitin (N-aceclyl-D-glucaosamine) holds the body in shape since it is a structural polymer with nutrient content proportional to worm age. Chitin is a source of insoluble fibre in fish feed that lacks quantifiable nutritional benefits in a diet. However, dietary fibre is important in regulating gut flow rate, intestinal growth and physiological state (Liu *et al*., 2022). The quantity of fibre in a diet has a proportionate effect on the intestinal chyme volume, viscosity and fermentation. Chitin is also hypothesised to decrease diet digestibility by impairing peptide breakdown by binding to digestive enzymes or limiting nutrient accessibility for digestion as chitin is embedded in lipids, proteins, and minerals (Abro *et al*., 2014). Enhanced chitin levels in a diet decrease nutrient absorption and digestibility by binding onto digestive enzymes and inhibiting their functionality. As a result, they hinder nutrient absorption and the availability of amines for growth (Liland *et al*., 2021). However, chitin derivatives (chitosan,

chitooligosaccharides, and glucosamines) are important in aquaculture (Abdel-Ghany & Salem, 2020; Eggink *et al*., 2022). Chitosan is an important derivative with valuable antimicrobial, immunostimulant and antioxidant activities. The chitosan combines with bacteria's DNA to inhibit mRNA and protein synthesis for bacteria's growth or combines with metal ions through chelation to enhance fish welfare (Belghit *et al*., 2018).

Ceolomic fluid maintains the worm's body shape and enables its borrowing activities. Additionally, the fluid boosts worm immunity and the immunity of animals treated with it due to its antibacterial activities (Bhuvaneswaran *et al*., 2019). The coelomic fluid proteins (lysenin, fetidin and eiseniapore) and anti-nutritional factors like lectin reduce diet palatability and present a challenge to worm use in aquafeed (Tacon, 1983; Kobayashi *et al*., 2001). However, these toxic proteins and antinutritional components are heat-labile and easy to minimise and their effects are minimised by heating, drying, or blanching in hot water. Therefore, processing and preservation methods greatly influence the quality of *E. fetida* meal in an aquafeed (Tacon, 1983; Pucher *et al.*, 2014).

Eisenia fetida is highly adaptable to different culture substrates with low mortalities due to its ability to balance energy expenditure (Musyoka *et al*., 2019). The species has a high feeding rate of 50% of its body size per day to enhance a high reproduction rate, hatching rates and growth rate (Vital *et al*., 2016).

Eisenia fetida transforms culture substrates into quality nutritional materials comparable to fishmeal (Tacon, 1983). Studies by Zakaria *et al*. (2013) reported

E. fetida meal as economically viable compared to soybean and chicken waste meals for *C. gariepinus* (Pucher *et al*., 2014). The species has a high protein content (50–70% dry weight), with balanced amino acids similar to fishmeal. It has fatty acids (5–10% of the dry weight), carbohydrates (2–21%), digestible energy (4000 kcal/kg), polyunsaturated fatty acids and vitamins and minerals (Jobling, 2016; Castro-Bedriñana *et al*., 2021). These nutritional qualities of worm meal depend on the species and feed substrate. However, worm use in aquaculture and livestock in some European countries is not accepted (Abdel-Azeem *et al.,* 2022). Nevertheless, studies on rats fed on *E. fetida* meal concluded that it is safe to use worms in human and animal feeds with no signs of morbidity, mortality or any health parameters reported (Tedesco *et al*., 2020). Additionally, *E. fetida* meal has low levels of chitin and high AA, fatty acids and, is highly palatable and digestible when grown on similar substrates (Parolina *et al*., 2020). However, in Musyoka *et al.* (2019), freeze-dried worm meals have been reported to be unpalatable to trout and guby due to lysis factors and the foul smell of the fluid. Therefore, the biological parameters of fish fed on *E. fetida* diets depend on the form in which it is presented to the fish.

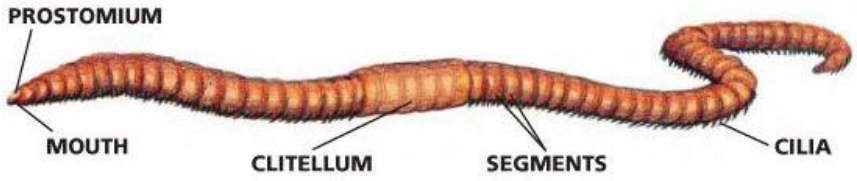

Figure 2.6: *Eisenia fetida* (Source: Google).

2.8 Effects of Alternative Protein Sources on *Clarias gariepinus* Larvae

2.8.1 Growth Performance

Protein efficiency ratio (PER), feed conversion ratio (FCR), and net protein utilisation (NPU) are indicators of feed quality, hatchery manager performance level and the effects of diet on the environment (De Silva & Anderson, 1994). A high FCR indicates feed waste and translates to increased production costs and environmental pollution. Therefore, fish larvae are fed at the correct feeding rate, frequency and timing to avoid feed wastage. However, there is limited information on the influence of formulated dry feed on the fish larvae's digestive enzyme activity.

Formulated diets have reported low fish larvae growth and survival, probably because of unstable feed particles resulting in increased loss of nutrients through leaching or nutrient imbalance during feed formulation (Hamre *et al*., 2013). Higher growth is reported on larvae fed live feed before weaning them to formulated dry animal or plant proteins (Stanković *et al*., 2013). According to Hamre *et al.* (2013), this might be because plant proteins have more dry matter and antinutritional factors that reduce their digestibility. Earlier studies on *C. gariepinus* larvae showed decreased growth with formulated dry feed (Dabrowski, 1984).

The use of *E. fetida* protein in fish feed improved the growth performance of *Tilapia mossambicus* fry (Chaves *et al.*, 2015), African catfish fingerlings, *C. gariepinus* (Zakaria *et al*., 2013; Dedeke *et al*., 2013; Djissou *et al*., 2016),

catfish, *Heterobranchus isopterus* (Nhi *et al*., 2000), *Clarias batrachus* (Bhuvaneshwaran *et al.,* 2019), *Parachanna obscura* (Vodounnou *et al.,* 2016), *Labeo rohita* fed pelleted *E. fétida* (Mohanta *et al*., 2016), eel, and carp (Knights, 1996).

2.8.2 Digestive Enzyme Activity

Digestive enzymes are protein molecules that catalyse the chemical breakdown of food into smaller, easily absorbable molecules. The categorisation of these digestive enzymes is determined by their respective sites of production: pancreatic enzymes (trypsin, chymotrypsin, amylase, and lipase) are synthesised by the exocrine pancreas; gastric enzymes (pepsin) are present in the stomach and intestinal mucosa enzymes (like isomaltase and trehalase) are situated in the intestine (Lazo *et al*., 2011). Fish larvae have different types and low amounts of digestive enzymes compared to adults of the same species (Kolkovski, 2001). These variations are among the difficulties fish larvae face when utilising compound feeds. It is, therefore, important to understand enzymatic activity in fish since it is integral in formulating age-specific diets, developing feeding procedures and assessing the nutritional status of fish larvae (Pradhan *et al*., 2014). Earlier studies have described the ontogeny of digestive enzymes in freshwater and marine fish larvae (Verreth *et al*., 1992; Zambonino-Infante 2008; Lazo *et al*., 2011; Pradhan *et al*., 2012). However, little is known about diet-induced digestive enzyme activity variations in the larvae of catfishes (Gisbert *et*

al., 2018). Thus, there is an urgent need for knowledge of digestive competence to aid in selecting appropriate feed ingredients that could lower the cost of aquafeed to replace fishmeal.

Pancreatic enzymes are the first to develop and their activity may be detected before the opening of the larvae's mouth (Rønnestad *et al*., 2013). Trypsin and chymotrypsin play a role in the breakdown of yolk protein (Gisbert *et al*., 2008). The activities of Trypsin are high in carnivorous and opportunistic feeders as compared to amylase in omnivores and herbivores (Uys & Hecht, 1987). Therefore, enzyme activity analysis reveals fish biology and trophic levels. Verreth *et al*. (1992), observed the presence of the pepsin enzyme activity to mark a functional stomach in *C. gariepinus* larvae. This observation was supported by a decrease in trypsin activities in his study. Catfish react to partial or full starvation by shrinking their exocrine and decreasing their enzyme activity like other teleosts (Zambonino-Infante *et al*., 2008). Carbohydrate diets stimulate high levels of α-amylase and suggest a high ability for fish larvae to digest carbohydrates (Giri *et al*., 2003). The problem with high carbohydrate diets is poor economic returns due to low larval growth and survival. Digestive enzyme activities depend on the type of feed and increase with the amount of feed given. For example, trypsin activities have been reported to increase while *C. gariepinus* is feeding but decreases after feeding in *C. gariepinus* (García-Ortega et al., 2000).

2.8.3 Stress Test

Stress is a general, non-specific response to any factor affecting an organism's normal physiological and metabolic functioning. Stress is responsible for disease outbreaks, reduced reproduction, increased mortality, poor food conversion ratio and decreased growth and productivity in an organism (Dhert *et al*., 1993; Crawley, 2013). During stress, the organism's energy is diverted from normal body processes by stress to stress-copying mechanisms until they are overstretching.

Stress test subjects test organisms like fish to a selected stressor to appraise the quality of a diet, the age at which fish larvae can stand different culture conditions with inherent stressors or document environmental changes. Stress tests are important in evaluating the nutritional requirements of fish species at various life stages and physiological conditions between treatments. Stress tests are a criterion for ranking different strains and hybrids of the other genera in aquaculture (Segner *et al*., 2012).

Pollution and natural decomposition of feed and organic matter in the aquatic environment enhance the occurrence and intensity of stressors like the osmotic shock in the culture system that compromises the welfare of fish (Ramos *et al*., 2021; Abdel-Latif *et al*., 2022). High ammonia levels in the environment enhance un-ionised ammonia uptake by fish gills and eventually increase larval epithelial permeability, convulsions, coma, and death (Randall & Tsui, 2002). Although stress effects are variable with age and are generally severe in starved fish larvae

or those fed on nutritionally imbalanced diets (Olivia *et al*., 2012). African catfish have a high tolerance to harsh environmental conditions, with adults enduring a toxicity range of 0.6-2 mg/l and 0.05–0.5 mg/l of unionised ammonia for larvae, depending on temperature and pH (Ngugi *et al*., 2007; Audu *et al*., 2017; Komugisha & Rajits, 2021). African catfish growth and production are compromised at 0.3 mg/l of unionised ammonia.

CHAPTER THREE

3.0 GENERAL MATERIALS AND METHODS

3.1 Larvae Production

Experimental larvae were produced in the aquaculture laboratory at the Department of Biology in the Faculty of Science and Technology, University of Nairobi (UoN). *Clarias gariepinus* brooders were sourced from the pre-evaluated Makindi fish farm, Murang'a County, Kenya. A total of three males (average weight 864 ± 5.4g) were initially selected based on the elongated red tip papilla behind the anus. Four females (average weight 1.04 ± 1.11kg) were also selected based on the swollen red genital opening and bloated belly. Each fish was placed in a separate 20-litre open plastic bucket filled up to one-third with culture water to avoid aggression and transported to the University of Nairobi, aquaculture laboratory. In the laboratory, the brooders were individually conditioned for two weeks in half-filled 100-litre plastic tanks at 27°C to acclimatise to the new culture conditions and recover from handling stress. During conditioning, the brooders were fed two times a day at 10 a.m. and 4 p.m. at 3% of body weight on commercial pellets (Skretting, Skretting Co. Israel) with 35% crude protein and a particle size of 5mm.

After two weeks of conditioning, one male brooder was randomly selected and anaesthetised in ice-cold water and sacrificed in preparation for the extraction of the pituitary gland. The extracted gland was crushed in a mortar and pestle before mixing the extract with 1.5 ml of normal saline water. A female (weighing

1.19kg) was randomly selected, anaesthetised in ice-cold water and injected with 1 ml/kg body weight of pituitary extract solution into its musculature lateral to the dorsal fin to induce ovulation. The female was injected at 9 p.m. and her eggs were stripped 12 hours later into a dry bowl. An hour before stripping the female, the second male was dissected, incisions made on the gonads, sperm squeezed and kept in a syringe with 1 ml of normal saline solution. A drop of sperm was placed on a petri dish with water to activate them before observing their motility under a microscope. The sperm moved actively and freely in the petri dish, which confirmed their viability. To fertilise the eggs, the stored sperm were spread over them in the bowl, slowly diluting them with water to promote sperm mobility while gently mixing with a finger for uniformity. Fertilised eggs were incubated on a Kakhaban mat in a 50-litre plastic basin holding 45 litres of water at 29°C (Figure 3.1). The incubator was aerated continuously with a single air stone in a flow-through system at a flow rate of 2.5 l/min to ensure a three-times-per-hour renewal rate. Hatching occurred 24 hours after incubation. The unhatched eggs, egg shells, and larvae were separated 24 hours after hatching.

Figure 3.1: *Clarias gariepinus* egg incubation set-up in the aquaculture laboratory, Department of Biology, University of Nairobi.

3.2 Feed Ingredients

Spirulina platensis and *E. fetida* were the protein alternatives that partially replaced *C. nilotica* in the *C. gariepinus* larval diets (Appendix 2). The *S. platensis* was sourced from Nasio Trust in Kakamega County, Kenya, where it had been cultured in an Orthophosphoric, Tetraphosphate, magnesium phosphate, EDTA salt, ferrous sulphate, and potassium salt medium for increased macronutrients (C, N, P, K, S, Mg, Ca, and Fe) and harvested every five days. After harvesting S. *platensis* was dried, ground into powder and stored until there

was enough for the fort his study. The powder was packed in air-tight zip-lock plastic bags and stored in closed carton boxes to maintain quality and consistency. *Eisenia fetida* had been cultured in a 90 cm x 60 cm x 30 cm woody structure with pre-composted goat manure mixed with potato peel substrate sourced from a reputable integrated fish farmer in Nyandarua County, Kenya. For quality consistency, *E. fetida* was maintained on the same moist substrate throughout the culture period at a temperature of 27°C and hand-picked within a uniform interval of 14 days. The worms were hand-picked from their substrate and transported to the UoN, Department of Biology, in open plastic buckets. The *E. fetida* were left in the buckets for 12 hours to allow recovery from handling stress and allow them time to empty their guts to reduce the foul smell. The unpleasant odour and off-flavours negatively affect the attractiveness and palatability of formulated diets., However, off-flavours are reduced by thoroughly cleaning and rinsing in clean water before being blanched in hot with a pinch of salt. Afterwards, *E. fetida* was batch-weighed and dried at 60°C in the oven to reduce heat-labile toxic proteins like lysenin and anti-nutritional factors for enhanced digestibility. The dry worms were stored at 4°C until feed formulation. The other feed ingredients sourced from reputable distributors were corn flour, wheat pollard (middles), dicalcium phosphate, ascorbic acid, methionine, lysine and vitamin-mineral premix.

The commercial feed of pellet size 100–500 µm (Gemma micro, Skretting Co., Italy) was obtained from a local feed importer, while *C. nilotica* was purchased from fish traders at the Kawangware open market in Nairobi County, Kenya. The

proportions of each ingredient in the formulation were based on their chemical composition summarised in Table 3.1 and analysed as described in Sub-section 3.4.

Table 3.1: Chemical composition of feed ingredients used in formulating *C. gariepinus* larval diets.

Ingredients	Moisture	% Dry matter (DM)				Minerals		
		Ash	CP	CF	EE	NFE	Ca2+	P3+
Wheat Pollard	5.00	3.97	16.1	7.30	5.93	66.7	0.26	1.44
DCP	1.00	100	-	-	-	-	20.1	18.1
Corn	6.10	1.331	7.14	2.93	5.50	83.1	0.04	0.33
C. nilotica	7.30	.0	63.9	1.47	3.93	18.7	3.49	1.46
**E. fetida*	2.50	6.71	64.3	1.29	11.0	16.7	1.25	0.78
S. platensis	9.90	13.8	61.8	1.43	2.67	20.3	1.87	0.81

**E. fetida= dried worm, +moisture on as is basis, CP =Crude Protein, CF= Crude Fibre, EE= Ether Extract (lipid), Ca^{2+} =calcium, P^{3+}=Phosphorous, NFE= Nitrogen-Free Extract, DCP =Dicalcium Phosphate, DM = Dry Matter.*

3.3 Diet Formulation

Dry ingredients were individually ground using a KD-318 kitchen blender (Ningbo Ambel International Co. Ltd., Guangdong, China) and sieved through a 125µm mesh to ease the handling. *Caridina nilotica* was partially replaced by either *S. platensis* or *E. fetida* to give three levels (25%, 50%, and 75%) of alternative protein inclusions to formulate approximately isonitrogenous (55% crude protein) and isocaloric (3800 kcal/kg digestible energy) diets with 20–21% nitrogen-free extract. These formulations for *C. gariepinus* larval diets (Table 3.2) were based on the reference diet described by Uys (1989). Digestible energies were calculated based on ingredient Digestible Energy (DE) book values in the

NRC (1993). Throughout the formulation process, the ingredient's nutritional content and price were considered in the nutritional requirements of *C. gariepinus* larvae at the lowest possible cost (least cost inclusion). Subsequently, the ingredients' proportions for each formulation were individually weighed before thoroughly mixing from the smallest proportions to the largest in a dry bucket using an egg beater hand whisk. This resulted in seven different homogeneous mixtures of seven diets: T_1 = 25%*S. platensis* + 75%*C. nilotica*, T_2 = 50%*S. platensis* + 50%*C. nilotica*, T_3 = 75%*S. platensis* + 25%*C. nilotica*, T_4=25%*E. fetida* + 75%*C. nilotica*, T_5 = 50%*E. fetida* +50%*C. nilotica*, T_6 = 75%*E. fetida* + 25%C. *nilotica* and T_7 = 100%*C. nilotica* (control). Equal proportions of vitamin-mineral premix, sodium chloride, ascorbic acid, dicalcium phosphate, methionine, and lysine were supplemented. Ten per cent of hot distilled water was added to each formulation blend to gelatinise carbohydrates before dough kneading. In the final stages of kneading the dough, varying levels of corn oil were added to raise crude lipid levels closer to the recommended range of 9% in *C. gariepinus* larvae diets (Uys, 1985). Diets were formulated once for consistency and fed to *C. gariepinus* larvae within the study period as illustrated in Appendix 3. The dough was pelletised using a manual pelletiser to make 200µm, 250µm, and 500µm pellet sizes for weeks one, two -three, and four to eight weeks respectively, assuming an increase in the mouth gape with age. Feed pellets were dried in the oven (Gallenhamp Catnoin Inc. 700110M, Gallenkamp and Company, Chester, United Kingdom) at 60°C to a constant dry weight and stored in plastic bags

tightly locked inside airtight plastic containers at room temperature. Previous research has reported limited growth with 100% of *E. fetida* or *S. platensis* therefore, 100% of *E. fetida* or *S. platensis* diet was not formulated in this study. The formulated diets were packed in zip-lock polythene bags in Appendix 4. A commercial (T_0) diet in Figure 3.2, called Gemma micro (Skretting Co. Netherlands), of particle size 100–200 µm was fed to 48–three-week-old larvae, while 300µm (size 300–500 µm) was fed to four–eight-week-old larvae. Despite the different pellet sizes of the commercial diet, its chemical composition (59% crude protein, 14% crude lipids, 14% ash content, 0.2% crude fibre, phosphorus of 1.3%, and calcium of 1.5%) was the same as per the labelling on the packet. The Gemma micro diet is designed for Zebrafish larvae and is mostly used for *C. gariepinus* larvae globally. Gemma micro was used in the current study because of its availability in the Kenyan market and high protein content for catfish's larval stage. The commercial diet had 100% fishmeal according to the manufacturer's descriptions. The diet was purchased from a reputable fish feed supplier in Muranga County, Kenya. The performance of larvae fed on formulated diets was compared with that of commercial diet (gemma micro) used in established hatcheries in Kenya

Table 3.2: Ingredient proportions (%) in *C. gariepinus* larvae formulated diets using *S. platensis* and *E. fetida* as partial protein alternatives (as is basis).

	Diets						
Ingredients	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Corn	3.62	2.21	0.12	9.38	9.94	10.1	4.54
Wheat pollard	7.03	8.17	9.13	2.49	1.50	1.06	5.12
Corn oil	3.29	3.56	4.69	2.07	2.50	2.78	4.28
C. nilotica	63.0	42.0	21.0	63.0	42.0	21.0	84.0
E. fetida	0.00	0.00	0.00	21.0	42.0	63.0	0.00
S. platensis	21.0	42.0	63.0	0.00	0.00	0.00	0.00
DCP	0.50	0.50	0.5	0.50	0.50	0.50	0.50
Lysine	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vit- mineral premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt (NaCl)	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Ascorbic acid	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Totals (%)	100	100	100	100	100	100	100
Cost (US$/kg)	13.8	26.0	38.3	3.27	5.02	6.78	1.52

DCP= dicalcium phosphate, T_1 = 25%S. platensis +75%C. nilotica, T_2 = 50%S. platensis +50%C. nilotica, T_3 = 75%S. platensis + 25%C. nilotica, T_4=25%E. fetida +75%C. nilotica, T_5 = 50%E. fetida +50%C. nilotica, T_6 = 75%E. fetida +25%C. nilotica, T_7 = 100%C. nilotica, Vit- mineral premix provided the following per kg of feed described on the packageas (vitamin A, 5000 IU; vitamin D3, 1000 IU; vitamin E, 150 IU; Vitamin B1, 10mg; Niacin, 100 mg; Pantothenic acid, 27.5mg; Biotin, 0.5mg; Folic acid, 3mg; Choline, 500 mg; vitamin C, 300 mg; manganese, 75 mg; iron, 20 mg; zinc, 22.5 mg; Copper, 2.5 mg; Cobalt, 0.1 mg; iodine, 0.7 mg; selenium, 0.06 mg). Note: Cost price of T_0 = US$/kg 59

A B

Figure 3.2: Commercial diet Gemma micro) from **Skretting Fish Feed Company, Netherlands a) Package and b) the powder.**

3.4 Chemical Analysis of Feed Ingredients and Formulated Diets

The nutritional value of dietary ingredients and all experimental (formulated and commercial) diet samples were determined at the University of Nairobi Animal Nutrition Laboratory using standard operating procedures developed by the Association of Analytical Chemists (1998), and their nutritional values are summarised in Tables 3.1 and 3.3, respectively. The expensive and time-consuming Kjeldahl method was used to determine the nitrogen (N) content in the samples and it was converted to crude protein using a Jones Factor of 6.25. The Kjeldahl method was used to ease comparison between researches as it is commonly used and reproducible between laboratories as opposed to the Biuret method which measures actual peptide ponds. In a micro-Kjeldahl (Gerhaelt Bonn 001460, Ultratronic GmbH, Germany) digester, a gramme of ingredient or

formulated diet was digested in sulfuric acid using a potassium sulphate catalyst to convert organic nitrogen in the test samples into inorganic ammonium sulphate. The addition of sodium hydroxide to the ammonium sulphate solution liberated ammonia. This ammonia was distilled in boric acid to a green ammonium borate solution for titration against a standard 0.1 N hydrochloric acid. Hydrochloric acid volume consumed during the titration was used to calculate the amount of nitrogen (N) liberated and crude protein was estimated using (N x 6.25) the Jones Factor (Equation 3.1). This factor was chosen because it is assumed that all proteins in the samples contained 16% nitrogen, and it is often used in quantifying crude protein. Soxhlet extraction method was used to determine ether extract (EE) or lipid with ether as an extraction solvent and quantified using Equation 3.2. The differentiation method determined the percentage (%) of nitrogen-free extracts (NFE) on a dry matter basis in Equation 3.3. The ash content of the samples was determined by incineration for eight hours at 550 °C in a Muffle furnace (Wc Heraeus, Hanau 170, 220v, Milan, Italy) to ensure minimal breakdown of calcium carbonate. Weende method was used to determine crude fibre in the feed ingredients and formulated feeds. Ten grams of feed or feed ingredient was digested by boiling it in sulphuric acid solution for 30 minutes, rinsed before reboiling it in sodium hydroxide solution and the residue was weighed. The difference between preweighed boiling and post-boiling weight represented the crude fibre content. An atomic absorption spectrophotometer (model 210, SpetraAA, 220FS FL, (fast sequential) Spectrometer, Varian, California, USA)

was used to measure calcium and phosphorus content in the samples at 420 nm and 220 nm, respectively, at the UoN, Department of Soil Science

$$\text{Crude protein} = (\%N) = \frac{Titre\ volume\ of\ 0.1N\ HCl\ x0.1\ x\ 0.014}{weight\ of\ the\ sample} * 100) * 6.25 \quad \text{Equation 3.1}$$

$$\text{Total lipid} = \frac{(weight\ of\ flask\ with\ lipid - weight\ of\ the\ flask)}{\text{Weight of the feed ingredient or formulated diet}} * 100 \quad \text{Equation 3.2}$$

%NFE= 100-(Ash+%CP+%EE+%CF) Equation3.3

(CP=Crude Protein, EE=Ether Extract, CF=Crude fiber)

Table 3.3: Chemical composition (% dry matter) of formulated and commercial diets fed *Clarias gariepinus* larvae in the hatchery.

Chemical components	**Diets**							
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_0**
Dry matter	88.6	91.79	94.59	94.3	94.1	94.97	93	97.3
CP	54.8	54.6	54.2	54.9	55.0	54.8	54.8	60.5
EE	8.82	8.00	7.57	8.97	8.96	10.4	8.81	10.1
CF	1.68	1.80	2.03	1.53	1.44	1.30	1.69	0.89
Ash	13.7	14.6	15.2	13.2	14.2	13.5	14.2	8.31
NFE	21.0	21.0	21.0	21.4	20.4	20.0	20.5	20.2
Calcium	2.71	2.36	2.02	2.47	1.89	1.31	3.03	1.41
Phosphorus	1.29	1.17	1.04	1.24	1.09	0.92	1.42	2.16
DEkCal/kg)	3804	3802	3801	3804	3800	3802	3802	-

CP= Crude Protein, EE=Ether Extract/lipids, CF=crdude fibre, NFE= Nitrogen Free Extract, DE=Digestible Energy (calculated), T_1 = 25%S. platensis +75%C. nilotica, T_2 = 50%S. platensis +50%C. nilotica, T_3 = 75%S. platensis + 25%C. nilotica, T_4 = 25%E. fetida + 75%C. nilotica, T_5 = 50%E. fetida +50%C. nilotica, T_6 = 75%E. fetida +25%C. nilotica, control (T_7) = 100%C. nilotica, T_0=Gemma micro.

3.5 Amino Acid in the Formulated Diets

The amino acid content of the formulated diets was investigated at Evonik Nutrition GMbH, Wolfgang, Germany (Table 3.4.). Performic acid oxidation was done before hydrolysis to oxidise methionine and Cysteine to methionine sulphate and Cysteic acid, according to the Association of Analytical Chemists (1998). Performic acid was neutralised as described by Greenfield & Southgate (2003). Protein was hydrolysed using 6M HCl to release amino acids. Individual amino acids were quantified using high-performance liquid chromatography (HPLC). Tryptophan was degraded during hydrolysis and tyrosine was destroyed by oxidation; these two amino acids were not quantified.

Table 3.4: Amino acid (g/100g diet) composition (as is basis) of different formulated diets used for feeding *C. gariepinus* larvae.

	Diets							
Amino acid	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_0**
Methionine	0.91	0.73	0.67	0.84	1.12	1.3	0.92	1.33
Cysteine	0.40	0.43	0.50	0.47	0.38	0.4	0.42	0.5
Met+ Cysteine	1.61	1.46	1.52	1.53	1.57	1.73	1.47	2.09
Lysine	2.42	2.49	2.61	2.14	2.88	2.97	2.25	3.29
Threonine	1.79	1.89	2.01	1.79	1.89	2.12	1.71	2.19
Arginine	2.35	2.37	2.22	2.04	2.46	1.17	2.23	3.20
Isoleucine	2.22	2.33	2.49	2.16	2.43	2.50	2.01	2.15
Leucine	3.80	3.9	4.15	3.55	3.93	4.10	3.39	3.82
Valine	3.12	3.33	3.43	2.82	2.69	2.46	2.67	2.58
Histidine	0.94	1.02	1.31	0.93	0.59	0.6	0.83	0.96
Phenylalanine	2.43	2.57	2.67	2.20	1.91	1.89	2.12	2.05
Glycine	2.65	3.10	2.95	2.45	2.55	2.52	2.71	3.54
Serine	1.72	1.65	1.63	1.58	1.75	1.98	1.57	2.13
Proline	2.67	2.97	3.04	2.31	1.79	1.80	2.28	2.76
Alanine	3.2	3.23	3.46	3.22	3.01	3.10	3.17	3.14
Aspartic acid	3.90	3.82	3.94	3.71	4.43	4.51	3.69	4.48
Glutamic acid	6.20	6.07	6.05	5.64	6.4	6.31	5.83	6.68

T_1 = 25%S. platensis +75%C. nilotica, T_2 = 50%S. platensis +50%C. nilotica, T_3 = 75%S. platensis + 25%C. nilotica, T_4 = 25%E. fetida +75%C. nilotica, T_5 = 50%E. fetida +50%C. nilotica, T_6 = 75%E. fetida +25%C. nilotica, control (T_7) = 100%C. nilotica, T_0 = Commercial diet (Gemma micro).

3.6 Performance of Larvae Fed on Formulated and Commercial Diets

Clarias gariepinus larvae produced in section 3.1 were used in the laboratory and grow-out experiments after approval by the Animal Use and Ethics Committee (Appendix 1). The larvae were fed on the formulated and gemma micro diets (section 3.3) for eight weeks to evaluate the effects of these diets on larval growth and nutrient utilisation (Chapter Four). During the feeding trial, larvae were sampled at first feeding, 7-, 14-, 21- and 28 days post-hatching (DPH) and assayed for selected digestive enzyme activity. The estimated enzyme activity indicated the effects of the diets on the digestive capacity of the fed larvae compared to the activity of the same enzymes at hatching in Chapter Five. The same fed larvae were also sampled and exposed to ammonia at weeks four and six of the feeding trial. The primary goal was to assess the quality of larvae fed on formulated diets and their ability to withstand ammonia stress under various culture conditions upon stocking for grow-out (Chapter Six). The ammonia stress test was founded on the assumption that quality diets produced large and optimally fed larvae with the highest survival and yield in the grow-out. After eight weeks of the feeding trial, larvae were transferred to grow-out tanks as fingerlings for stocking to evaluate the effects of hatchery nutrition on the growout performance in chapter seven.

3.7 Statistical Analysis

Before statistical analysis, all data sets, except for partial economic analysis, were checked for normality and homogeneity of variance using Kolmogorov-Smirnov's (Zar, 1999) and Levene's (Levene, 1961) respectively. Data were considered normal and homogeneous at $p > 0.05$. Except for stress tolerance, which was analysed by the GenStat 13.3 edition software program, all other statistical analyses were determined using IBM SPSS Version 20. The effects of dietary treatments on water quality, growth, nutrient utilisation survival, stress tolerance and digestive enzymes were determined using a one-way analysis of variance (ANOVA). A t-test was used to compare means between time points in stress tolerance and diet in the grow-out. When significant differences were discerned, treatment means were compared using Tukey's Honest Significant Difference post hoc test (Tukey, 1977) and considered different at $p<0.05$. The standard Error was used to indicate variability sampling distribution. All estimated values were presented as mean ± Standard Error (SE) or ± Standard Error Mean (SEM).

CHAPTER FOUR

4.0 GROWTH, NUTRIENT UTILISATION AND SURVIVAL OF AFRICAN CATFISH LARVAE FED ON SPIRULINA AND REDWORM IN FORMULATED DIETS

4.1 Introduction

African catfish (*C. gariepinus*) aquaculture production is expected to increase by 65% globally and 54% in Sub-Saharan Africa by 2030 (Troell *et al*., 2019). To realise and maintain this Upward aquaculture production path requires an optimal supply of farm inputs including quality feed and seed. Optimal growth, survival and welfare of fish larvae depend on many factors, like culture conditions, species genetics, fish physiological status, dietary protein composition and quality., The nutritional needs of fish change depending on the species and stage of development. The nutritional requirements and feed formulations for different fish species exist, though relatively limited for the larval stage (Roo *et al*., 2023). This challenge is associated with difficulties working with small-sized larvae, inducing fish larvae to ingest dry diets, and the high larval sensitivity to handling.

These limitations notwithstanding, there is a consensus amongst fish nutritionists that the larval stage requires quality dry feed to replace live feed as a starter diet in the hatchery. The dry feed should be cost-effective, easily digestible, and with minimal negative impacts on the environment. Researchers have fed *C. gariepinus* larvae on dry-formulated diets that contain aquatic animal proteins.

However, the results have been inconsistent, with low survival rates ranging from 20 - 60% (Verreth *et al.,* 1992; Kpogue *et al*., 2013; El-Sebaie *et al*., 2014; Nyonje *et al.,* 2018). Further exploitation of aquatic animal proteins for fish feed competes with other livestock feed industries. Intensive use of aquatic animal proteins in aquaculture disrupts food chains that support wild fisheries. As a result, there has been a consistent shortage of high-quality and quantity *C. gariepinus* fingerlings to meet aquaculture intensification demand for stocking (Coffey *et al*., 2016; Han *et al*., 2018; Obiero *et al*., 2019). Continuous search for sustainable, economical, and readily available protein sources that ensure feed nutritional quality and aquatic resource integrity is of urgency.

Unfortunately, plant protein alternatives to aquatic animal proteins like fishmeal and *C. nilotica* are limited due to the presence of anti-nutritional factors that reduce diet digestibility, non-soluble carbohydrates, and fibre, which also increase aquatic pollution (Ytrestøyl *et al.,* 2015; Montoya-Camacho *et al*., 2019). However, animal protein alternatives to fishmeal have challenges like higher levels of microbial contamination, zoonotic infections, and fatty acid rancidity (Kobayashi *et al*., 2015). Inconsistent results in fish larvae growth fed on aquatic animal protein alternatives have further made research on feasible protein alternatives in the fish feed industry a must. As such, non-conventional protein sources of *Spirulina* (*S. platensis*, Geitler, 1925) and earthworms (*Eisenia fetida*, Savigny, 1826) have gained interest lately through innovation, science, and technology.

Spirulina platensis and *E. fetida* have the potential to replace fishmeal in aquaculture as their nutritional composition is comparable to that of yeast, milk powder, and fishmeal (Jobling, 2012; Abd-El Alim *et al*., 2018). They have a protein content of 43–70% depending on the methods of culture and analysis, 4–10% lipids with total fatty acids, 15–25% carbohydrates, and high levels of vitamins and minerals (Radhakrisnan *et al*., 2014; Guedes *et al*., 2015; Liestianty *et al*., 2019; Antonova *et al*., 2021; Gunya & Masika, 2021).

Both *S. platensis* and *E. fetida* are highly digestible. The *S. platensis* is a superior plant protein with high levels of carotenoids, antioxidants, and a fragile murein envelope covering instead of the cell wall. *Spirulina platensis* species exhibits a significant total fatty acid content of 81.2 mg/g of dry matter. Additionally, the species possesses a gross energy value of 4777 kcal/kg and shows no detectable presence of heavy metal contamination (Liesstianty *et al*., 2019; Tibbetts *et al*., 2023). *Eisenia fetida* is a good feed ingredient for the aquafeed industry because it has a lot of methionine and lysine, 6.6–10.5 mg/g protein of total fatty acid, and enough polyunsaturated fatty acids, with a gross energy value of 4000 kcal/kg (Salmean *et al*., 2015; Castro-Bedriñana *et al*., 2020; and Antonova *et al*., 2021). Therefore, *E. fetida* is an efficient feed ingredient in fish nutrition, though its use in larval feed is limited.

In earlier studies, replacing fishmeal with *S. platensis* or *E. fetida* had variable effects on the growth and performance of fish larvae. *Spirulina platensis* enhanced growth, feed utilisation, disease resistance and stress tolerance

in *Pangasianodon gigas* (Tongsiri *et al.*, 2010), *C. gariepinus fry* (Promya & Chitmanat, 2011), and *O. nilotica* (Velasquez *et al*., 2016). On the other hand, variable growth and nutrient utilisation have been reported in *Heterobranchus isopterus* (Nhi *et al*., 2000), *C. gariepinus* (Dedeke *et al*., 2013; Djissou *et al*., 2016), *Labeo rohita* (Mohanta *et al*., 2016), *Parachanna obsura* (Vadounnou *et al*., 2016), and *C. batrachus* (Bhuvaneshwaran *et al*., 2019) fed on *E. fetida*. However, their performance depends on the age, form in which they are fed, processing, and inclusion levels (Sonani *et al*., 2015; Mohanta *et al.*, 2016). Though *S. platensis* and *E. fetida* are easier to digest, there is still not enough research on their use in starter diets that do not include live feed at the beginning of the feeding trials in larval nutrition. This objective intends to contribute to the pool of science guiding the formulations of high-quality and cost-effective *C. gariepinus* starter feed for enhanced aquaculture production and food security for the global population. The specific objectives towards the contribution were:

i. To determine the growth performance of *Claris gariepinus* larvae fed on *Spirulina platensis* and *Eisenia fetida* as partial protein alternatives to *Caridina nilotica* in formulated diets.
ii. To determine the nutrient utilisation of *Claris gariepinus* larvae fed on *Spirulina platensis* and *Eisenia fetida* as partial protein alternatives to *Caridina nilotica* in formulated diets.

iii. To evaluate the survival of *Claris gariepinus* larvae fed on *Spirulina platensis* and *Eisenia fetida* as partial protein alternatives to *Caridina nilotica* in formulated diets.

iv. To estimate a partial cost analysis of *Spirulina platensis* and *Eisenia fetida* as partial protein alternatives to *Carida nilotica* in *Clarias gariepinus* larvae-formulated diets.

4.2 Materials and Methods

4.2.1 Experimental Design for *Clarias gariepinus* Larvae Feeding Trial

Feeding trials were for eight weeks using 24 glass aquaria of 50-litre capacity, filled with 45 litres of standing tap water at 28°C (Figure 4.1). Forty-eight hours post-hatching, and allowing a 10% mortality rate, 25 larvae/litre (0.002 g/larvae) were randomly distributed (complete random design) into each glass aquarium. Larvae in the aquarium were fed one of the experimental diets randomly assigned in triplicate, 10 hours after stocking. This was to allow the larvae time to recover from handling stress. On the first day of feeding, larvae were fed on a 100% commercial diet (Gemma micro, Skretting Fish Feed Co., Netherlands) before consecutively decreasing by 25%, with the respective formulated diet increasing in equal proportions. From four days post-feeding, larvae were fed on 100% of the assigned test diet until the end of eight weeks. The diet particle size progressively increased from 200µm at first feeding to 500µm in the fourth week, assuming an increase in the mouth gape. Subsequently, *C. gariepinus* larvae were

fed on the 500µm particle size of the formulated diets until the end of the eight-week feeding. Larvae were hand-fed at 20% wet body weight (instead of satiation for optimal performance) for the first two weeks to avoid larvae starvation (Verreth *et al*., 1992) and to enable quantifying of the feed consumed for nutrient utilisation processing. For economic reasons, this feeding rate was reduced to 10% of body weight until the end of eight weeks. A feeding frequency of five times a day was maintained throughout the feeding trial, with a weekly feeding rate adjustment based on the sample wet body weight. Each aquarium was aerated using a single airstone while its water temperature was maintained at 28℃ using a thermostat throughout the experimental period. All aquaria were cleaned using cotton wool before siphoning out particles like excess feed and faeces that had settled at the bottom with approximately 50% of the bottom water every morning before feeding. To maintain water quality, an equal volume of fresh tap water was added to replace that siphoned water. Excess light into the room was controlled by closing all windows to establish and maintain a 12-hour light and 12-hour dark photoperiod regime during the study period.

Figure 4.1: Experimental set-up for the eight dietary treatments in *C. gariepinus* feeding trials at the Department of Biology, University of Nairobi.

4.2.2 Data Collection

Every morning before feeding, dissolved oxygen, temperature, and pH were measured in each aquarium using a multi-parameter water quality measuring meter (Multi WTW 3630 IDS, Xylem Analytics, Brandenburg, Germany). A hundred millilitre of sample water from each aquarium was placed in plastic bottles and kept at -18°C until total NH4-N analysis using a Hanna Ammonia Medium Range meter (ISM HI96715C, Hanna Instruments, Waitakere, New Zealand). The total ammonia nitrogen was converted to un-ionised ammonia by a factor of 1.216 as described in the manufacturer's instructions.

Thirty C. *gariepinus* larvae were weighed at stocking (initial weight), weekly for the first month and every two weeks until the end of the experiment., fish larvae were sub-sampled using a scoop net from each aquarium (with replacement) and weighed individually to near 0.001g in an ASB-220-C2 analytical weighing balance (Shambhavi Impex, Mumbai, India). The total body length was measured on a wet measuring board to the nearest 0.1 cm at stocking (initial body length), weekly for the first month and every fortnight.

To determine the Specific Growth Rate (SGR), 30 larvae per replica (90 per treatment) were removed (without replacement) at stocking and, at the end of the experiment, weighed and oven-dried using the model TS 8000S, Termaks, Bergen, Norway, at 104°C for four hours to a constant weight. Total larvae count in each aquarium was recorded at the end of the 1^{st}, 4^{th}, and 8^{th} weeks of the experimental period, and live individuals were presented as % survival compared to stocking densities. Larvae removed for SGR determination were added to the surviving larval numbers. Equations 4.1 and 4.2 were utilised to compute the growth of *C. gariepinus* larvae, specifically their total length and weight gain. The daily growth rate was calculated using formula 4.3 SGR used 4.4 and survival was determined using formula 4.5.

Wet body weight gain (g) = final weight (FW) – initial weight (IW) 4.1

Length gain (cm) = final length (FL) – initial length (IL) 4.2

$$\text{Daily growth rate (\%g/day)} = 100 * \frac{\text{final weight(g) – initial weight(g)}}{number\ of\ experimental\ days} \quad 4.3$$

$$\text{Specific growth rate(\%/day)} = 100 * \frac{\text{Ln final dry weight (g) – Ln initial dry weight (g)}}{number\ of\ experimental\ days} \quad 4.4$$

$$\text{\% Survival} = 100 * \frac{total\ larvae\ count}{total\ stocked\ larvae} \quad 4.5$$

Where L_n = Natural Logarithm

The Feed Conversion Ratio (FCR) was estimated as feed consumption (g) per wet weight gain (g), and the Protein Efficiency Ratio (PER) was assessed by (% diet protein*weight of diet consumed)/ total protein consumed (g).

4.3 Partial Cost Analysis of the Formulated Diets

Aquaculture is an economic enterprise therefore; a partial economic analysis was conducted to assess the cost-effectiveness of *S. platensis* and *E. fetida* diets in *C. gariepinus* larvae diets. Transportation costs and the price of ingredients used in the formulation were the only economic criteria in estimating the performance of formulated starter diets. All the other support operations and processing costs were assumed to be constant in all diet formulations. All estimations were based on the Kenyan market retail prices in USD (1 USD = Ksh. 100 by the time of purchase). The economic performance of the formulated diets considered the formulation cost of each formulated diet, larval survival and weight gain.

However, the market price of the commercial diet was assumed to be the formulation cost. To assess the general performance of the enterprises' returns on investment, the price index and incidence cost were estimated as indicators of the economic efficiency of the formulated diets. Incidence costs and price indexes were appraised to indicate the cost and profit of producing a fish unit for all diets tested. Returns were the total sales compared to the cost of feed. The price of the larvae was based on the market selling price (USD 0.1) in Kenya.

$$\text{Incidence cost (IC)} = \frac{cost\ of\ feed\ (\text{USD})}{weight\ gain\ of\ produced\ fish\ (g)} \quad \text{Equation 4.6}$$

$$\text{Returns on diet (USD)} = \text{priceoflarvae*survival} \quad \text{Equation 4.7}$$

$$\text{Returns on investment (USD)} = \frac{returns\ on\ dietary\ treatment}{cost\ of\ feed\ of\ dietary\ treatment} \quad \text{Equation 4.8}$$

$$\text{Price index (PI)} = \frac{total\ number\ of\ larvae*selling\ price}{cost\ of\ feed!} \quad \text{Equation 4.9}$$

4.4 Results

4.4.1 Growth Performance

The weight gain of *C. gariepinus* larvae fed on experimental diets progressively increased throughout the experimental period (Figure 4.2). Larvae fed on the commercial (Gemma micro) diet exhibited the highest and significantly different ($p < 0.05$) weight gain of 0.51±0.02g compared to all other diets evaluated. Larvae fed on 50%*S. platensis* and 50%*C. nilotica* (T_2) recorded significantly different ($p < 0.05$) and the lowest weight gain of 0.20 ± 0.04g. Noticeable disparities were initially detected in the third week of the study period, persisting

until the end of the study. *Clarias gariepinus* larvae fed on diet T_5 (50%*E. fetida* and 50%*C. nilotica*) had significantly different ($p < 0.05$) and the highest weight gain of 0.43±0.01g compared to all other formulated diets in the study period. The weight gain of *C. gariepinus* larvae fed 100%*C. nilotica* (T_7) was 0.26±0.00g and was not significantly different ($p = 0.341$) from 0.25±0.01g posted by larvae fed on diet T_2 (25%*S. platensis* and 75%*C. nilotica*) at the end of the study. The dietary treatments T_1 and T_4 had a similar proportion (25%) of *C. nilotica* meal replaced by either *S. platensis* or *E. fetida*, respectively. However, larvae fed on diet T_4 had a high and significantly different ($p<0.05$) weight gain of 0.35g compared to 0.26g observed in the larvae fed on T_1. A similar daily growth rate was recorded in T_2 and T_3-fed larvae at the end of the study. Larvae fed on diet T_6 (75% *E. fetida*+25%*C. nilotica*) had high and significantly different ($p<0.05$) weight gain of 0.29g compared to 0.23g posted by larvae fed on diet.

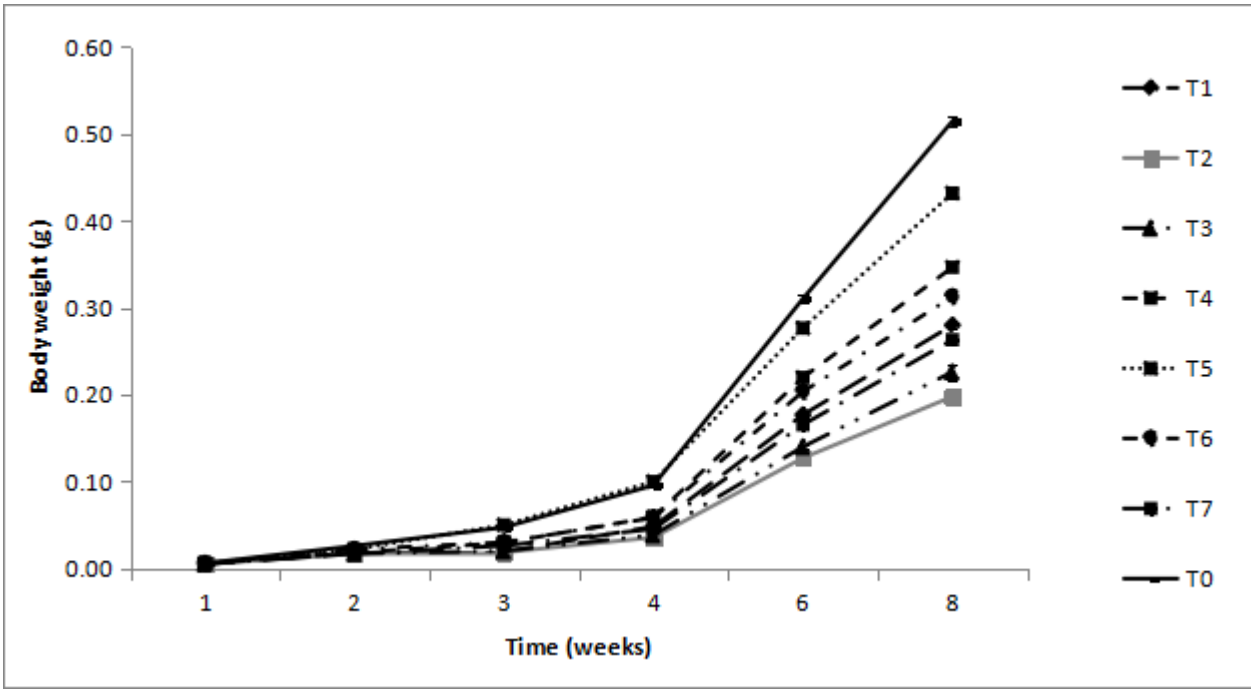

Figure 4.2: *Clarias gariepinus* larvae weight gain (mean ±SE, n = 90) fed *S. platensis* and *E. fetida* as partial alternatives in formulated diets.
(T_1 = 25% S. platensis +75% C. nilotica, T_2 = 50% S. platensis +50% C. nilotica, T3 = 75% S. platensis + 25% C. nilotica, T_4 = 25% E. fetida +75% C. nilotica, T_5 = 50% E. fetida +50% C. nilotica, T_6 = 75% E. fetida +25% C. nilotica, control (T_7) = 100% C. nilotica, T_0=commercial diet)

Formulated diets' effects on the other growth parameters had a similar trend as the body weight gain for the *E. fetida* or *S. platensis* diet at the end of the study period. Compared to all other diets, diet T_5 (of 50%*E. fetida* and 50%*C. nilotica*)-fed larvae grew the most to attain 0.82% g/day daily growth rate, total body length of 4.27 cm, and specific growth rate (SGR) of 9.57%/day). In contrast, diet T_2 (50% *S. platensis* + 50% *C. nilotica*) fed larvae posted the lowest and significantly different ($p < 0.05$) daily growth rate (0.38% g/day), total body length (2.97 cm), and SGR (7.31%/day) compared to all the other test diets. This growth performance of larvae fed on T_2 was not significantly different ($p < 0.05$) from that of those fed diet T_3 (75% *S. platensis* and 25%*C. nilotica*). The growth

parameters of larvae fed on the 100% *C. nilotica* (T_7) diet were similar to those recorded for larvae fed on diet T_1 (25%*S. platensis* and 75%*C. nilotica*) throughout the experiment. Growth parameters of total body lengths, DGR, or SGR were not significantly different between larvae fed 50%*E. fetida* and 50%*C. nilotica* (T_5) and diet T_0 (commercial diets) in Table 4.1. Feeding larvae on 25% *S. platensis* posted lower growth than *E. fetida* in a diet. Also, all diets containing *E. fetida* protein had higher growth values than *C. nilotica* as a single protein source.

Table 4.1: Growth performance (mean ± SE, n = 90) in *C. gariepinus* larvae fed *S. platensis* and *E. fetida* as partial alternatives to *C. nilotica* in formulated diets.

	Diets									
Parameter	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_0**	**F**	**p**
DGR (%g/day)	$0.50\pm0.02b^{c}$	0.38 ± 0.00^{a}	0.44 ± 0.01^{a}	0.67 ± 0.04^{d}	0.82 ± 0.01^{e}	0.56 ± 0.01^{c}	0.50 ± 0.02^{bc}	0.83 ± 0.02^{e}	94.52	0.01
IL (cm)	0.60 ± 0.01^{a}	0.60 ± 0.01^{a}	0.60 ± 0.04^{a}	0.6 ± 0.01^{a}	0.6 ± 0.01^{a}	0.6±0.01a	0.60±0.01a	0.6±0.01a	-	-
FL (cm)	3.41 ± 0.02^{bc}	2.97 ± 0.20^{a}	3.33 ± 0.16^{ab}	3.80 ± 0.00^{d}	4.27 ± 0.27^{e}	3.60 ± 0.20^{d}	3.40 ± 0.03^{c}	4.51±0.03e	146.42	0.01
SGR(%/day)	8.29±0.03b	7.31 ± 0.40^{a}	7.82 ± 0.16^{a}	8.82 ± 0.03^{c}	9.57 ± 0.30^{d}	8.43 ± 0.14^{b}	8.27 ± 0.03^{b}	9.92 ± 0.07^{d}	359.16	0.00

means in the same row without common letters are different $p<0.05$, T_1 = 25% S. platensis +75% C. nilotica, T_2 = 50% S. platensis +50% C. nilotica, T3 = 75% S. platensis + 25% C. nilotica, T_4 = 25% E. fetida +75% C. nilotica, T_5 = 50% E. fetida +50% C. nilotica, T_6 = 75% E. fetida +25% C. nilotica, control (T_7) = 100% C. nilotica, T_0=commercial diet, DGR=daily growth rate, SGR= specific growth rate, IL= initial length, FL= final length, SE = standard error

4.4.2 *Clarias gariepinus* Larvae Nutrient Utilization

Nutrients were utilised differently based on the protein source and inclusion levels. However, differences in utilising diets were first observed in week two of the study period. However, there was a general decrease in FCR to the lowest points before a steady rise in *C. gariepinus* larvae regardless of the diet used, though specific to the protein source (Figure 4.3). *Spirulina platensis* diets had the best FCR and PER in week two of the study. A similar trend was observed in week three for *E. fetida*-fed larvae. At the end of the study period, *C. gariepinus* larvae effectively utilised the commercial diet to post significantly different ($p<0.05$) and the best FCR of 1.48 compared to all other diets tested. Larvae fed on diet T_5 (50%*E. fetida* and 50%*C. nilotica*) utilised feed efficiently to post the best FCR of 1.28 and 1.74±0.03g in weeks three and eight respectively compared to all other formulated. Larvae fed on 50%*S. platensis* and 50%*C. nilotica* (T_2) were less efficient in utilising the diet nutrients to significantly differently ($p<0.05$) and had poor FCR of 1.66 and 2.97 in weeks two and eight of the study period respectively. Nutrient utilisation by larvae fed on 100%C. *nilotica* was similar to that of those fed on 25%*S. platensis* and 75%*C. nilotica*, with an FCR of 2.3 at the end of the study. However, *S. platensis* diets posted poor FCR in the range of 2–3 at the end of the experimental period (Figure 4.3).,

Commercial (Gemma micro) postest the highest and significantly different ($p<0.05$) PER of s 1.55 at the end of the study compared to all other test diets.

This was closely followed by significantly different ($p<0.05$) and the best PER of 1.30 posted by larvae fed on T_5 (50%*E. fetida* and 50%*C. nilotica*) compared to all formulated test diets... Larvae fed on the commercial diet posted a significant ($p = 0.00$) difference and the highest PER of 1.55 at the end of the study compared to all other test diets. However, larvae fed on 50%*S. platensis* and 50%*C. nilotica* (T_2) had the lowest PER of 0.68 by week eight of the study

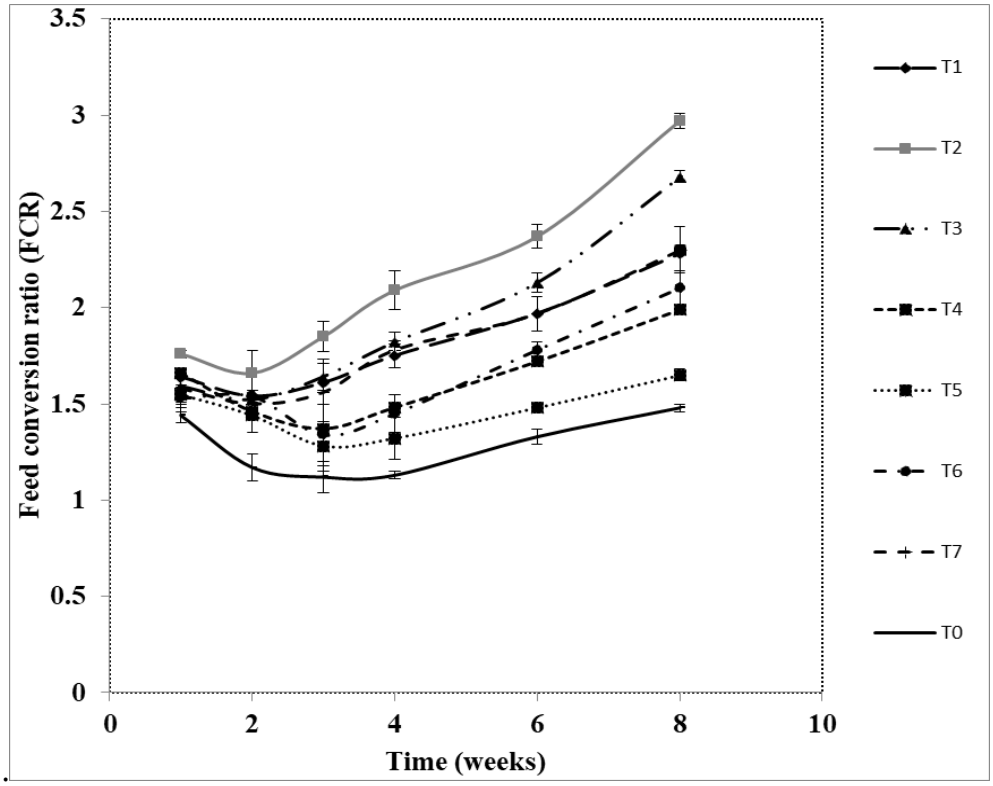

Figure 4.3: Feed Conversion Ratio (Mean ± SE) for *C. gariepinus* larvae fed *S. platensis* and *E. fetida* as partial alternatives in formulated diets.

(T_1 = 25%S. platensis +75%C. nilotica, T_2 = 50%S. platensis +50%C. nilotica, T3 = 75%S. platensis + 25%C. nilotica, T_4 = 25%E. fetida +75%C. nilotica, T_5 = 50%E. fetida +50%C. nilotica, T_6 = 75%E. fetida +25%C. nilotica, control (T_7) = 100%C. nilotica, T_0=commercial diet)

Table 4.2: Protein efficiency ratio (mean ± SE, n = 30) in *C. gariepinus* larvae fed *S. platensis* and *E. fetida* as partial protein alternatives in formulated diets.

Time	Diets									
weeks	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_0	F	P
1	1.12 ± 0.08^{a}	1.04 ± 0.01^{a}	1.14 ± 0.03^{a}	1.10 ± 0.03^{a}	1.18 ± 0.03^{a}	1.19 ± 0.05^{a}	1.16 ± 0.06^{a}	1.26 ± 0.03^{a}	2.15	.10
2	2.62 ± 0.02^{ab}	2.37 ± 0.02^{a}	2.48 ± 0.03^{a}	2.97 ± 0.56^{ab}	3.8 ± 0.29^{ab}	3.83 ± 0.10^{ab}	2.60 ± 0.36^{ab}	4.02 ± 0.26^{ab}	4.16	.009
3	1.23 ± 0.04^{ab}	0.94 ± 0.04^{a}	1.11 ± 0.05^{a}	1.33 ± 0.04^{b}	1.46 ± 0.17^{bc}	1.40 ± 0.15^{bc}	1.20 ± 0.15^{ab}	1.79 ± 0.14^{c}	5.48	.002
4	1.07 ± 0.01^{ab}	0.87 ± 0.04^{a}	0.95 ± 0.01^{a}	1.23 ± 0.01^{bc}	1.40 ± 0.13^{c}	1.24 ± 0.08^{bc}	1.03 ± 0.05^{ab}	1.63 ± 0.03^{d}	12.2	0.00
6	0.99 ± 0.03^{ab}	0.84 ± 0.02^{a}	0.90 ± 0.02^{a}	1.05 ± 0.02^{b}	1.33 ± 0.02^{c}	1.13 ± 0.02^{bc}	0.96 ± 0.08^{ab}	1.56 ± 0.04^{d}	21.0	0.00
8	1.00 ± 0.03^{b}	0.68 ± 0.04^{a}	0.87 ± 0.01^{a}	1.03 ± 0.05^{b}	1.30 ± 0.03^{c}	1.08 ± 0.11^{b}	1.01 ± 0.08^{b}	1.55 ± 0.02^{d}	27.5	0.00

Means in the same row without common letters are different $p<0.05$, T_1 = 25%S. platensis +75%C. nilotica, T_2 = 50%S. platensis +50%C. nilotica, T3 = 75%S. platensis + 25%C. nilotica, T_4 = 25%E. fetida +75%C. nilotica, T_5 = 50%E. fetida +50%C. nilotica, T_6 = 75%E. fetida +25%C. nilotica, control (T_7) = 100%C. nilotica, T_0=commercial diet, DGR=daily growth rate, SGR= specific growth rate, IL= initial length, FL= final length, SE = standard error

4.4.3 *Clarias gariepinus* Larval Survival

All dietary treatments tested on *C. gariepinus* larvae had a percentage survival within a range of 60–81% at the end of the study (Figure 4.4). Earlier in the study, there progressive decline in the observed survival. The highest mortalities (12–30%) were recorded in week one of the experimental period compared to the subsequent weeks of the study. *Clarias gariepinus* larvae fed on diet T_5 recorded significantly different ($p<0.05$) and the highest survival percentage of 81% compared to all the other test diets. The lowest survival of 60% was observed in larvae fed on diet T_1 (25%*S. platensis* and 75%*C. nilotica*) at the end of the study period. However, larvae fed on diet T_4 reported low survival compared to all other *E. fetida* diets. Larvae fed on *E. fetida* diets generally had higher survival percentages than those fed on a similar *S. platensis* inclusion level.

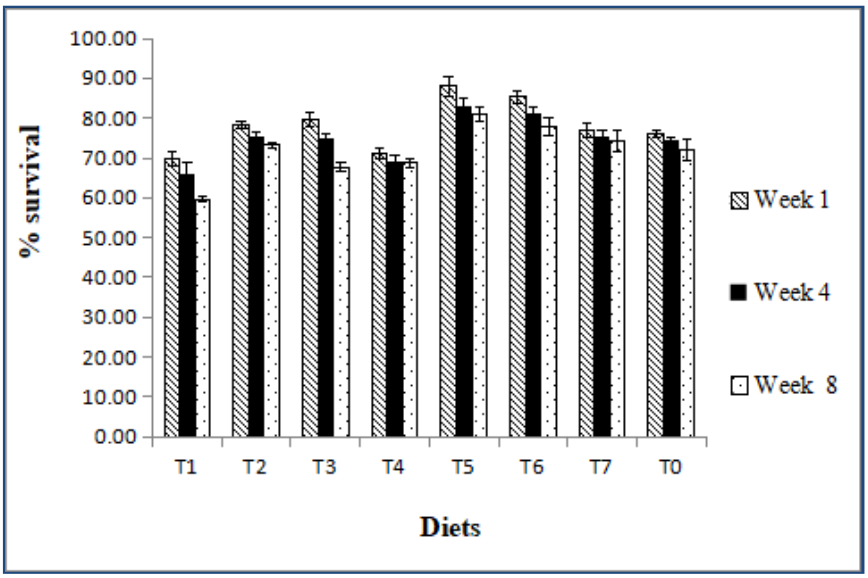

Figure 4.4: Survival (Mean ±SE, n=3) Percentage of *C. gariepinus* larvae fed *S. platensis* and *E. fetida* as partial alternatives in formulated diets.

(*T_1 = 25%S. platensis +75%C. nilotica, T_2 = 50%S. platensis +50%C. nilotica, T3 = 75%S. platensis + 25%C. nilotica, T_4 = 25%E. fetida +75%C. nilotica, T_5 = 50%E. fetida +50%C. nilotica, T_6 = 75% E. fetida +25%C. nilotica, control (T_7) = 100%C. nilotica, T_0=commercial diet)*

4.4.4 Water Quality

Water quality parameters (temperature, pH, dissolved oxygen, and un-ionised ammonia) remained relatively constant throughout the experimental period (Table 4.3). No statistical differences ($p<0.05$) were observed in all the water quality parameters monitored. Un-ionised ammonia was slightly high when larvae were fed on the *E. fetida* diet (0.04 mg/l), though it was not significantly ($p < 0.05$) different from all other experimental diets.

Table 4.3: Mean water quality parameters (mean ± SE, n = 3) for *C. gariepinus* larvae fed *S. platensis* and *E. fetida* as partial alternatives in formulated diets.

Parameter	Diets								F	P
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_0		
Temp(°C)	26.74±0.22	26.94±0.27	26.84±0.29	26.95±0.24	26.98±0.29	26.84±0.26	26.88±0.29	26.94±0.29	0.95	0.55
pH	7.29±0.04	7.31±0.02	7.30±0.03	7.32±0.01	7.33±0.03	7.33±0.04	7.30±0.02	7.30±0.01	1.32	0.33
NH3(mg/l)	0.03±0.00	0.04±0.01	0.03±0.00	0.04±0.00	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.01	0.8	0.59
DO (mg/l)	5.40±0.15	5.42±0.13	5.26±0.18	5.46±0.08	5.42±0.13	5.36±0.19	5.51±0.12	5.41±0.14	1.4	0.12

Means in the same row without common letters are different $p<0.05$, T_1 = 25%S. platensis +75%C. nilotica, T_2 = 50%S. platensis +50%C. nilotica, T3 = 75%S. platensis + 25%C. nilotica, T_4 = 25%E. fetida +75%C. nilotica, T_5 = 50%E. fetida +50%C. nilotica, T_6 = 75%E. fetida +25%C. nilotica, control (T_7) = 100%C. nilotica, SE = standard error

4.4.5 Partial Cost Analysis of the Formulated Diet Fed *Clarias gariepinus* Larvae

Feeding *C. gariepinus* larvae on *S. platensis* or *E. fetida* proteins had variable effects on the feed cost, returns on diet, return on investment (ROI), incidence cost and price index (Table 4.4). Feed and incidence costs increased as the proportions of *S. platensis* or *E. fetida* replacing *C. nilotica* increased (from 25% to 75%) in a diet. On the other hand, the price indices decreased as the proportion of S. platensis or *E. fetida* in a diet increased. Of all the diets formulated, diet T_3 (75%*S. platensis* + *25%C. nilotica*) was the most expensive (US$ 38.30/kg) compared to all other diets formulated. Larvae fed on this diet (T_3) also had the highest and significantly different incidence cost (IC) of 174 and the lowest returns on investment (ROI) of US$ 0.18. However, diet T_7 (100% *C. nilotica*) had the lowest and significantly different ($p<0.05$) feed cost of US$ 1.52±0.23, 6.08 incidence cost and the highest price (12.06) index and ROI (US$ 4.87±0.02). The accrued ROI for *Spirulina* diets and those of the commercial diet were less than one. However, those of *E. fetida* diets had their ROI being greater than one though they decreased from 25% - 75% of the inclusion level in a diet.

Table 4.4: Partial cost analysis (mean SE, n = 30) for *C. gariepinus* larvae fed *S. platensis* and *E. fetida* as partial alternatives in formulated diets.

Parameter	Diets								F	P
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_0		
W(g)	0.30 ± 0.0^{c}	0.2 ± 0.0^{a}	0.20 ± 0.01^{b}	0.30 ± 0.02^{d}	0.4 ± 0.01^{e}	0.30 ± 0.01^{c}	0.3 ± 0.02^{bc}	0.50 ± 0.01^{f}	94.5	0.01
%Survival	60 ± 3.03^{a}	73.0 ± 3.5^{ab}	68.0 ± 3.0^{ab}	69.0 ± 2.3^{ab}	81.0 ± 1.39^{b}	77.0 ± 1.70^{b}	71.0 ± 2.40^{ab}	72.0 ± 2.10^{ab}	21.6	0.00
cost (US$)	13.8±1.0	26.0±0.3	38.30±0.5	3.27±0.7	5.02±0.4	6.78±0.30	1.52±0.20	59.00	-	-
R. (USD$)	6.00 ± 2.7^{c}	7.30 ± 1.2^{b}	6.80 ± 6.2^{b}	6.92 ± 2.6^{b}	8.10 ± 1.3^{a}	7.70 ± 3.27^{ab}	7.10 ± 0.9^{b}	7.20 ± 1.9^{b}	3.70	0.03
ROI(US$)	0.43 ± 0.0^{b}	0.28 ± 0.0^{b}	0.18 ± 0.0^{a}	2.11 ± 0.0^{e}	1.61 ± 0.0^{d}	1.13 ± 0.01^{c}	4.87 ± 0.0^{f}	0.12 ± 0.06^{a}	591	0.01
IC	53.1 ± 0.9^{c}	137 ± 2.0^{d}	$174\pm1.\ 8^{e}$	9.62 ± 0.1^{a}	12.6 ± 0.1^{b}	25.1 ± 0.1^{bc}	6.08 ± 0.1^{a}	120.4 ± 0.2^{d}	229	0.00
PI	1.34 ± 0.2^{ab}	0.54 ± 0.1^{a}	0.39 ± 0.0^{a}	5.86 ± 0.2^{d}	4.46 ± 0.1^{c}	2.39 ± 0.0^{b}	12.1 ± 0.3^{e}	1.86 ± 0.0^{b}	719	0.00

Wg=weight gain over the 60 days, ROI= returns on investment, IC= incidence of cost, PI= price index, US$ American dollar, means in the same row without common letters are different p<0.05, T_1 = 25%S. platensis +75%C. nilotica, T_2 = 50%S. platensis +50%C. nilotica, T_3 = 75%S. platensis + 25% C. nilotica, T_4 = 25%E. fetida +75%C. nilotica, T_5 = 50%E. fetida +50%C. nilotica, T_6= 75%E. fetida +25%C. nilotica, control (T_7) = 100%C. nilotica, SE = standard error

4.5 Discussion

4.5.1 Growth Performance in *Clarias* gariepinus Larvae

Growth is an increase in size and assumes a specific relationship between time and magnitude. Genetics, environmental factors, dietary intake and an organism's physiological state all play a role in influencing growth. For improved productivity, sustainability and profitability, it is important to understand growth to ease its transformation and application in aquaculture. Growth indicators included specific growth rate (SGR), daily growth rate, mean body weight, and total body length (Figure 4.2 and Table 4.1).

Clarias gariepinus larvae fed on diet T_5 (50% *E. fetida* + 50%*C. nilotica*) grew the fastest, gaining 0.43±0.1g in weight. This showed how easily the diet was digested, absorbed and incorporated into body biomass. Enhanced growth observed in larvae fed on diet T_5 was attributed to the combined advantages of utilising diverse animal protein qualities, which are determined by specific amino acids, energy content, lipid types, and mineral composition. Increased growth in diet-fed T_5 larvae was also attributed to high levels of the limiting methionine (1.12 g/100g diet) and lysine (2.88 g/100 g), though lower than those reported for dietsT_6 and commercial. Lysine and methionine are building blocks for proteins that eventually increase muscle growth in the larvae in diet T5-fed larvae (Fang *et al*., 2021). Diet T_5 also had high levels of glutamic and aspartic acids, suggesting increased digestion and absorption of nutrients while ensuring the general health of diet-T_5-fed larvae. In addition, high aspartic acid levels in diet T_5 suggest low

energy use in protein synthesis. Therefore, most of the diet's energy was translated to growth (Andersen *et al.,* 2016).

The highest growth was expected in larvae fed on diet T_6 (75%*E. fetida* +25%*C. nilotica*) because of its high levels of methionine and lysine is responsible for enhanced muscle build-up. Nevertheless, previous reviews conducted by Musyoka *et al*. (2019), documented a reduction in palatability and digestibility at high *E. fetida* inclusion levels in a diet. High *E. fetida* levels in a diet suggest proportionately high coelomic fluid substances like lysenin and indigestible chitin content. Increased lysenin and chitin reduce diet palatability and digestibility respectively (Liu *et al*., 2022). Therefore, 75%*E. fetida* diet introduced increased proportions of chitin in this diet which reduced *C. gariepinus* larvae's ability to utilise it. Chitin reduces the digestive enzyme's ability to hydrolysing feed by binding to the digestive enzymes (Abro *et al*., 2014). So, the poor growth observed in larvae fed diet T_6 might have been due to limited nutrient utilisation or unknown experimental factors in this study.

Relatively high levels of limiting amino acids (though methionine was less than 2.5% of dietary protein recommended for *C. gariepinus* larvae by Uys, 1989) in diets T_5 and T_0 compared to other test diets provides a possible explanation for the improved growth with no significant differences in the daily growth rate, final body lengths and specific growth rates in larvae fed on these two diets. This is because high levels of these amino acids might have enhanced growth, digestive enzyme activity, absorption, and general welfare in fish larvae (Hien *et al.,* 2018).

However, the current study could not compare the recommended methionine levels because of the limited literature on fish larvae amino acid requirements (Jobling, 2012). Therefore, the study proposes studies to determine levels of methionine requirement by *C. gariepinus* larvae for optimal growth. Despite this challenge, methionine can be supplemented in diets and therefore should not be a criterion in selecting a feed ingredient for larval feed. Despite age and species differences, 50% of *E. fetida* (T_5) growth is comparable to previous research. The highest growth of 2.20g was reported for *Heterclarias* larvae (Olele, 2011), 179.58g in rohu (Beg *et al.*, 2016), 2.85g for *Labeo rohita* fry (Mohant *et al*., 2016), and 54.33g for *Parachanna obscura* fry (Kpogue *et al*., 2013). However, these past findings were higher than the current study report. The observation was not anticipated given the assumed higher feed ingestion due to the feeding rate of 20% decreasing to 10% which has been reported to enhance feed utilisation Chepkirui-Boit *et al*., 2011). Therefore, these observed differences were attributed to the feeding regime because earlier studies used live feed before a dry diet, culture conditions, stocking density, and the nutritional status of broodstock. In the current study, larvae were first fed on dry diets therefore, they did not have the advantage of exogenous enzymes from live feed (Verreth *et al*., 1992). Additionally, disparities might have been due to *E. fetida* meal quality differences depending on worm culture period, substrate, and meal processing (Tacon *et al*., 1983).

Larvae fed on *50% S. platensis* and 50% *C. nilotica* (T_2) reported the lowest (0.20±0.04g) growth at the end of the study compared to all the other test diets. Low growth in diet T_2-fed larvae was not expected since the diet had high levels of alanine, glycine, and arginine amino acids which are responsible for enhanced feeding activity, digestion and development in fish larvae (Kolkovski *et al.*, 2009). Indication of limited availability of these amino acids for *C. gariepinus* utilisation to enhance body mass despite, their abundance in the diet. An observation attributed to the increased presence of trypsin and amylase inhibitors in high *Spirulina* diets decreases their digestibility (Nandeesha *et al*., 1998; Nagappan *et al*., 2021). This study's findings of low growth in *C. gariepinus* larvae fed on a diet T_2 (50% *S. platensis* and 50%*C. niloticus*) was comparable to what was reported for *Carassius auratus* larvae where 50% *S. platensis* was used to replace fishmeal in a diet fed under similar culture conditions (Gowsalya & Kumar, 2018). The weight gain and SGR for larvae fed on diet T_2 in the current study were lower than those reported for *O. niloticus* fed on freeze-dried *Chlorella* species (Badwy *et al*., 2008). This suggests that different drying methods result in variable nutrient utilisation. In the current study, sun-drying *S. platensis* retained fewer nutrients for optimal growth than freeze-drying in the previous research (Musyoka *et al*., 2019).

Proximate analysis of the formulated diets revealed relatively low lipid content of 7.57 – 8.82% dry matter lipid content that decreased with increasing *S. platensis* levels in a diet compared to *E. fetida* where lipid content increased with its

increased level in a diet. Therefore, low growth performance in diets T_2 and T_3 was attributed to possibly reduced diet's palatability, attractiveness, energy supply for cell functioning and essential fatty acids necessary for catfish optimal larval growth and development (Radhakrishnan *et al*., 2016). However, low lipid content in *S. platensis* diets may not be enough reason to exclude them in larval nutrition because their lipid content may be increased through the manipulation of their culture medium

4.5.2 Nutrient Utilisation

A reduction of feed conversion ratio (FCR) in all diets from week one to their respective low values before increasing until the end of the experiment suggested different nutrient utilisation based on the protein source and was comparable to earlier reviews on larval nutrition (Hamre *et al.*, 2013; Rønnestad *et al*., 2013). One-week-old larvae with high FCR meant feed wastage which was attributed to poor feed utilisation due to undifferentiated feed acquisition processes, underdeveloped digestive system, slow and drastic intestinal development in addition to absence or reduced protease activity in fish larvae at the start of feeding (Verreth *et al*., 1992; Hamre *et al.*, 2013). Despite using high-feeding *C. gariepinus* larvae at a rate of 20%, they posted a high FCR in the first week of feeding. Indicating low feed ingestion rates and utilisation which led to starvation or underfeeding. This finding is supported by earlier findings of underfeeding when *C. gariepinus* larvae were first fed at 25% of their body weight (Uys, 1989).

Possibly because of *C. gariepinus* larvae's huge nutrient demand for effective utilisation to match its faster growth of up to 100% of its body weight at its initial life stage (Hamre *et al*., 2013).

Low FCR was recorded in week two of feeding *C. gariepinus* larvae on *S. platensis* and 100. *nilotica* compared to week three for *E. fetida* diets. An indication of the variable abilities of the formulated diets in improving digestive capacity in *C. gariepinus* larvae. Suggesting *S. platensis* and 100%*C. niloticas'* ability to enhance the maturation of digestive morphology and physiology earlier for effective diet utilisation compared to *E. fetida* diets. However, larvae fed on *S. platensis* and 100%*C. nilotica* diets posted the poorest FCR at the end of the study compared to those fed on *E. fetida*. This variability over time was attributed to the fish larvae intestine's ability to self-renew in response to various feed particle sizes (Rønnestad *et al*., 2013).

Of the formulated diets, diet T_5-fed larvae converted the diet efficiently to attain the best FCR of 1.74±0.03 at the end of the study period. A sign of *C. gariepinus'* larval ability to digest and absorb combined nutrients from 50%*E. fetida* and 50% *C. nilotica* compared to all other formulated diets. This meant improved growth, *C. gariepinus* larva welfare and quality for subsequent life stages. Larvae fed on diet T_5 FCR were comparable to 1.58 for *Labeo rohita* fry (Mohanta *et al*., 2016). The similarities observed were attributed to the similar processing of *E. fetida* meal and the utilisation of high dietary lipids (9% in both studies) for enhanced diet acceptability and promoted positive utilisation.

However, diet T_5-fed larvae FCR was much higher than 1.29 reported for *Parachanna obscura* (Vital *et al*., 2016) though, it was lower than 2.5 and 5 reported *C. gariepinus* larvae when larvae were fed on 50% garden snail meal and Eudrilid earthworm in a diet respectively (Sogbesan *et al*., 2006; Dedeke *et al*., 2013). The differences between these studies were a surprise since they had approximately 55% crude protein and 9% crude lipid in their diets. Therefore, differences were attributed to variable age, worm species, quality of culture substrate, meal digestibility, dietary chitin and feeding protocol (Patil & Biradar, 2017). This is because the quality of the culture substrate affects the quantities of amino acids, types of carbohydrates and metabolisable biomass in worm meals (Dietz & Liebert, 2018; Sharma & Garg 2018). Further, the age at which worms are harvested for feed processing determines their digestibility. In the current study, *E. fetida* was harvested every fortnight from a soft recomposed substrate compared to river-sourced Eudrilid earthworm with unidentified ages in Dedeke *et al.* (2013). Thus, worm meals had variable digestibility and utilisation due to chitin content differences proportional to worm age and culture substrate. (Bhuvaneshwara *et al*., 2019).

High and poorer FCR posted by larvae fed on diets T_2 and T_3 were attributed to high crude fibre of 1.78 and 2.03% dry matter. The posted crude fibre for diets T_2 and T_3 was above 1.6$ dry matter recommended Uys (1989). High dietary fibre makes the diet less digestible and reduces the nutrients available for utilisation (Sivakumar *et al*., 2018). This explains the reduced growth observed in larvae fed

on diets T_2 and T_3. However, high crude fibre improves larval intestinal health and stability and reduces gut evacuation rate. Fibre in the gut increases the intestinal chyme viscosity and bulkiness while producing beneficial gut prebiotics during their fermentation (Rønnestad *et al*., 2013). However, the gut evacuation rate increases with high feeding frequency (Ahmad *et al*., 2021). Therefore, the high feeding frequency of five times per day in the current study might have compromised the advantages of crude fibre viscosity during diet T_2 digestion. This reduced contact time between feed, intestinal surface, digestive enzymes, and fluids for effective diet digestion, absorption and utilisation.

Despite differences in fish larvae age, feeding rate and frequency, the FCR is 50%. *S. platensis* and 50%*C. nilotica*-fed larvae were comparable to 2.33 reported for *O. niloticus* juveniles (Badwy *et al*., 2008; Velasquez *et al*., 2016) and 2.5 for *O. mossambicus* fry (Olvera-Novoa *et al*., 1998). This similarity was in line with earlier reports of poor FCR in diets having high *Spirulina* levels (Sivakumar *et al.*, 2018). However, the current study FCR for larvae fed on 50%*S. platensis* was higher than 1.7 for *Cyprinus carpio* (Abdulrahman *et al*., 2013), 1.45, and 0.76 for *C. gariepinus* larvae, reported by Gisbert *et al*., (2022) and Raji *et al*., (2018), respectively. Observed differences in nutrient utilisation were due to differences in meal drying, kneading during formulation and age of spirulina used (Shah *et al*., 2018). In the current study, Sun drying of S. platensis might have denatured the meal proteins and encouraged the loss of amino acids, making diets indigestible and unpalatable (Musyoka *et al*., 2019). *Spirulina platensis* was

observed to have low crude lipid and high fibre crude content. This is possibly because low lipid content increases dustness which might have enhanced nutrient loss through leaching associated with its low stability in water (Radhakrishnan *et al*., 2016). The loss of nutrients in diets T_2 and T_3 was supported by the rough feel of these diets between fingers and their fast colouring of the aquarium water during feeding compared to all other diets. Faster diet disintegration and loss of nutrients in these two diets. Previous research has also reported poor FCR in fish larvae fed on high-*Spirulina* diets due to low water stability, low dietary lipids, small particle sizes and high crude fibre in the diet (Hamre *et al*., 2013).

Clarias gariepinus larvae fed on diet T_5 posted a PER of 1.3, suggesting efficient dietary protein utilisation for increased growth compared to all other formulated diets tested. This finding was comparable to earlier research in which 50% fishmeal was replaced by *E. fetida* (1.36 for *Labeo rohita* fry, 1.29 for *Parachanna obscura* (Mohanta *et al*., 2016; Vital *et al*., 2016) and 1.25 for *C. gariepinus* fed on 50% garden snails (Vital *et al*., 2016). Similarities were attributed to methods for worm processing and the use of a uniform drying temperature of 60℃ in both studies. However, this PER posted by larvae fed on diet T_5 was lower than 1.57 PER observed in *Poecilia reticulata* fed 50%*E. fetida* larval diet (Mohanta *et al*., 2016). The PER differences between these studies were attributed to variable lipid content in the diets used. Mohanta *et al*. (2016), used a diet lipid content of 15%, which enhanced diet palatability in the earlier study compared to 8.98% in the current study.

The protein efficiency ratio was reduced to its lowest of 0.68 in larvae fed on diet T_2. This presented diet T_2 as a low-quality diet and this observation was supported by its low methionine and lysine levels. This study PER for 50%S. *platensis*-fed larvae were lower than the 3.13 reported for *C. gariepinus* larvae fed on a diet containing 50% *S. platensis* (Raji *et al*., 2018) and 2.93 at 40% *S. platensis* for *O. niloticus* juveniles (Badwy *et al*., 2008; Velasquez *et al*., 2016). The variations were attributed to diet processing methods that affect diet digestibility differently between the studies (Boney & Moritz, 2017).

4.5.3 *Clarias gariepinus* Larval Survival

The survival of *C. gariepinus* larvae is a challenge with the species having a mortality record of up to 80% during the initial weeks of feeding (Hecht & Appelbaum, 1987; El-Sabaie *et al*., 2014; Nyonje *et al*., 2018). In the current study, test diets affected *Clarias gariepinus* larvae survival giving a survival percentage between 60 - 81%. This survival percentage was not attributed to water-quality parameters. No significant differences ($p<0.05$) were observed in all water quality parameters evaluated between dietary treatments. Therefore, survival differences were not attributed to water quality effects. Larvae fed on diet T_5 revealed the highest and significantly different ($p<0.05$) larval survival, attributed to high levels of methionine, lysine, and glutamic acid. (Andersen *et al*., 2016). These amino acids have been reported to activate intestinal digestive enzyme activities, enhance the development of intestinal structures for improved

digestion and absorption, and improve cell survival (Mukhtar *et al*., 2017). Further, increased lipid content (though lower than that in diet T_6), in diet T_5 provided the required energy and fatty acids for survival and might have spared proteins for enhanced cell growth and survival (Bell *et al.,* 2003). The observed survival declines in larvae fed on a commercial diet and 75%*E. fetida* compared to T_5-fed larvae was attributed to high lipid levels (energy-dense diets) compared to the recommended 9% dietary lipids for optimal survival. Energy-dense diets and a high feeding frequency of five times a day increase gut evacuation (Wu *et al*., 2017). This results in sustained feed intake but low absorption and utilisation of nutrients, amino acids and macronutrients necessary for larval survival. However, the highest survival of 81% in larvae fed on 50%*E. fetida* diet in the present study was lower than the 99% reported for *Parachanna obscura* (Vital *et al*., 2016) and *C. gariepinus* (Djissou *et al*., 2016). Nonetheless, this study's survival of 81% was higher than the 74% reported for *C. gariepinus* fry (Dedeke *et al*., 2013). The observed differences were attributed to the variable composition of amino acids, fatty acids, lysenin and the garlic smell associated with worm processing methods (Gunya *et al*., 2016).

The lowest survival of 60% was recorded in larvae fed on 25. *platensis* and 75%C. *nilotica* (T_1) despite, its lipid level of 8.82% dry matter and relatively high levels of arginine, lysine, and glutamic acid. However, s, diet (T_1) had the highest moisture content of 11.4%, compared to less than 10% recommended in fish diets (Tacon & Metian, 2015). Moisture does not contribute to the nutritional content of

a diet but it reduces the diet's shelf-life by encouraging bacterial growth and the production of aflatoxins. Microbial growth on such a diet enhances larvae handling and physiological stress (Jobling, 2016). High moisture content also reduces diet digestibility due to changes in diet-specific dynamics necessary for breaking the feed during digestion (Oehme *et al.,* 2014). The survival in diet T_1 (25%*S. platensis* + 75%*C. nilotica)* fed larvae was not similar to the 77% reported for *C. gariepinus* larvae fed on a diet having *25%S. platensis (De Chavez et al., 2018)* and *O. mossambicus* fry (Olvera-Novea *et al., 1998).* The differences were attributed to the nutrient composition of *S. platensis* as influenced by its growth phase (Guedes *et al.,* 2015). Lower survival with *Spirulina* diets than worms-fed larvae has also been reported in *Chitala chitala* fed on *S. platensis* and *Tubifex tubifex* meal (Sarkar *et al*., 2006). An indication of compromised nutritional value in *S. platensis* diets compared to *E. fetida*. Possibly because of the limited *S. platensis* digestibility of high ash content, which hardens a diet (Hua *et al.,* 2019; Seghiri *et al*., 2019; Koli *et al*., 2022). Low survival with *S. platensis* diets was reported despite high levels of glycine and alanine (Table 3.4). Observation contradicted the report of Dabrowski (1984), who related increased survival to high levels of glycine and alanine in fish larvae. Histidine has also been reported to improve survival by providing important energy in metabolic processes (Liaqat *et al*. 2017). The current study could not support this observation because *S. platensis*-fed larvae had low survival despite having higher histidine than *E. fetida* diets.

4.5.4 Partial Cost Analysis for *C. gariepinus* Experimental Diets

The profitability of aquaculture enterprises relies on effective feeding for enhanced survival, growth and well-being of fish. Therefore, the objective of any fish entrepreneur is to reduce feeding costs while improving the survival and overall performance of cultured fish species. This is because the feeding cost is an important part of aquaculture production and accounts for 50–70% of the operation costs, depending on the production system. Larvae fed on *E. fetida* diets had high economic efficiency owing to their low incidence costs and increased price indices, though lower than those of 100. *nilotica*. The attractiveness of *E. fetida* diets is also attributed to their high price index and ROI of greater than 2 and 1, respectively. High PI and ROI were indicators of cheaper, viable, and profitable investments likely to take a shorter time to break even. Larvae fed on *S. platensis* diets were not economical because they all posted an ROI of less than 1. This observation was attributed to the exploitative prices of *S. platensis* in the Kenyan market at the time of this study. This exploitative *Spirulina* price is attributed to limited technical skill in the culture of *S. platensis* associated with the complex culture medium (Pelizer *et al*., 2015). Regardless of *E*. *fetida* or *S. platensis,* the feed cost increased as the proportions of protein increased in a diet, while ROI investment declined. A similar increase in the cost of feed with a respective decline in ROI has also been reported by Mugo-Bundi *et al*. (2015).

4.6 Conclusion and Recommendations

It is feasible to partially replace C. nilotica with 25% *S. platensis* and 75% *E. fetida* in a diet to boost aquaculture productivity and resilience though with reduced survival. Fishmeal in commercial diet (gemma micro) could also be replaced with 50%*E. fetida,* without negative effects on specific growth rate (SGR), daily growth rate and survival. For economic efficiency, *E. fetida* diets were most viable because of high returns on investment and higher price indices compared to *S. platensis* diets and commercial diets. However, investing in feed with *C. nilotica* projected superior profits owing to its two-digit price index and the highest returns on investment, though with reduced growth and nutrient utilisation. Larvae fed on diet T_5 (50%*C. nilotica* and 50%*E. fetida)* performed the best in all biological parameters evaluated on *C. gariepinus* larvae at a relatively low formulation cost. Larvae fed on high *S. platensi*s diets poorly utilised their nutrient to give low growth due to their high crude fibre. *Spirulina platensis* diets were costly and the least profitable. The study recommends an evaluation of anti-nutritional factors, palatability and digestibility in *E. fetida* diets of importance in fish larval nutrition.

CHAPTER FIVE

5.0 DIGESTIVE CAPACITY OF AFRICAN CATFISH LARVAE FED ON SPIRULINA AND REDWORM IN FORMULATED DIETS

5.1 Introduction

Digestive capacity in any organism is defined by its anatomical features, digestive enzymes, and species-specific nutritional requirements**.** Fish larvae's digestive ability and nutritional requirements differ substantially from their respective adults due to age-specific transition functions (Rathore *et al*., 2016). Digestive enzymes form the chemical component of the digestive physiology of an organism and vary with and among species to result in digestive capacity differences. These differences resulted from variable enzyme activity levels, presence, absence or distribution of enzymes. These enzyme inconsistencies are responsible for age-specific abilities in digesting and absorbing feedstuffs.

Digestive enzyme activity changes occur in successive order from pancreatic, intestinal then gastric in all teleost (Lazo *et al*., 2011). This is influenced by the age of a species, hunger, food quality and movement, and the functionality of digestive accessory organs (Rønnestad *et al*., 2013). Therefore, there is a need to understand species-specific larval digestive functionality as an indicator of its ability to digest different feedstuffs and nutritional requirements. To appreciate fish digestive variability, digestive enzyme activities have been analysed to provide information on species feeding ecology in the natural environment, feed ingredient composition, and digestive competencies to determine weaning time

(Hani *et al*., 2018). Furthermore, estimates of digestive enzyme activity have provided an understanding of limiting factors in larval rearing, feeding regimes, and the optimisation of rearing practices (Gisbert *et al*., 2018).

Earlier research has analysed digestive enzyme activities using different techniques. Histochemical techniques measure enzyme activity in the tissue based on substrate or dye absorption rate (Govoni *et al*., 1986; Verreth *et al*., 1992). The calorimetric method quantifies enzyme activity by analysing the optical densities of released products (Iijima *et al*., 1998). The electrophoretic technique estimates enzyme activities depending on their size and a specific electrical charge. High-performance liquid chromatography (HPLC) or thin-layer chromatography has also been used in estimating digestive enzymes in chromatography assays based on the differential movement of digestive molecules (Lambeth & Muhonen, 1994). Light pulse radioactive substrates released during radiometric assays have also been used to estimate digestive enzyme activity (Zajicek *et al*., 2005). Through these techniques, digestive enzyme activity has been analysed in sheatfish, butter catfish, porthole shovelnose catfish, and spotted tiger catfish (Liu *et al*., 2010; Pradhan *et al*., 2013; Castro-Ruiz *et al*., 2019).

For optimal survival and development, *C. gariepinus* larvae rely on the costly *Artemia* nauplii with variable nutritional content that is influenced by the geographical location of its origin. This is because of its ability to donate its digestive enzymes to *C. gariepinus* larvae, which increases the digestion of feed that the larvae ingest. Also, the donated digestive enzymes stimulate the

production of *C. gariepinus* larvae endogenous digestive enzymes. (Sontakke *et al*., 2019; Lipscomb *et al*., 2020). However, dry-formulated use in larval feed is on the rise because of its storage ease and lower production cost though with limited success. This has been attributed to the variable and late maturation of digestive physiology in the larvae when they are first fed on dry diets (Govoni *et al*., 1986; Onura *et al*., 2018). Furthermore, fish larvae have difficulties digesting and absorbing dry diets because of their immature digestive physiology. Maturation delays in fish lare are occasioned by dry diets' reduced ability to stimulate endogenous digestive enzymes through digestive zymogens unknown growth factors provided by live feed (Gisbert *et al*., 2022).

Despite challenges in fish larval digestive physiology, larvae starter dry diet formulations depend on expensive fishmeal with supply challenges and without aquaculture and environmental sustainability guarantee. This has provided the incentives for the continuous need to develop digestible, cost-effective and environmentally friendly new dry starter diets for sustained *C. gariepinus* larvae production in aquaculture. To achieve these, studies on protein sources/alternatives have been conducted with limited considerations of the diet's influence on larval digestive capacity. This is despite digestive capacity's importance in formulating species- and age-specific diets. This has resulted in a mismatch between diet formulation and the level of knowledge on functional digestive systems in fish larvae. This is notwithstanding the understanding that effective feed management must match larval digestive physiology. Given the

information above, the purpose of the study was to contribute to the existing scientific knowledge on the digestive capacity and nutritional physiology of *C. gariepinus* larvae. The study also aims to appraise formulated diets' influence on the digestive competencies in *C. gariepinus* larvae and enhance the development of practical feed formulations. This was achieved through the following specific objectives;

i. To determine the digestive enzyme activity (total protease, trypsin, alpha-amylase, and lipase) of *Clarais gariepinus* larvae fed on *Spirulina platensis* and *Eisenia fetida* formulated diets.
ii. To evaluate the effects of protein sources on the digestive enzyme activity of *Clarias gariepinus* larvae fed on *Spirulina platensis* and *Eisenia fetida* in formulated diets.

5.2 Materials and Methods

5.2.1 Sampling and Sample Preservation

One gram of larvae was randomly sampled at zero (hatching) and 2 (first feeding/48 hours), 50 larvae at 7 and 14 days, and 30 larvae at 21- and 28-days post-hatching (DPH) from each dietary replicate glass aquaria. Sampling was done in the morning before feeding to ensure gut emptiness and optimal stimulation of digestive enzyme activity (García-Ortega *et al*., 2000). All sampled larvae were euthanised in ice-cold water and rinsed in distilled water to remove excess feed. Rinsed larvae were dried on a paper towel to remove excess water,

weighed, and immediately placed in 2–5 ml plastic vials for fixing in liquid nitrogen for at least 24 hours. Subsequently, the frozen samples were stored at -20°C pending determination of digestive enzyme activity.

5.2.2 Tissue Extraction

Sampled C. *gariepinus* larvae were too small for dissection therefore, whole-body larvae were used to obtain crude enzyme extracts for assaying Weighed and frozen replicate samples were homogenised in an ice-cold extraction solution (0.1% triton X-100 mixed with a buffer of 100 mM Tris-HCl and 0.1 mM EDTA in the ratio of 1:9, respectively) to a 10% homogenate. The 0.1% triton X-100 was to disintegrate tissue membranes, while the Tris-HCl buffer (pH 7.8) prevented any changes in pH. Ethylenediaminetetraacetic acid (EDTA) in the buffer reacted with calcium and magnesium in the sample cells to make the cell membranes less stable to ease, protein extraction. Frozen samples were homogenised in a cold mortar and pestle with a few glass beads before centrifuging them in a Hanil-Sc-industrial supra-22k centrifuge at 20000 x g for 20 minutes at 4°C. The resulting supernatant was portioned and transferred into five Eppendorf tubes of 2 ml for storage at -20°C until assaying for protein and enzyme activities at the Department of Biochemistry, UoN. All samples were homogenised three years after fixation and storage at -20°C.

Digestive enzymes total protease, trypsin, alpha-amylase and lipase activities were estimated for they are primarily responsible for the breakdown of major

macronutrients in a diet. All assays were based on spectrophotometric procedures, in which the substrate rate of disappearance or formation represented the quantity of digestive enzyme activity. The intensity of all substrates in the reaction solutions for each assay was measured in the spectrophotometer (mini-1240, Shinshu, Japan) using enzyme-specific wavelengths in triplicate at room temperature and a length of 1 cm

5.3 Sample Processing

5.3.1 Protein Content

Protein content was determined by the quick and sensitive Bradford (1976) method using bovine serum albumin (BSA) as a standard. A 2 mg/ml stock solution of BSA was used to prepare the standard curve. The BSA solution was pipetted into eleven test tubes of 0 - 100µl in triplicate and made to an equal volume of 100µl using the extraction buffer. Subsequently, 100 µl of diluted protein sample replicates were transferred into these reacting test tubes, followed by 5 ml of Bradford reagent. The reacting mixture was vigorously mixed before incubation at room temperature for 5 minutes. Its absorbance was read at 595 nm against a sample blank. The protein concentration was determined by comparing sample absorbance against the BSA standard curve and expressed in mg protein/mg tissue homogenate at 595nm.

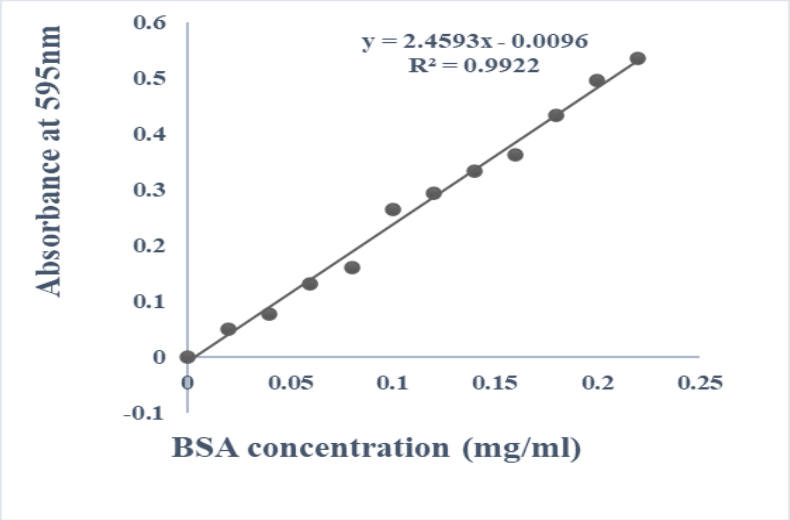

Figure 5.1: Bovine serum albumin standard curve for protein content in this study.

5.3.2 Total Protease Activity

Total protease activity was determined as described in García-Ortega *et al*., (2000). The method used readily available casein as a substrate and L-tyrosine as a standard. Casein solution was prepared by dissolving 6.5mg of casein in 1 ml of 50 mM potassium phosphate buffer at a pH of 8.0 and gently stirring to a homogenous dispersion at a temperature of 80℃. Subsequently, 800µl of the casein solution, 800µl of the buffer, and 400µl of the supernatant sample solution were reacted in the test tubes. The reacting test tubes were swirled and incubated at 37℃ for 10 minutes incubation allowed the enzyme to break down the casein and free the tyrosine for estimation. During this time, 1.1 mM L-tyrosine standard stock was prepared by dissolving 0.2 mg/ml in distilled water. The l-tyrosine standard curve was prepared by pipetting volumes of 0 to 0.50 ml into seven test

tubes, and the volumes were made to 2 ml using distilled water. Afterwards, 800 µl of a 110 mM trichloroacetic acid (TCA) solution was added to each standard or sample test tube. The TCA reacts with tyrosine to stop the reaction and precipitates substrate residuals. The subsequent solution was whirled and incubated at 37℃ for 30 minutes. Afterwards, the absorbance of the standard and sample was read at 280nm. ProteaseProtease activity units of tyrosine liberated/mg protein/minute.

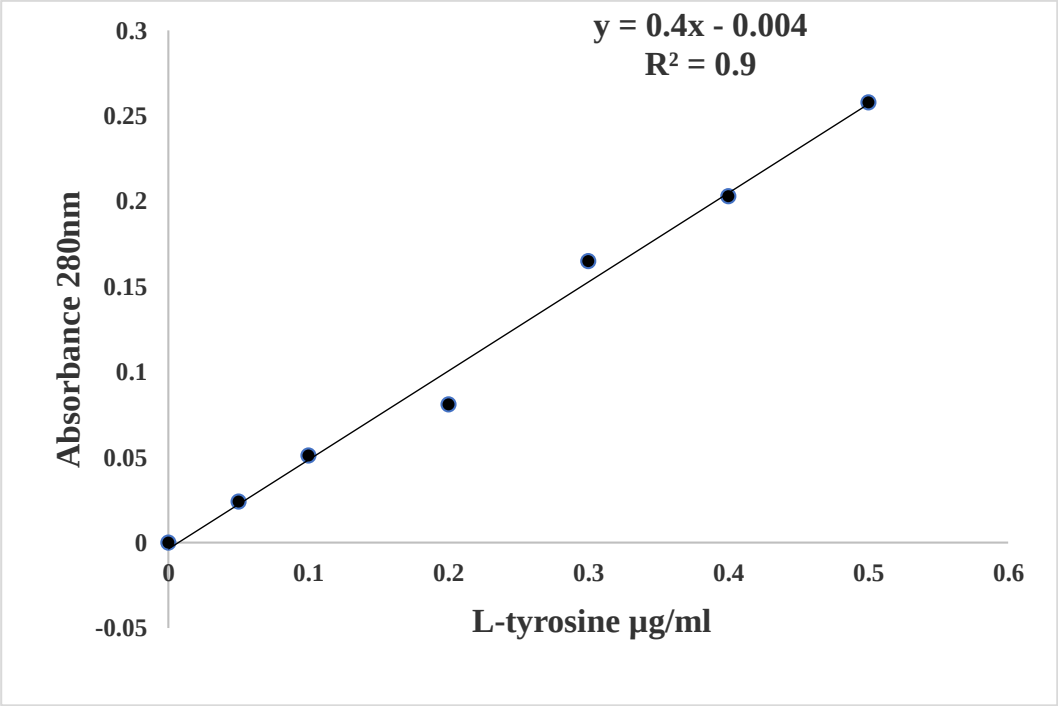

Figure 5.2: L-tyrosine standard curve for total protease activity in this study.

5.3.3 Trypsin Activity

This study used the modified Erlanger *et al*., (1961), method in determining trypsin activity, and BAPNA (N-α-Benzoyl-DL-arginine 4-nitroanilide hydrochloride) as a substrate. The substrate solution was prepared by dissolving 0.43 milligrams of BAPNA in 1 millilitre dimethyl sulfoxide (DMSO). After that, the prepared substrate was diluted to 100 ml using pre-heated buffer (10 mM $CaCl_2$ and 50 mM Tris-base mixture) in a ratio of 1:99 at 37℃ and pH 7.9. A total volume of 1.5 ml (consisting of 1.25 ml of prepared BAPNA and 25 µl of diluted supernatant enzyme extract) was incubated at 37℃ for 15 minutes. The reaction was halted by adding 2 ml of 30% acetic acid. The yellow colour of the nitroanilide was measured by comparing it to a reference blank at a wavelength of 410 nm. Trypsin activity µmol hydrolysed BAPNA/mg protein/min and demonstrated in the following equation.

$$\text{Trypsinactivity} = \frac{410\text{ nm sample Absorbance change} * 1000 * \text{vol of reacting mixture}}{\text{min} * 8800 * \text{mg protein in reacting vol}} \quad \text{Equation 5.1}$$

5.3.4 Alpha-Amylase Activity

Following the Miller (1959) method, a 1% (w/v) starch solution was used as a substrate and maltose was used as a standard to measure alpha-amylase activity. The 1% starch solution was prepared in sodium phosphate (Na_2HPO_4) buffer (pH 7.0) containing 0.01M $NaCl_2$ to activate amylase activity. Equal volumes of starch (1 ml) and enzyme extract (1 ml) for all formulated diets were incubated at 37℃

for 15 minutes to activate the breakdown of starch into sugars. A double volume of freshly prepared 3, 5-di-nitro-salicylic acid (DNS) reagent (a mixture of 1.5 g of DNS dissolved in 20 ml of 2M NaOH to stabilise the reagent mixture and 45 ml of sodium potassium tartrate tetrahydrate in 75 ml of water) was added to stop the reaction (maltose reacted with DNS, reducing it to orange-red NS i.e nitro-salicylic acid). The reacting mixture was further re-incubated in a boiling water bath for 5 minutes. Consequently, the reacting mixtures were allowed to cool to room temperature before diluting them with 6 ml of distilled water. During this time, a maltose standard curve was prepared from dilutions of maltose stock solution in a range of 0 to 1 ml. The amount of soluble dye (orange-red) produced corresponded with amylase activity from the maltose standard curve. A 1 mg/ml maltose stock solution was prepared in 0.1M sodium phosphate buffer at pH 7. The amylase absorbance was read at 540nm against a sample blank as an increase in maltose through the hydrolysis of starch and recorded as units of maltose liberated from starch/mg protein/min at 37℃.

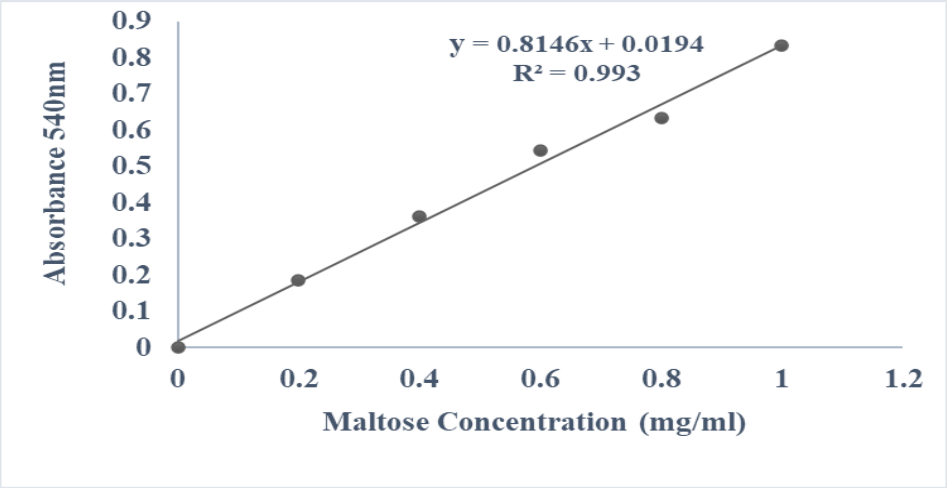

Figure 5.3: Maltose standard curve for amylase activity in this study.

5.3.5 Lipase Activity

Lipase activity was determined by the hydrolysis of the 4-nitrophenyl myristate substrate to 4-nitrophenol, as described in Iijima *et al.* (1998). A 0.2 ml sample and 0.08 ml of 10 mM 2-methoxyethanol were put into test tubes with 2.87 ml of 5.2 mM sodium cholate dissolved in 0.25 mM Tris-HCl at pH 9. The tubes were then left to sit at room temperature for 15 minutes so that lipase could start working. After that, 0.18 ml of the substrate (10 mM p-nitrophenyl myristate dissolved in 100% ethanol) was added. The mixture was then left to sit at 15℃ for another 2 minutes. Adding 4.67 ml acetone-n-heptane (5:2, v/v) into the test tubes with the mixture stopped the reaction. The resultant homogeneous mixture was shaken thoroughly before centrifuging at 6100g for 2 min using a bench centrifuge. The absorbance of the reacting mixture was read at 405 nm. The p-nitrophenol standard curve was used to determine lipase activity, which was presented as units of p-nitrophenol/mg protein/min at 15°C.

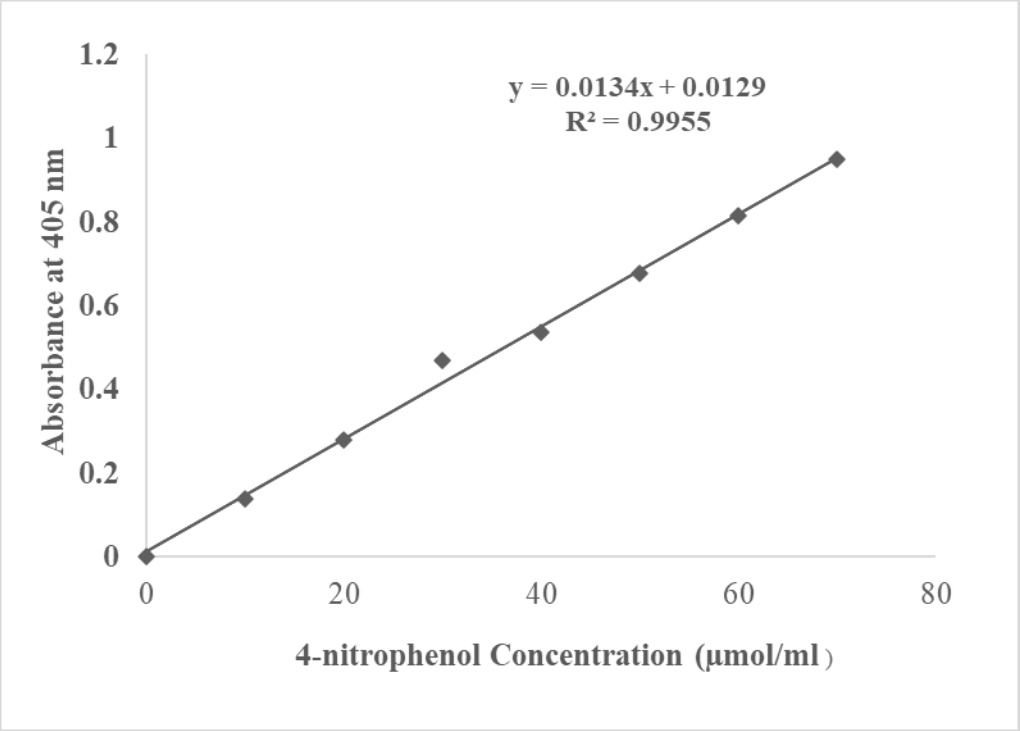

Figure 5.4: 4-nitrophenol standard curve for lipase activity in this study.

5.4 Results

5.4.1 Introduction

The protein content and digestive enzyme activities of *C. gariepinus* larvae fed on formulated diets were analysed at various feeding times (0, 2, 7, 14, 21, and 28 days after hatching). All enzymes' activities evaluated in this study were quantifiable at hatching and increased in enzyme-specific patterns until the 28th DPH. No significant ($p < 0.05$) differences in all digestive enzyme activities between dietary treatments were discerned from 0 to 7 DPH.

5.4.2 Total Protein

Clarias gariepinus larvae fed on all test diets progressively increased their protein concentration and significant differences ($p< 0.05$) were first observed at the age of 14 DPH (Table 5.1). *Clarias gariepinus* larvae fed on diet T_5 (50% *E. fetida* and 50% *C. nilotica*) had the highest and significantly different ($p<0.05$) protein content of 1.77±0.03 mg/ml tissue homogenate was not different from 1.74 mg/l homogenate posted by larvae fed on a commercial diet. However, the lowest protein content of 1.11±0.03 mg/ml tissue homogenate at 28 DPH was posted by those fed on diet T_2 (50% *S. platensis* and 50% C. *nilotica*).

Table 5.1: Total protein content (mg/ml of tissue homogenate ± SE; n=3) in *C. gariepinus* larvae fed *S. platensis* and *E. fetida* as partial alternatives in formulated diets.

	Diets									
DPH	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_0**	**F**	**p**
0	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00	-	-
2	0.18±0.01	0.18±0.01	0.18±0.01	0.18±0.01	0.18±0.01	0.18±0.01	0.18±0.01	0.18±0.01	-	-
7	0.26±0.02	0.25±0.00	0.25±0.03	0.25±0.03	0.36±0.03	0.26±0.03	0.28±0.03	0.39±0.03	3.56	0.05
14	0.46 ± 0.02^{bc}	0.45 ± 0.01^{c}	0.46 ± 0.01^{bc}	0.49 ± 0.02^{bc}	0.55 ± 0.02^{b}	0.49 ± 0.02^{bc}	0.49 ± 0.02^{bc}	0.73 ± 0.02^{a}	24.35	0.00
21	0.66 ± 0.02^{bc}	0.60 ± 0.02^{c}	0.57 ± 0.02^{c}	0.66 ± 0.02^{bc}	0.75 ± 0.02^{b}	0.64 ± 0.02^{c}	0.64 ± 0.03^{c}	0.89 ± 0.02^{a}	20.36	0.00
28	1.54 ± 0.03^{b}	1.11 ± 0.03^{e}	1.26 ± 0.03^{de}	1.41 ± 0.02^{c}	1.77 ± 0.04^{a}	1.42 ± 0.03^{c}	1.41 ± 0.03^{c}	1.74 ± 0.03^{a}	48.38	0.00

DPH = Days Post Hatching; ^{abcde} Means in the same row with different superscripts are significantly different ($p<0.05$); T_1 = 25%S. platensis +75%C. nilotica; T_2 = 50%S. platensis +50%C. nilotica; T_3 = 75%S. platensis + 25%C. nilotica; T_4 = 25%E. fetida +75%C. nilotica; T_5 = 50%E. fetida +50%C. nilotica; T_6 = 75%E. fetida +25% C. nilotica; control (T_7) = 100%C. nilotica; T_0= commercial diet (Gemma micro); SE = standard error

5.4.3 Digestive Enzyme Specific Activity

5.4.3.1 Total Protease

A gradual increase in total protease activity in larvae fed on test diets was recorded (Table 5.2). Commercial (T_0) diet-fed larvae had the highest protease activity of 0.92±0.03 units/mg protein/min, which was similar ($p = 0.11$) to the 0.89±0.03 units/mg protein/min posted by those larvae fed on diet T_5 (50% *E. fetida* and 50% *C. nilotica*) and T_6 (75% *E. fetida and 25% C. nilotica*). The lowest (0.67±0.03 and 0.68±0.03 units/mg protein/min) total protease activity was recorded in larvae fed on diets T_2 (50% *S. platensis* and 50% *C. nilotica*) and T_3 (75% *S. platensis and 25% C. nilotica*), respectively. The commercial (Gemma micro), 100% *C. nilotica*, and *E. fetida* diets had higher total protease activities than the *S. platensis* diets.

Table 5.2: Total protease-specific activity (Units/mg protein/min ±SE; n=3) of *C. gariepinus* larvae fed *S. platensis* and *E. fetida* as partial alternatives in formulated diets.

Time	Diets									
(DPH)	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_0	F	p
0	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00	0.20±0.00	-	-
2	0.47±0.01	0.47±0.01	0.47±0.01	0.47±0.01	0.47±0.01	0.47±0.01	0.47±0.01	0.47±0.01	-	-
7	0.57±0.01	0.54±0.03	0.56±0.03	0.60±0.01	0.62±0.01	0.63±0.01	0.59±0.01	0.64±0.01	2.32	0.076
14	0.65±0.03	0.62±0.03	0.62±0.03	0.65±0.03	0.69±0.02	0.65±0.02	0.65±0.03	0.69±0.03	0.844	0.568
21	0.69±0.03	0.65±0.03	0.66±0.03	0.70±0.03	0.76±0.03	0.68±0.03	0.68±0.03	0.78±0.03	2.255	0.084
28	$0.74±0.03^{abc}$	$0.67±0.03^{c}$	$0.68±0.02^{c}$	$0.82±0.03^{ab}$	$0.89±0.06^{a}$	$0.82±0.03^{ab}$	$0.85±0.03^{a}$	$0.92±0.03^{a}$	8.617	0.00

0dph= hatching, 2dph= first feeding0dph= hatching, 2dph= first feeding, means in the same row without common letters are different $p<0.05$, T_1 = 25%S. platensis +75%C. nilotica, T_2 = 50%S. platensis +50%C. nilotica, T_3 = 75%S. platensis + 25%C. nilotica, T_4 = 25%E. fetida +75%C. nilotica, T_5 = 50%E. fetida +50%C. nilotica, T_6 = 75%E. fetida +25%C. nilotica, control (T_7) = 100%C. nilotica, SE = standard error

5.4.3.2 Alpha-amylase Activity

Activity of Alpha-amylase in *C. gariepinus* larvae was 0.05±0.02 units/mg protein/min at hatching. Alpha-amylase activity remained consistently below 0.11 units/mg protein/min at 2 days post-hatching (Table 5.3) before a progressive increase up to 21 DPH before a slight decline at 28 DPH in all dietary treatments. The highest and ($p < 0.05$) alpha-amylase activity of 0.39±0.04 units/protein/min was found in larvae fed on diet T_5 at 21 DPH. Larvae fed on diet 75%*S. platensis* (T_3) posted the lowest alpha-amylase activity of 0.20±0.03 units/mg protein/min at 28 DPH.

Table 5.3: Alpha-amylase specific activity (Units/mg protein/min ± SE; n=3) of *C. gariepinus* larvae fed *S. platensis* and *E. fetida* as partial alternatives in formulated diets.

	Diets									
DPH	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_0**	**F**	**p**
0	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.05±0.01	-	-
2	0.06+0.03	0.06+0.04	0.06+0.05	0.06+0.06	0.06+0.07	0.06+0.08	0.06+0.09	0.06+0.10	-	-
7	0.21±0.04	0.17±0.05	0.17±0.02	0.20±0.05	0.18±0.08	0.22±0.02	0.18±0.05	0.18±0.05	1.7	0.18
14	$0.32±0.00^{a}$	$0.19±0.02^{b}$	$0.20±0.03^{b}$	$0.32±0.02^{a}$	$0.33±0.01^{a}$	$0.31±0.04^{b}$	$0.30±0.02^{b}$	$0.30±0.01^{b}$	8.73	0
21	$0.33±0.01^{b}$	$0.30±0.03^{b}$	$0.24±0.01^{c}$	$0.35±0.00^{b}$	$0.39±0.00^{a}$	$0.35±00^{b}$	$0.33±0.03^{b}$	$0.34±0.01^{b}$	25.08	0
28	$0.28±0.03^{b}$	$0.22±0.01^{c}$	$0.20±0.03^{c}$	$0.32±0.00^{b}$	$0.35±0.00^{a}$	$0.30±0.01^{b}$	$0.29±0.04^{b}$	$0.33±0.02^{a}$	78.21	0

DPH = Days Post Hatching; ^{abc}Means in the same row with different superscripts are significantly different ($p<0.05$) t; T_1 = 25%S. platensis +75%C. nilotica; T_2 = 50%S. platensis +50%C. nilotica; T_3 = 75%S. platensis + 25%C. nilotica; T_4 = 25%E. fetida +75%C. nilotica; T_5 = 50%E. fetida +50%C. nilotica; T_6 = 75%E. fetida +25% C. nilotica; control (T_7) = 100%C. nilotica; T_0= commercial diet (Gemma micro); SE = standard error

5.4.3.3 Trypsin Activity

At 0 DPH, the trypsin activity in *C. gariepinus* larvae was 0.33±0.01 units/mg protein/min (Table 5.4). At 2 DPH, it dropped to its lowest level of 0.27 units/mg protein/min (Table 5.4). Subsequently, trypsin activity progressively increased to a peak at 21 DPH before a decline at 28 DPH in all dietary treatments. However, the increase between 7 and 14 DPH was minimal in all *S. platensis* diets. The *C. gariepinus* larvae fed on diet T_6 had the highest trypsin activity (1.24±0.03 units/mg protein/min) at 21 DPH. At the age of 28 DPH, *C. gariepinus* larvae fed on high *S. platensis* diets posted the lowest trypsin activities of 0.80 - 0.88 units/mg protein/min. At 28 DPH, trypsin activity in *C. gariepinus* larvae fed on high *E. fetida* diets (50% and 75%E. *fetida*) was the highest and significantly different ($p<0.05$) from 50% and 75% *S. platensis* diets. Diets with *25% E. fetida or S. platensis* had similar ($p = 0.11$) effects on trypsin activities at age 28 DPH.

Table 5.4: Trypsin-specific activity (Units/mg protein/min ±SE; n=3) of C. gariepinus larvae fed S. platensis and E. fetida as partial alternatives in formulated diets.

	Diets									
DPH	T1	T2	T3	T4	T5	T6	T7	T0	F	p
0	0.33±0.01	0.33±0.01	0.33±0.01	0.33±0.01	0.33±0.01	0.33±0.01	0.33±0.01	0.33±0.01	-	-
`2	0.27±0.02	0.27±0.02	0.27±0.02	0.27±0.02	0.27±0.02	0.27±0.02	0.27±0.02	0.27±0.02	-	-
7	0.37±0.02	0.37±0.03	0.34±0.05	0.36±0.03	0.39±0.02	0.38±0.02	0.37±0.03	0.392±0.00	0.95	0.5
14	0.38±0.02c	0.40±0.02c	0.38±0.01c	0.42±0.02c	0.54±0.01a	0.55±0.01a	0.46±0.05ab	0.47±0.03ab	2.21	0.05
21	0.94±0.15abc	0.82±0.02bc	0.87±0.08ab	0.96±0.07abc	1.21±0.02a	1.24±0.03a	1.05±0.00b	1.13±0.06a	6.56	0.01
28	0.88±0.05ab	0.80±0.01b	0.80±0.03b	0.91±0.01ab	0.99±0.04a	1.02±0.00a	0.90±0.01ab	1.06±0.00a	10.86	0.00

DPH = Days Post Hatching; [abc]Means in the same row with different superscripts are significantly different ($p<0.05$); T_1 = 25%S. platensis +75%C. nilotica; T_2 = 50%S. platensis +50%C. nilotica; T_3 = 75%S. platensis + 25%C. nilotica; T_4 = 25%E. fetida +75%C. nilotica; T_5 = 50%E. fetida +50%C. nilotica; T_6 = 75%E. fetida +25% C. nilotica; control (T_7) = 100%C. nilotica; T_0= commercial diet (Gemma micro); SE = standard error

5.4.3.4 Lipase Activity

Table 5.5 shows that the lipase activity in *C. gariepinus* larvae was 0.94±0.38 units/mg protein/min at 0 DPH. Lipase activity increased with age until 28 DPH in all dietary treatments. The highest lipase activity of 60 units/mg protein/min was observed in larvae fed high *E. fetida* diets (50% and 75%*E. fetida*). However, *C. gariepinus* larvae fed on a 75% S. *platensis* diet had the lowest lipase activity of 36.58±0.07 units/mg protein/min at the age of 28 DPH. This lipase activity for 75%*S. platensis-fed* larvae were not significantly different (p = 0.08) from 38.05±0.54 units/mg protein/min posted by larvae fed on 50%*S. platensis.*

Table 5.5: Lipase-specific activity (Units/mg protein/min ± SE; n=3) of *C. gariepinus* larvae fed *S. platensis* and *E. fetida* as partial alternatives in formulated diets.

	Diets									
DPH	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_0**	**F**	**P**
0	0.94±0.38	0.94±0.38	0.94±0.38	0.94±0.38	0.94±0.38	0.94±0.38	0.94±0.38	0.94±0.38	-	-
2	4.85±0.90	4.85±0.90	4.85±0.90	4.85±0.90	4.85±0.90	4.85±0.90	4.85±0.90	4.85±0.92	-	-
7	$8.545±1.42^{a}$	$9.46±6.42^{a}$	$9.87±0.52^{a}$	$9.78±0.42^{a}$	$9.81±0.32^{a}$	$9.76±0.22^{a}$	10.03±0.00a	$9.93±0.00^{a}$	1.92	0.35
14	$16.52±3.71^{abc}$	$13.44±2.79^{de}$	$11.29±1.14^{e}$	$14.38±1.00^{cd}$	$19.07±1.18^{b}$	$17.95±2.30^{abc}$	$20.24±1.17^{b}$	$28.08±2.94^{a}$	2.96	0.01
21	$33.39±0.94^{Cd}$	$29.93±3.12^{d}$	$30.04±1.03^{d}$	$33.96±0.65^{cd}$	$34.95±2.45^{c}$	$36.57±0.91^{b}$	$32.55±0.86^{cd}$	$41.12±0.92^{a}$	26.51	0.00
28	$43.38±2.90^{bc}$	$38.05±0.54^{c}$	$36.58±0.07^{c}$	$46.86±1.20^{bc}$	$60.23±3.95^{a}$	$60.79±3.53^{a}$	$50.35±3.91^{bc}$	59.2. ±0.04[a]	30.74	0.00

DPH = Days Post Hatching; ^{abc}Means in the same row with different superscripts are significantly different ($p<0.05$); T_1 = 25%S. platensis +75%C. nilotica; T_2 = 50%S. platensis +50%C. nilotica; T_3 = 75%S. platensis + 25%C. nilotica; T_4 = 25%E. fetida +75%C. nilotica; T_5 = 50%E. fetida +50%C. nilotica; T_6 = 75%E. fetida +25% C. nilotica; control (T_7) = 100%C. nilotica; T_0= commercial diet (Gemma micro); SE = standard error

5.5 Discussion

Diet formulation demands insightful information and an understanding of digestive physiology. This is important in selecting suitable feed types corresponding to the age of the fish. However, many studies on fish-formulated diets have mostly ignored the effects of such diets on fish digestive physiology. Therefore, physiological responses to explain fish larvae performance, feed acceptance, nutrient utilisation, and growth of individual fish observed in such studies are overlooked (Srichanum *et al*., 2012; Rønnestad *et al*., 2013).

The presence of all digestive enzyme activities in *C. gariepinus* larvae at hatching indicated the larvae's ability to hydrolyse the diet's macronutrients like proteins, carbohydrates and lipids. Macronutrient hydrolysis is important in providing energy for normal growth and development in an organism. The presence of digestive enzyme activities at 0 DPH (hatching) suggested the existence of functional liver and exocrine pancreatic cells. Exocrine pancreas cells secreted pancreatic enzymes to digest yolk sac nutrients, while the liver cells' secretions (bile salts) activated digestive enzymes to enhance feed utilisation. Researchers have found digestive enzyme activities in *C. batrachus*, butter catfish, and pimelodid catfish at hatching (Verreth *et al*., 1993; Radhan *et al*., 2013; Castro-Ruiz *et al*., 2019). This suggests a genetic predisposition for digestive processes.

Digestive enzyme activities are products of fish larvae' responses to factors including genetics, temperature and feedstuffs or diets (Goswami *et al*., 2020). As a result, diets T_5 (50%*E. fetida* and 50%*C. nilotica*) and T_0 (commercial Gemma micro) fed larvae had the highest significantly different ($p<0.05$) responses to their diets to posthigh enzyme activities compared to all other experimental diets. This observation relates to the low FCR in larvae fed diet T_5 and enhanced growth (Figures 4.3 and 4.2, respectively). An indication of optimal digestion and nutrient utilisation of diets T_5 and T_0 fed larvae. Hence, low enzyme activities in larvae fed on high (having 50% and 75%) *S. platensis* diets explain their low growth performance (in Chapter 4).

However, low enzyme activities in larvae fed on diets having 50% *S. platensis* or 75% *S. platensis* did not imply their absence. Enzyme activity might have been present but inhibited by enzyme inhibitors (like phytate) which bind onto enzymes to inhibit their activities in *Spirulina* diets (Chen *et al*., 2021). Furthermore, high crude fibre (Table 3.3) in diets T_2 (50%*S. platensis and 50%C. nilotica)* and T_3 (75%*S. platensis and 25%C. nilotica*). The high fibre increased digestion chyme viscosity and inhibited enzyme stimulation through reduced feed movement along the intestinal tract. Alternatively, low enzyme activity levels in *S. platensis* diets were possibly attributed to enzyme inactivation by trypsin and amylase inhibitors (Liu *et al*., 2022)

Despite use of different protein sources, all evaluated digestive enzyme activities were not significantly different ($p < 0.05$) at 7 DPH. The observation was

attributed to the dry diet's reduced ability to stimulate enzyme activities. Fish larvae fed on dry diets do have the advantage of donated exogenous enzymes to stimulate their endogenous enzymes. In addition, larvae were fed a mixture of the formulated and commercial diets in the first four days of exogeneous feed which might have masked the individual formulated diet's enzyme-specific stimulation effects in larval fish. Observation supported by earlier reports of constant enzyme activity in the initial days of feeding, irrespective of the diet used (Wei *et al*., 2010). This directs study inferences to genetic programming rather than feedback responses

Protease activity in *C. gariepinus* larvae was detected at hatching and progressively increased until 28 DPH in all test diets to indicate the larvae gained body mass over time. However, low protease activities in *S. platensis*-fed larvae explain their low weight gains (Figure 4.1) due to slow digestive physiology This finding was comparable with the steady protease rise reports for the *Ompok hypothalamus* (Tang *et al.,* 2020). Low protease activity during the early days of *C. gariepinus* development indicated an immature digestive system during yolk sac absorption and protein hydrolysis (Liu *et al.,* 2010). All diets tested did not exhibit significant differences in protease activities from 0 to 21 DPH (Table 4.2). Probably because the diets used were approximately isonitrogenous (about 55% crude protein) and the inclusion of all other proteases like chymotrypsin and other protease-like enzymes in the assay (Ren *et al*., 2012). However, a steady but

differential increase of total protease activities from age 0 - 28 DPH in all dietary treatments suggested enhanced growth and digestive physiology over time.

The presence of alpha-amylase at hatching signalled the presence of glycogen deposits in the yolk sac. A steady increase of alpha-amylase activity from 0 – 21 DPH suggested larvaes' increased ability to utilise carbohydrates. The current study findings compare with alpha-amylase increase with age and feeding in butter catfish (Pradhan *et al*., 2013), striped catfish (Rangasin *et al*., 2012), and Acipenseids (Babaei *et al*., 2011). The upward trend for alpha-amylase activities in the current study contradicted the decreasing alpha-amylase activities in response to feed composition and carbohydrate type in pimelodid catfish larvae (Castro-Ruiz *et al*., 2019) and Sheatfish fed on earthworms (Liu *et al*., 2010).

Clarias gariepinus fed on T_2 and T_3 diets had low alpha-amylase activity. An indication of protein dependence in providing energy for metabolic activities and low protein availability for biomass buildup. The estimated amounts of alpha-amylase activities were high and significantly different ($p < 0.05$) in larvae fed on *E. fetida* diets compared to those fed on S. *platensis*. This suggests alpha-amylase activity sensitivity to carbohydrate sources rather than carbohydrate amounts in a diet. However, this observation did not compare with reports of alpha-amylase activity dependence on substrate concentration in *O. niloticus* larvae (Yue *et al*., 2019). The observed difference was e attributed to variances in feeding habits between the evaluated fish species and feed quality (Mir *et al*., 2018). In addition,

the current study parameters did not reveal any evidence to explain the slight drop in alpha-amylase activities at the age of 28 DPH in all dietary treatments.

Trypsin is a universal and pancreas-specific enzyme. This enzyme is responsible for the hydrolysis of proteins into amino acids. Amino acids are building blocks in an organism's biomass and provide energy for body functioning. The progressive increase in trypsin activity marked the development of the pancreas (Segner *et al*., 2012; Gisbert *et al*., 2014). Trypsin activity at hatching was a sign of protein digestion and a drop at 2DPH suggested exhaustion of yolk sac proteins and growth. Trypsin activities steadily increased from 7 - 21 DPH before a decrease at 28 DPH marked the presence of a functional stomach and improved acid digestion. However, earlier studies have reported the highest trypsin activities at 14 DPH in *C. gariepinus* larvae compared to 21 DPH in the current study (Shan *et al*., 2009; Pradhan *et al*., 2013). The observed delay in attaining optimal trypsin activity in the current study was a response to a dry diet's limited ability to stimulate digestive physiology. This means that the larval stage in the current study ended at 21 DPH

Trypsin activity barely increased in larvae fed *S. platensis* diets between 14 - 21 DPH, while the reverse was observed for *E. fetida* diets. This suggests protein sources' variable ability to stimulate trypsin activity. The steady rise in trypsin activity did not match the low and steady trypsin activities found in Pimelodid catfish from first feeding until they were 20 DPH old (Castro-Ruiz et al., 2019). The differences were possible because of dissimilarities in the culture conditions,

dietary protein, feeding regime, or contamination during enzyme sampling or assaying. This is because Pimelodid catfish larvae were fed diets with 45% crude protein compared to approximately 55% crude protein in the current study. Proposing a difference in optimal protein requirements in catfishes. The study hypothesises that low trypsin activity in Pimelodid catfish was a result of high dietary protein levels above optimal, which resulted in trypsin activity inhibition due to auto-hydrolysis of the excess protein (Lazo *et al*., 2011).

It was not expected that the diets could influence trypsin activities differently since they were approximately isonitrogenous (Table 3.3). *Clarias gariepinus* larvae fed on diets with high levels of *S. platensis* (T_2) and 75% *S. platensis* (T_3) had the least trypsin activity. This observation suggests insufficient protein ingestion for digestion or limited absorption of nutrients. Increased fibre in *S. platensis* diets (Table 3.3) might have inactivated trypsin enzyme activities by providing a feeling of satiation in the larvae and inhibiting hunger (Srichanun *et al*., 2012).

The study of lipases in fish larval development is key to optimising dietary lipid utilisation and determining lipid levels in diet formulations. In the current study, a progressive increase in lipase activity in all treatments suggested steady lipolytic efficiency with age and dietary lipids. This trend deviated from reports on striped catfish (Rangsin *et al*., 2012), sheatfish (Tang *et al*., 2020), and marine fish species (Zammbonino-Infante *et al*., 2008). This observation also deviated from

the histological development of *C. gariepinus* larvae reports in Verreth *et al*., (1993). The divergent observations are attributed to differences in the accuracy and specificity of estimation methods. The current study used a spectrophotometer that was not specific therefore, a lipase overestimation was possible due to the quantification of both esterases and all other lipases.

Clarias gariepinus larvae fed on diets T_2 and T_3 (50% *S. platensis* and 75%*S. platensis)* had the lowest (36–38 units/mg protein/min) and significantly different ($p<0.05$) lipase activity. In contrast to *S. platensis, C. gariepinus* larvae fed on diets having high *E. fetida* levels (50% *E. fetida* and 75% *E. fetida)* recorded the highest lipase activity of about 60 units/mg protein/min compared to all other test diets. The variations were attributed to differences in lipid levels (Table 3.3) and diet composition. The difference in lipase activities in *C. gariepinus* larvae signalled different abilities to break down *S. platensis* and *E. fetida* lipids. m. This study's findings did not compare with those reported for *Silurus soldatovi* (Liu *et al*., 2010). The variations could be due to differences in lipid composition and levels (Ren *et al*., 2012). It is important to note that differences in diets used, assay temperature and pH, reporting units, instruments and methods used in the analysis made it challenging to compare the current study enzyme activities with earlier research.

5.6 Conclusion and Recommendation

It is possible to replace up to 75% of the *C. nilotica* in the starter diet for *C. gariepinus* larvae with *E. fetida* or 25% with *S. platensis* to improve their digestive capacity. A formulated diet had variable effects on the *C. gariepinus* larvae's digestive capacity. *Clarias gariepinus* larvae diet T_6 (75%*E. fetida* and 25%*C. nilotica*) utilised dietary lipids most efficiently to exhibit the highest lipase activity despite its low growth. Protein sources influenced digestive capacity differently, with larvae fed on *E. fetida* diets revealing enhanced digestive enzyme activity compared to those fed on *S. platensis* diets. The study recommends different protocols to verify digestive capacity variability through enzyme activities.

CHAPTER SIX

6.0 STRESS TOLERANCE OF AFRICAN CATFISH LARVAE FED ON SPIRULINA AND REDWORM IN FORMULATED DIETS

6.1 Introduction

Improved growth and survival of fish larvae are indicators of a balanced diet (among other factors) that meets the species' nutritional demands. However, a balanced diet is not an assurance of quality larvae because it does not reveal the physiological state of the fish (Dhert *et al*., 1993). Fish larvae quality and physiological well-being are products of environmental changes, management practices, stocking density, prey abundance, species genotype, and nutritional condition (Oliva-Teles, 2012; Chase *et al*., 2016; Rehman *et al*., 2017). As a result, larval robustness has remained a limiting factor in seed production and a barrier to aquaculture development (Ramaswamy *et al*., 2013; Mejri *et al*., 2021). Therefore, there is a need to evaluate fish larval conditions in different environments with inherent stressors.

Proteins and lipids are important nutrients in a fish diet because they affect fish responses to growth, stress, survival, and health (Mesa-Rodriguez *et al*., 2018). Fishmeal is a high-quality protein source for livestock and fish feeds because of its balanced amino acid profile, which is important for fish larvae growth, though it is expensive (Cashions *et al*., 2017). However, potential fishmeal alternatives in larval nutrition have variably influenced fish larvae' growth, disease resistance, health and overall welfare (Rehman *et al*., 2017). Any changes in fish larvae'

responses to stress from handling, low oxygen, and chemical shock are due to the diet's nutritional content. The larvae' diet is the most important in influencing fish larvae's energy content, fatty acid profiles, phospholipids and antioxidant levels which affect their well-being. The amount and quality of protein in an organism's diet also determines the intensity of stress responses towards a stressor. Proteins are key in larval nutrition for they determine nitrogen deposition, utilisation and the energy required to excrete nitrogenous wastes (Aragão *et al*., 2022).

Generally, stress manifests through changes in an organism's metabolic and biochemical functions, hydromineral levels, hormonal balance and mortality (Segner *et al*., 2012). Analysis of stress in fish larvae has been conducted using various methods, including enzyme-linked immunosorbent assay (ELISA) to quantify hormones like cortisol, which produces reliable results but is prohibitively expensive for most hatchery managers (Du *et al*., 2020; Samaras & Pavlidis, 2020; Kim *et al.,* 2022). Fish larvae exposed to air for a set period revealed no correlation between test results and growth performance (Oliva- *et al.*, 2012). Quantification of yolk sac vitellin reveals the nutritional quality of fish larvae and indicates the amount of energy reserves during their developmental phase (Mhadhbi *et al*., 2010). Larval growth, deformities, colour, and appearance are morphological criteria of larval quality (Dhert *et al*., 1993). Gross or microscopic examinations reveal morphological responses in fish. However, morphological analysis is time-consuming and requires expertise without a guarantee of accuracy, leading to difficulties in distinguishing tissue rapture and

adaptation to a specific stressor (Harper & Wolf, 2009). In addition, the effects of stress caused by overcrowding and handling of fish larvae are subjective and have challenges standardising and evaluating using correlation and statistical analysis (Fontana *et al*., 2021). Hatchery personnel have successfully separated high-quality catfish larvae from low-quality ones by exposing batches of hatchlings to light, with photonegative individuals considered higher in quality (Schreck & Tort, 2016). This is because energetic larvae cross barriers in the trough to the dark areas in search of shelter. This method is important to hatchery managers because it is simple and appropriate for use at an early stage of larval growth but before exogenous feeding. This method compares batches of larvae from different fish brooders though it is not applicable in comparing the effects of dietary treatments. Furthermore, hatchlings' quality is evaluated based on their ability to survive starvation in specific activity indices (Aristizabal *et al*., 2009). The development of salinity and chemical stress shocks (such as ethanol and ammonia tests) has provided simple standards for assessing larval capacity to resist inherent stressors in culture conditions both in fish and shrimp (Liu *et al*., 2020). These tests are sensitive and easily reproducible in establishing larval quality within minimal time and financial costs (Ricotta *et al.*, 2003). As an alternative, Dhert *et al*. (1993) developed a stress index using cumulative mortality which is simple and easily reproducible.

Ammonia is a common pollutant in the aquatic environment that is associated with metabolic activities and organic disintegration rates. Ammonia in culture

water compromises fish welfare depending on its concentration and the form in which it exists (Boyd, 2017; Ramos *et al*., 2021). According to Abdel-Latif et al., 2022), carbon (IV) oxide, salinity, temperature, pH, and dissolved oxygen affect fish larvae responses to ammonia effects in the culture system. Exposure of fish larvae to high levels of unionised ammonia causes gill lesions and enlargement, blood vessel congestion, disorganisation of lamellae and increased endocrine secretion of cortisol and catecholamine hormones to indicate stress (Fletcher, 1997). These hormones ensure the release of glucose in body tissues to provide the extra energy demands required to sustain metabolic adjustments in response to stress. Stress responses are diverse and include recovery from stress effects or death in extreme stress conditions. Death due to unionised ammonia is possible through the displacement of K^+ ions in tissues and the depolarisation of neurons (Wendelaar, 1997). This activates N-methyl D-aspartate glutamate-gated channels to permit the overflow of Ca^{2+} ions. Despite the importance of cortisol and catecholamine hormones in reversing stress effects, high hormone levels in the bloodstream demand and consume high energy levels in fish larvae. Excess use of body energy in the cortisol and catecholamine processes results in compromised growth, and immune system and, disrupts body hydromineral balance due to changes in gill structure through inflammatory reactions (Boerrigter, 2015). However, the extent of growth reduction of hydromineral disruption depends on the larval age, nutritional status and immune capacity of the fish larvae.

Ordinarily, a quality diet should provide extra energy and essential fatty acids for fish larvae to adjust and adapt to rearing conditions. However, free amino acids and fatty acids in a diet are expensive therefore, different amino acid and fatty acid alternatives have been explored with variable results (Mejri *et al*., 2021). Plant and animal protein alternatives to fishmeal influence how fish larvae react to stress differently based on the amounts of lipids, highly unsaturated fatty acids and dietary nutrients with their digestibility. Crude lipid in a diet determines the amount of free fatty acids and dietary energy available for enhanced stress tolerance.

There is evidence of crustacean and animal stress, though this information is rarely applied in fish larval nutrition (Baßmann *et al*., 2017; Liu *et al*., 2020). An observation attributed to limited data on fish stress and a lack of consensus among fish larval nutritionists in protocols of assessing fish larvae quality despite, increased larval susceptibility to environmental stress. As a result, it is necessary to evaluate fish larvae's stress tolerance to validate the effectiveness of hatchery management practices, appraise diet nutritional content and document changes in environmental conditions.

Clarias gariepinus is an eco-toxicological model organism whose biology is well understood and documented (Nguyen & Janssen, 2002; Kreutz *et al*., 2008). The species has great tolerance to different environmental conditions because of its ability to breathe atmospheric air (air breather). The species larval stage has an un-ionised ammonia tolerance in the range of 0.00–0.5 mg/l (Ngugi *et al*., 2007;

Irina, 2014; Audu *et al*., 2017; Komugisha & Rajts, 2021). This species' un-ionised ammonia tolerance range is attributed to its undefined larval stage-specific attributes (Verreth *et al*., 1992;). However, *C. gariepinus* larvae are stress-sensitive and require an optimal diet and water quality for maximum performance (Schreck & Tort, 2016).

The future success of *C. gariepinus* in aquaculture depends on cost-effective and quality starter diets. For this reason, fish larvae nutritionists endeavour to develop environmentally friendly starter diets for enhanced growth, survival, and larval welfare. The effectiveness of these diets has been demonstrated, and their use has intensified in recent years (Kong *et al*., 2020). However, data on the performance of these formulated diets in fish larvae beyond growth and survival rates is limited. This is notwithstanding the importance of the information from such evaluations in developing fish feeds that guarantee fish larval health and quality. Dietary nutritional content diet has possible proportionate responses to its physiological status.

Hatchery managers supply fish farmers with larvae based on size without considering the larvae’s ability to withstand different culture conditions dependent on feed type and fertiliser used during pond management. Consequently, farmers are left with the challenge of larval quality, resulting in economic losses due to increased mortalities related to stocking in different culture conditions. The current study intends to contribute to a pool of science that improves understanding of diet's influence on *C. gariepinus* larvae robustness in

culture systems. Study findings will enable hatchery managers to compare different fish larvae' qualities as influenced by larval nutrition and rearing conditions. To achieve this, specific objectives were:

1. To determine the total mortalities of *Clarias gariepinus* larvae exposed to different un-ionised ammonia concentrations.
2. To estimate survival time until fifty per cent of *Clarias gariepinus* larvae exposed to the different un-ionised ammonia concentrations died.
3. To determine the stress index of *Clarias gariepinus* larvae exposed to the different un-ionised ammonia concentrations.

6.2 Materials and Methods

6.2.1 Preliminary Un-ionised Ammonia Stress Test

Un-ionised ammonia stress test solutions were made by dissolving 0.00 (culture water), 0.1, 0.5, 1, 1.5, and 2 g/l of ammonium chloride salt (batch number 213330, 99.9% purity) obtained from a Nairobi, Kenya, laboratory chemical supplier. Each test solution was dissolved in five litres of tap water in plastic buckets at 28°C and a pH of 7.6. Test solutions were stirred and left standing for 2 hours in the dissolving plastic buckets

For uniformity and stabilisation. The pH of the prepared total ammonia solutions was maintained at or above 7.6 by a few drops of ammonium hydroxide (1N NH_4OH) to ensure total ammonia equilibrium tilted to unionised ammonia. The concentrations of un-ionized ammonia in the prepared ammonium chloride solutions of 0.0 (culture water), 0.1, 0.5, 1, 1.5, and 2 g/l of ammonium chloride

salt were 0.00 (control), 0.00, 0.01, 0.03, 0.04, and 0.05 mg/l, respectively considering recommended limits in Kamugisha & Rajts, (2021). These were direct estimates considering temperature and pH in the ammonia conversion Table by Francis-Floyd *et al*., (2009). Subsequently, 200 ml of each ammonia solution was transferred into 250 ml disposable plastic cups in triplicate. The disposable cups with ammonia solution were in a 100-litre water bath at 28°C until their content temperatures matched those in the water bath.

To select appropriate un-ionized ammonia levels for use in the subsequent stress test, four-week-old *C. gariepinus* larvae were starved for 18 hours to ensure a uniform physiological state before subjecting them to the variable un-ionised ammonia concentrations described above. Subsequently, the larvae were randomly sampled using a scoop net and concentrated in a strainer without exposure to air to minimise stress from sampling. Eighteen larvae per replicate and 54 per treatment were randomly placed in each cup with un-ionised ammonia solutions for 12 hours. All test cup solutions were not aerated to avoid the introduction of other gases like carbon (IV) oxide that could lower the pH to lethal levels. In addition, there was no feeding or handling during the trials to reduce the nitrogenous waste and handling stress on the larvae, respectively. Every 30 minutes, the death of *C. gariepinus* larvae in each test solution replicate was observed and recorded. Death was defined as a lack of response to a plastic ruler's touch.

After 12 hours of larvae exposure, the number of deaths was observed and counted in all test solutions. The death counts and the start of the deaths were variable and depended on the un-ionised ammonia level. All larvae exposed to 0.05mg/l died while no deaths were recorded in those larvae exposed to 0.00mg/l un-ionised ammonia. Compared to all other unionised ammonia solutions, larvae exposed to 0.05 mg/l began to die after four hours of exposure. Total deaths in each ammonia solution and the time of the deaths informed the choice of 0.0 mg/l (control) and higher concentrations of 0.04 and 0.05 mg/l, with an intermediate 0.03 mg/l concentration for the un-ionised ammonia stress experiment. *Clarias gariepinus* larvae aged six weeks were exposed to the same unionised concentrations chosen for the four-week-old larvae.

6.2.2 Experimental Setup for ammonia stress test

According to the preliminary trial, a stock solution of 20 litres of the un-ionised ammonia solutions selected for the stress test (0.00, 0.03, 0.04 and 0.05mg/l) was prepared in plastic buckets. Afterwards, 350 ml of each ammonia solution was transferred into 24 disposable plastic cups of 500 ml capacity and suspended in a water bath randomly assigned respective un-ionised ammonia in replicates. The disposable cups with ammonia solution were suspended in four different water baths improvised using 100-litre glass aquaria (Figure 6.1) at 28°C until their temperatures matched those of the water bath. Subsequently, forty *C. gariepinus* larvae per replicate and 120 per dietary treatment were randomly

selected using a scoop net. Of these, 10 larvae per replicate were randomly placed in the disposal cups in each of the specific un-ionised ammonia solutions as described in the preliminary trial.

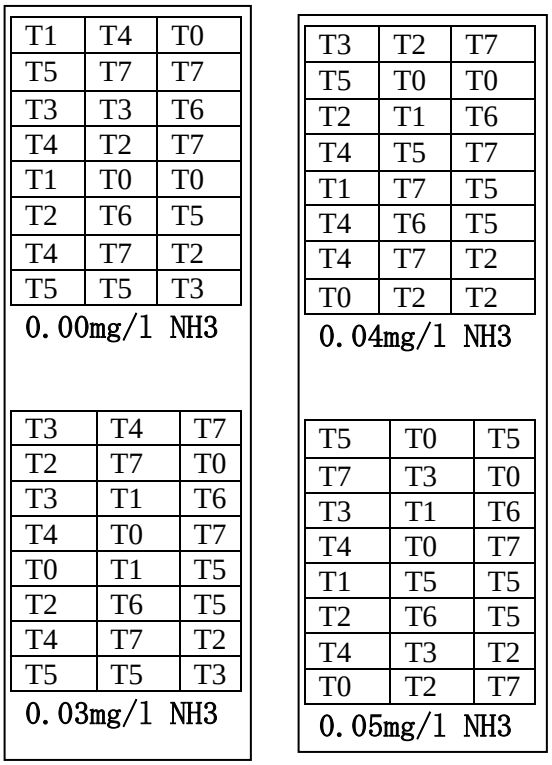

Figure 6.1: Amodel Showing the Four Aquaria for Stress Test Experimental Set-up.

6.3 Data Collection

Clarias gariepinus larvae deaths were recorded every 30 min over 24 hours. Deaths were counted per replicate and used to calculate total mortality. The time it took for half of the exposed larvae to die was observed and used in calculating survival time until 50% of the exposed larvae died in respective unionised

ammonia solutions. To calculate cumulative mortality/stress indices, Time at the start of deaths, progressive deaths and total mortality were considered as described in Dhert *et al*. (1993). A higher cumulative death/stress index indicated poor-quality larvae.

6.4 Results

6.4.1 *Clarias gariepinus* Larvae Total Mortality during Unionized Ammonia Exposure

Total mortality increased with the increase in unionised ammonia concentration but decreased with larval developmental age. The control (0.0 mg/l unionised ammonia) had a total mortality of 10%–16% for both four- and six-week-old *C. gariepinus* larvae. Larvae exposed to 0.05 mg/l unionised ammonia registered 100% mortality within 24 hours, regardless of dietary treatments in the two larval ages (Figures 6.2a and 6.2b). *Clarias gariepinus* larvae fed on diet T_5 (50% *E. fetida* and +50% *C. nilotica)* had the lowest and significantly different ($p<0.05$) total mortalities of 78% and 52% for four and six-week-old larvae respectively when exposed to 0.04 mg/l unionised ammonia. On the other hand, larvae fed on diet T_2 (50%*S. platensis* and 50% *C. nilotica*) posted the highest total mortalities of 97% and 73% for four and six-week-old larvae respectively when exposed to 0.04 mg/l unionised ammonia within 24 hours. However, diet T_3 (75%*S. platensis and 25% C. nilotica)* had the lowest mortality of 65% compared to all other larvae fed on *Spirulina* diets. Generally, *S. platensis* diets had higher total mortalities than *E. fetida*-fed larvae exposed to all un-ionised ammonia concentrations and at

different ages. Six-week-old larvae had low total mortalities regardless of the diets they were fed on compared to four-week-old larvae.

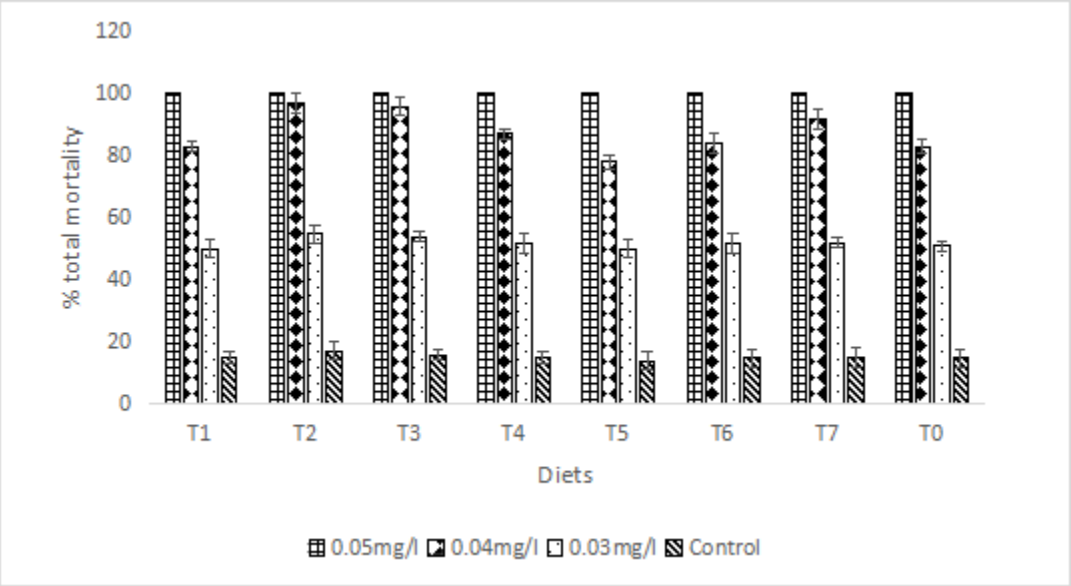

Figure 6.2a: Four-week-old *Clarias gariepinus* larvae total mortality (mean ± SE, n=3) during un-ionized ammonia exposure.
(Note: T_1 = 25%*S. platensis* + 75%*C. nilotica*; T_2 = 50%*S. platensis* + 50%*C. nilotica*; T_3 = 75%*S. platensis* + 25%*C. nilotica*; T_4 = 25%*E. fetida* + 75%*C. nilotica*; T_5 = 50%*E. fetida* + 50%*C. nilotica*; T_6 = 75%*E. fetida* + 25%*C. nilotica*; *control* (T_7) = 100%*C. nilotica*; T_0 = Commercial diet (Gemma micro); SE = Standard Error)

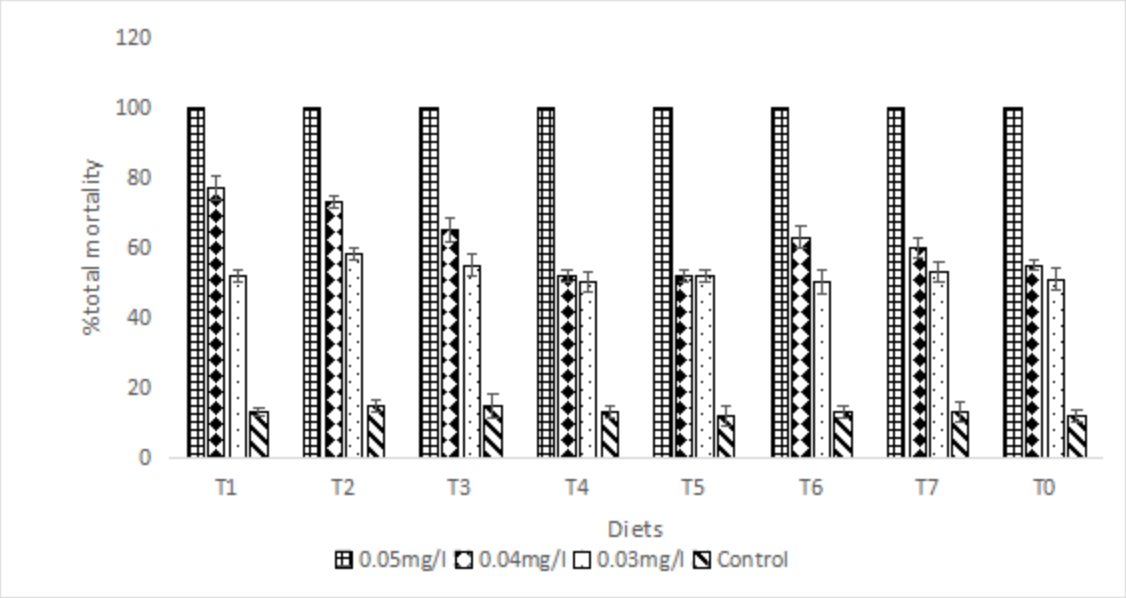

Figure 6.2b: Six-week-old *Clarias gariepinus* larvae total mortality (mean ± SE, n=3) during un-ionized ammonia exposure.
(Note: T_1 = 25%*S. platensis* + 75%*C. nilotica*; T_2 = 50%*S. platensis* + 50%*C. nilotica*; T_3 = 75%*S. platensis* + 25%*C. nilotica*; T_4 = 25%*E. fetida* + 75%*C. nilotica*; T_5 = 50%*E. fetida* + 50%*C. nilotica*; T_6 = 75%*E. fetida* + 25%*C. nilotica*; *control* (T_7) = 100%*C. nilotica*; T_0 = Commercial diet (Gemma micro); SE = Standard Error)

6.4.2 Survival Time to the Death of Fifty Percent of the Exposed C*larias gariepinus* Larvae in Un-ionized Ammonia

The survival time for half the number of exposed *C. gariepinus* larvae fed on *S. platensis* or *E. fetida* diets was influenced by un-ionised ammonia concentrations (Table 6.1). The survival time in unionised ammonia (until 50% of the exposed larvae died) decreased as the un-ionised ammonia concentration increased in all test diets. Six-week-old larvae survived longer hours before 50% of the exposed larvae died compared to four-week-old larvae for both *S. platensis* and *E. fetida* diets. Larvae fed on diets T_2 and T_3 (50% and 75% *S. platensis*, respectively) had

a shorter survival time before 50% of the larvae exposed to 0.05 mg/l un-ionised ammonia died compared to those fed on T_5 and T_6 (having 50% and 75% *E. fetida*, respectively) for both four- and six-week-old larvae. Also, larvae fed on diets T_5 and T_6 had the longest and significantly different ($p<0.05$) survival time from all other test diets in week six-old larvae when exposed to 0.03 and 0.04 mg/l unionised ammonia.

Four- and six-week-old larvae fed on 50% *E. fetida* and 50% *C. nilotica* (T_5) recorded the longest and significantly different ($p<0.05$) survival times of 12.7 and 18.7 hours, respectively was recorded in 0.05 mg/l unionised ammonia. On the other hand, four- and six-week-old larvae fed on a combination of 50% *S. platensis* and 50% *C. nilotica* (T_2) were more sensitive to the un-ionised ammonia concentrations post the shortest and significantly different ($p<0.05$) survival time of 8 and 10.3 hours respectively before 50% of the exposed larvae to die in 0.05 mg/l un-ionised ammonia.

Table 6.1: Survival time (mean hours, SEM, n=40) until the death of fifty per cent of C. *gariepinus* larvae in un-ionised ammonia.

Age	Ammonia	Diets										
(weeks)	levels	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_0	LSD	SEM	p
4	0.05mg/l	9.0 ± 0.30^{b}	8.0 ± 0.23^{a}	8.2 ± 0.1^{a}	9.30 ± 0.46^{b}	12.7 ± 0.40^{d}	10.8 ± 0.10^{c}	9.83 ± 0.12^{b}	10.7 ± 0.29^{c}	32.43	10.69	<.001
	0.04mg/l	11.7 ± 0.42^{b}	10.8 ± 0.19^{a}	10.8 ± 0.21^{a}	13.3 ± 0.51^{c}	15.7 ± 0.22^{d}	13.8 ± 0.10^{c}	12.8 ± 0.31^{bc}	14.8 ± 0.31^{d}	45.86	15.12	<.001
	0.03mg/l	23.7 ± 0.20^{ab}	23.3 ± 0.00^{a}	23 ± 0.22^{a}	23.8 ± 0.00^{ab}	24 ± 0.00^{b}	23.8 ± 0.10^{ab}	23.8 ± 0.00^{ab}	23.8 ± 0.16^{ab}	25.35	8.36	0.012
	Control	-	-	-	-	-	-	-	-	-	-	-
6	0.05mg/l	11.7 ± 0.31^{b}	10.3 ± 0.43^{a}	10.7 ± 0.25^{a}	15.3 ± 0.32^{c}	18.7 ± 0.43^{e}	16.7 ± 0.31^{d}	14.8 ± 0.30^{c}	167 ± 0.34^{d}	36.25	11.95	<.001
	0.04mg/l	17.3 ± 0.21^{b}	15.0 ± 0.51^{a}	15.7 ± 0.16^{a}	19.8 ± 0.10^{cb}	23.8 ± 0.21^{d}	24.0 ± 0.35^{d}	19.2 ± 0.54^{b}	21.8 ± 0.22^{c}	32.43	10.69	<.001
	0.03mg/l	21.3 ± 0.45^{bc}	20.0 ± 0.28^{a}	20.3 ± 0.10^{a}	21.8 ± 0.23^{bc}	24.0 ± 0.00^{d}	23.5 ± 0.25^{d}	22.2 ± 0.43^{c}	22.8 ± 0.10^{c}	42.9	14.14	<.001
	Control	-	-	-	-	-	-	-		-	-	-

^{abcde}Means in the same row with different superscripts are significantly different ($p<0.05$)*; T_1 = 25% S. platensis +75% C. nilotica; T_2 = 50%S. platensis +50%C. nilotica; T_3 = 75%S. platensis + 25%C. nilotica; T_4 = 25%E. fetida +75%C. nilotica; T_5 = 50%E. fetida +50%C. nilotica; T_6 = 75%E. fetida +25%C. nilotica; control (T_7) = 100%C. nilotica; T_0 = Commercial diet (Gemma micro); SEM =Standard Error Mean; LSD=Least Significance Difference; time in 24hrs*

6.4.3: *Clarias gariepinus* Larvae Stress Indices during Un-ionised Ammonia Exposure

For both four- and six-week-old *C. gariepinus* larvae, the stress index increased as the concentration of unionised ammonia increased (Table 6.2). Larvae fed on diets T_2 (50% *S. platensis* and *C. nilotica*) and T_3 (75%*S. platensis* and *C. nilotica*) diets reported the highest stress indices in all un-ionised ammonia concentrations compared to those posted by larvae fed on *E. fetida* diets with similar inclusion proportions. Larvae fed on diet T_5 (50% *E. fetida* and 50% *C. nilotica)* had the lowest and significantly different ($p<0.05$) stress index of 342 in 0.05 mg/l un-ionised ammonia concentration at the age of six weeks, compared to all other diets tested. *Clarias gariepinus* larvae fed on the 50% *S. platensis* and 50% *C. nilotica diet* (T_2) had the highest and significantly different ($p<0.05$) stress index of 586 and 500 in 0.05 mg/l un-ionised ammonia at the age of four and six weeks, respectively. *Clarias gariepinus* larvae fed on *S. platensis* diets had stress indices in all un-ionised ammonia solutions compared to corresponding *E. fetida* diets.

Table 6.2: Stress indices for (mean, SEM, n=40) four- and six-week-old *C. gariepinus* larvae fed *S. platensis* and *E. fetida* as partial protein alternatives in formulated diets.

Ammonia level	Diets								LSD	SEM	p
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_0			
control	89.3±0.54	89.0±0.54	88.0±0.54	87.0±0.10	83.7±6.20	84.0±4.12	85.3±3.94	83.0±4.32	5.994	1.98	0.07
0.03mg/l	194±4.21^{d}	265±0.54^{e}	265±0.45^{e}	156±2.56^{c}	115±4.21^{a}	147±2.00^{b}	153±2.36^{c}	121±2.45^{a}	5.2	1.74	0.001
0.04mg/l	475±0.43^{d}	531±3.23^{f}	497±2.31^{e}	443±5.00^{c}	371±3.34^{a}	394±1.25^{b}	451±7.00^{c}	366±5.10^{a}	8.88	2.93	0.001
0.05mg/l	554±2.54^{d}	586±2.45^{f}	574±3.35^{e}	522±1.45^{c}	457±8.24^{a}	483±1.00^{b}	517±3.00^{c}	468±3.45^{a}	5.212	1.72	0.001
Control	82.0±2.50	83±2.50	84±2.50	81.7±2.50	83.3±2.50	82.0±2.50	82.3±2.50	82.1±2.50	2.729	0.90	0.53
0.03mg/l	170±2.50^{d}	220±3.33^{f}	212±7.12^{e}	142±4.12^{b}	111±4.00^{a}	127±3.54^{b}	151±5.21^{bc}	128±3.45^{b}	4.633	1.52	0.001
0.04mg/l	259±5.12^{d}	334±2.45^{f}	318±2.25^{e}	240±3.12^{c}	185±2.54^{a}	211±3.27^{b}	236±4.40^{c}	219±3.23^{b}	6.474	2.13	0.001
0.05mg/l	430±3.23^{e}	500±4.32^{g}	476±3.23^{f}	406±2.22^{c}	342±0.00^{a}	375±3.54^{b}	419±2.43^{d}	378±3.23^{b}	6.018	1.98	0.001

^{abcdefg}*Means in the same row with different superscripts are significantly different* ($p<0.05$)*; T_1 = 25% S. platensis +75% C. nilotica; T_2 = 50% S. platensis +50% C. nilotica; T_3 = 75% S. platensis + 25% C. nilotica; T_4 = 25% E. fetida +75% C. nilotica; T_5 = 50% E. fetida +50% C. nilotica; T_6 = 75%E. fetida +25%C. nilotica; control (T_7) = 100% C. nilotica; T_0 = Commercial diet (Gemma micro); SEM =Standard Error Mean; LSD=Least Significance Difference*

6.4.4 A Comparison of Four- and Six-week-old *C. gariepinus* Larvae Stress Indices in Different Un-ionized Ammonia Concentrations

In all diets evaluated, six-week-old larvae exhibited a lower stress index compared to four-week-old larvae as shown in Table 6.3. Larvae fed on 50% *E. fetida* and 50% *C. nilotica* (diet T_5) recorded significantly ($p<0.05$) lower stress indices of 414 and 264 for four- and six-week-old larvae, respectively, when compared to all other diets tested using t-test. Nonetheless, larvae fed on T_2 (50%*S. platensis* and 50%*C. nilotica*) recorded the highest stress indices of 545 and 417 in four- and six-week-old larvae, respectively though, it was not significantly ($p = 0.07$) different from 536 and 414 posted by larvae fed on diet 75% *S. platensis* and 25% *C. nilotica* (T_3). Both four- and six-week-old larvae fed on *S. platensis* had higher stress indices than those fed on *E. fetida.*

Table 6.3: Stress indices (Equality test, mean ± SEM, DF=58, SED, t-value) compared between four- and six-weeks-old *C. gariepinus* larvae fed on *S. platensis* and *E. fetida* as partial protein alternatives in formulated diets.

Diets	Equality of variance	week 4	week 6	SED	t-value	p-value
	p-value	mean	mean			
T_1	0.80	479±19.8^{c}	345±38.1^{e}	50.9	2.64	0.02
T_2	0.15	545±18.3^{d}	417±37.1^{f}	41.4	3.10	0.011
T_3	0.12	536±17.3^{d}	414±37.1^{f}	40.9	2.98	0.014
T_4	0.13	482±17.6^{c}	323±37.3^{d}	41.2	3.86	0.003
T_5	0.22	414±16.4^{a}	264±35.2^{a}	40.2	3.75	0.004
T_6	0.14	443±17.7^{b}	293±36.6^{c}	40.7	3.70	0.004
T_7	0.20	490±12.0^{c}	327±40.9^{d}	42.6	3.82	0.003
T_0	0.13	400±12.0^{a}	270±16.1^{b}	40.2	3.00	0.012

^{abcdef}*Means in the same row with different superscripts are significantly ($p<0.05$) different; T_1 = 25%S. platensis +75%C. nilotica; T_2 = 50%S. platensis +50%C. nilotica; T_3 = 75%S. platensis + 25%C. nilotica; T_4 = 25%E. fetida +75%C. nilotica; T_5 = 50%E. fetida +50%C. nilotica; T_6 = 75%E. fetida +25%C. nilotica; control (T_7) = 100%C. nilotica; T_0 = Commercial diet (Gemma micro); SEM =Standard Error Mean; SED =Standard Error Difference; LSD=Least Significance Difference; DF = Degrees of Freedom.*

6.5 Discussion

Four- and six-week-old *C. gariepinus* larvae that were exposed to the control (0.00 mg/l) died (highest mortality of 16%) irrespective of the diet they were fed on (Figures 6.2a and 6.2b). An indication of incidental mortalities due to natural factors or experimental settings that possibly compromised oxygen and energy distribution to vital organs for the efficient functioning and survival of the larvae in the control solution. The results of this study were similar to those found (1–21%) for *C. gariepinus* larvae in a control group (Nguyen & Jassen, 2002). This serves as a warning to farmers and hatchery managers that even in the absence of toxic substances in the culture water, mortalities may still occur.

In a 0.05 mg/l unionised ammonia solution, all *C. gariepinus* larvae died irrespective of

age and diet that they are fed on. Suggesting proportional un-ionised ammonia concentration effects and all diet's reduced ability to enhance *C. gariepinus* larvae tolerance in 0.05 mg/l of un-ionised ammonia. Possibly because of severe gill and internal organ damage by un-ionised ammonia which results in the reduction of ammonia excretion and detoxification site surface areas. These findings did not compare with the recommended 0.05 mg/l optimal unionised ammonia concentration for *C. gariepinus* larvae by Komugisha & Rajts (2021). This could be due to differences in larval quality influenced by brooder nutrition and culture conditions. This study, therefore, hypothesises the optimal un-ionised ammonia tolerance level for *C. gariepinus* larvae to be less than 0.05 mg/l.

In low unionised ammonia concentrations, all larvae fed formulated diets had low total mortalities (Figures 6.2a and 6.2b). This finding was attributed to earned tolerance through ammonia exposure in the culture water. This is because there are always low levels of un-ionised ammonia in the culture water due to the consistent decomposition of faeces and uneaten feed. Accordingly, low unionised ammonia concentrations stimulated *C. gariepinus* larvae's adaptive immunity, allowing a faster cellular response against low ammonia toxicity exposures. Earlier research by Terjesen *et al.* (2001) and Kreutz *et al*. (2008) reported a comparable trend for *C. gariepinus* larvae at low ammonia concentrations. This resemblance could be attributed to *C. gariepinus* larvae's ability to down-regulate stress effects (Sayed & Authman, 2018).

Stress indices reported over 24 hours revealed the variable influence of formulated diets on the nutritional status and physiological well-being of *C. gariepinus* larvae (Table 6.2). High-stress indices in larvae exposed to 0.05 mg/l un-ionised ammonia, regardless of the diet they were fed on. This suggested reduced resilience of *C. gariepinus* at high un-ionised ammonia concentrations. This was probable because of the impaired ammonia excretion due to histological gill damage occasioned by increased un-ionised ammonia uptake. This led to the destabilisation of gaseous exchange, ion exchange and acid-base regulation through reduced gill surface area. Therefore, high-stress indices in the 0.05 mg/l unionised ammonia suggested excessive oxidative stress due to liver dilations and cell inflammations (Mesa-Rodriguez *et al*., 2018).

Four- and six-week-old *C. gariepinus* larvae that were fed diet T_5 (50%*E. fetida* and 50%*C. nilotica*) had low-stress indices, implying that they were of a better quality since they survived unionised ammonia stress compared to all other formulated diet-fed larvae. This was possible due to the high energy reserves provided by the high lipid content of 8.96% (Table 3.3) and their enhanced growth. High lipids provide essential fatty acids, phospholipids and neutral lipids for enhanced immunity, survival, and stress tolerance (Abaho *et al*., 2016). Phospholipids ensured electrolyte balance by reducing membrane permeability to ammonia, while neutral lipids provided energy through oxidative phosphorylation. This was important in sustaining hormone balance for effective resistance to ammonia stress effects. Furthermore, the physiological state of the

fish larvae is a product of its diet therefore, high levels of glutamic acid in diet T_5 might have been oxidised to provide the extra energy required during stress and in detoxifying ammonia to glutamate (Conceição *et al*., 2012). High levels of methionine and threonine in diet T_5 stabilised body muscle and mucin to reduce excretory site cell damage. As a result, *C. gariepinus* larvae resistance to ammonia stress might have been improved through enhanced ion exchange and acid-base balance. The relatively high methionine (1.12 g/100 g diet) and lysine (2.88 g/100 g diet) were responsible for excretory site muscle growth and development for improved ammonia excretion. The present study findings on the quality of diet in T_5-fed larvae were in agreement with enhanced quality reported for long-fin yellow tail (*S. rivoliana*) larvae when *E. fetida* replaced 50% fishmeal in a diet (Mesa-Rodriguez *et al.,* 2018). the similarity was attributed to the use of 9% DM lipid diets, which provided essential fatty acids and fat-soluble nutrients for efficient physiological processes in managing ammonia effects. The highest stress indices in diet-T_2-fed larvae indicated reduced stress resistance to un-ionised ammonia exposure. This may be because of a reduction in immunity or inadequate ammonia stress coping strategies, poor growth, and low methionine and lysine levels, which limit the growth and development of ammonia excretory sites for enhanced deamination of ammonia. This is because stress sensitivity in a species is proportional to its nutritional standing and body size (Mattos *et al*., 2019). Therefore, larvae fed on diet T_2 may have had fewer cells because of their small body size for efficient cellular homeostasis. This resulted in larvae fed on

diet T_2 being weak and vulnerable to ammonia stress (Sushma *et al*., 2021). Additionally, low growth in larvae fed on diet T_2 indicated low energy reserves for effective oxygen uptake required to minimise stress effects during un-ionised ammonia exposure through ammonia oxidation processes. However, this study's findings did not compare with reports for *Lates calcarifer* after 40–50% of the fishmeal in the species' diet was replaced by *S. platensis* (Abdel-Latif *et al*., 2022). The differences were attributed to body sizes, species-specific responses to stressors and lipid types.

There are reports of increased cellular responses in fish fed on high-lipid diets due to the provision of sufficient fatty acids for enhanced survival and tolerance to stress (Mesa-Rodriguez *et al*., 2018; Esmaeili *et al*., 2022). Therefore, *C. gariepinus* larvae were fed on a diet of 75%*E. fetida* were expected to be less sensitive to un-ionised ammonia stress because of their high lipid levels (10.4% dry matter). Nevertheless, their low growth suggested a reduced energy supply or reserve for enhanced stress tolerance. A signal of low lipid utilisation despite high lipase activity (chapter 5). Therefore, the supply of cellular metabolite activities to enhance environmental stress tolerance in *C. gariepinus* larvae was limited during the study period. This study's findings did not allude to the fact that the availability of dietary lipids for physiological processes depends on the feed quality and quantity consumed (Kumlu *et al*., 2021).

Low-stress indices in larvae fed on *E. fetida* compared to those fed on equal proportions of *S. platensis* replacing *C. nilotica* in a diet demonstrated *C.*

gariepinus larvae variable abilities in utilising different protein and lipid sources to enhance stress tolerance. Variable utilisation of lipids in the current study to enhance stress tolerance in larvae fed on both *E. fetida* or *S. platensis* suggests differences in lipid classes in these diets (Awed *et al*., 2020; Sushma *et al*., 2021; Abdel-Latif *et al*., 2022). Consequently, *the S. platensis* diets possibly provided sub-optimal HUFA for enhanced ammonia stress tolerance.

A comparison of stress indices between four- and six-weeks-old *C. gariepinus* using a t-test revealed reduced stress indices in older larvae to suggest reduced sensitivity to un-ionised ammonia or improved larvae robustness (Table 6.3). This was attributed to enhanced gill functionality over time. At the initial weeks of culture, larval gills were small-sized for un-ionised ammonia excretion efficiency. Therefore, four-week-old larvae's ammonia excretion relied on cutaneous simple diffusion or active transport for ion exchange since excretory and detoxifying sites were inferior in clearing ammonia. Furthermore, the current study findings were similar to those reported for earlier life stages of *C. gariepinus* exposed to different toxicants (Nguyen & Janssen, 2002). This is in line with earlier reports of increased stress sensitivity at earlier life stages of an organism because of their high mass-specific metabolic rates (Esmaeili *et al*., 2022). Therefore, the current study proposes *E. fetida* diets in larval nutrition for improved larvae quality at a low feed cost compared to *S. platensis* and current commercial (gemma micro) diets. However, the study finding deviated from increased stress tolerance reported for five-day-old tilapia larvae compared to 25-day-old tilapia (Luz *et al*.,

2012). The increased stress tolerance in the fifth-day-old tilapia suggested possible variabilities in ammonia levels during yolk deamination and species-specific responses to ammonia stress.

Spirulina platensis diets were characterised by high total mortalities (Figures 6.2a and 6.2b), stress indices (Table 6.2), and a shorter survival time for 50% of *C. gariepinus* larvae to die when exposed to un-ionised ammonia concentrations compared to *E. fetida* diets (Table 6.1). This was attributed to variable lipid classes, nutrient alteration in the process of drying (sun and oven drying) before diet formulation, and differences in digestibility and assimilation of plant and animal protein in the gastrointestinal tract of larvae (Verreth *et al*., 1992; Munguti *et al*., 2021). This finding contradicted previous findings that *S. platensis* reduces toxic effects in *C. gariepinus* through the neutralisation activities of antioxidants (Sayed & Authman, 2018; Awed *et al*., 2020). This might be because of the differences in *S. platensis* used in both studies depending on their different culture media, age at harvesting, and whether fish larvae earned immunity from previous exposure to toxicants.

Clarias gariepinus larvae quality was enhanced by *E. fetida* diets and comparable to those reported for the Caspian roach (*Rutilus caspicus*) larvae and mirror carp, where *E. fetida* replaced herring meal (Rawling *et al*., 2014; Rufchaei *et al*., 2019). The similarity was attributed to bioactive molecules like peroxidase and lysozymes in *E. fetida* meal, which improve stress tolerance and growth in fish larvae (Tacon *et al*., 1983).

6.6 Conclusions and Recommendations

The study demonstrated that incidental mortalities are possible during stocking regardless of the larval diet used in the hatchery. Replacing *C. nilotica* in the diet with 50% and 75% of *E. fetida* reduced total mortality in *C. gariepinus* larvae exposed to unionised ammonia. In addition, *C. gariepinus* larvae fed on *S. platensis* were less tolerant, as they took a shorter survival time before 50% of the larvae exposed to unionised ammonia died. *Clarias gariepinus* larvae were more robust when fed on *E. fetida* diets, as evidenced by lower stress indices, though this was reduced at 75% *E. fetida* diet. *Clarias gariepinus* larvae fed on 50%*E. fetida* and 50% C. *nilotica* were less (low-stress index) sensitive to unionised ammonia. *Clarias gariepinus* larvae ammonia stress tolerance increased over the growth period regardless of the diet used. The study suggests *E. fetida* diets for *C. gariepinus* larvae and extends the hatchery time for improved larvae quality and survival at a low feed cost. The findings will help farmers develop criteria for evaluating larval quality before stocking. More research is needed to determine whether 0.05 mg/l unionised ammonia concentration is above optimal unionised ammonia tolerance levels for *C. gariepinus* larvae.

CHAPTER SEVEN

7.0 EFFECTS OF HATCHERY NUTRITION ON NUTRIENT UTILIZATION, GROWTH PERFORMANCE AND SURVIVAL OF AFRICAN CATFISH FINGERLINGS IN THE GROW-OUT

7.1 Introduction

Aquaculture is the fastest-growing food industry globally, with 55 million metric tonnes from inland aquaculture in 2020 (FAO, 2022). However, the irregular and inadequate supply of fingerlings for stocking poses a significant threat to its upward trajectory (Estensoro *et al*., 2011; Kumar *et al*., 2022). A challenge associated with the dilution of larvae quality and increased inbreeding due to broodstock recycling over the years by hatchery managers.

Fish larvae quality challenges are often occasioned by reliance on live feed. Attempts to use dry diets in larval nutrition have not been fully realised because of the increased mortality and compromised fingerling quality associated with nutritional deficiencies in dry diets (Ali *et al.,* 2003). Low fish larvae quality and increased mortalities are influenced by many factors including individual fish attributes, feeding regime, feed quality, quantity, and feed availability (Pepin *et al*., 2014; Herawati *et al*., 2016; Rehman *et al*., 2017). Furthermore, failure to attain optimal fish growth may occur because of the high risk of starvation occasioned by the high nutritional demand of larvae (100% of their biomass per day). This has exacerbated the elusive economic viability of using nutritionally balanced, conveniently available, cost-effective and eco-friendly dry feed (Jena *et*

al., 2017; Joffre & Verdegem, 2019). Therefore, there is a serious push from fish nutritionists to find protein alternatives to formulate dry diets and the eventual replacement of both live and expensive dry feed in larviculture. However, the quality and composition of the formulated diets vary according to protein sources and their formulation process. Consequently, the performance of dry-formulated diets is variable and has resulted in discrepancies in fish larval performance and development.

Hatchery nutrition is important in the early life stages of fish and is key in determining the growth, quality and welfare of the organism's later life stages. This nutrition regulates larvae physiological functions and gene expression (Hvas *et al*., 2022). Supplementing commercial diets with *S. platensis* increased intestinal growth and absorptive surface in Tilapia fingerlings (Khalila *et al*., 2018). A similar trend was observed in African catfish larvae fed on mixed diets of equal proportions of live feed and commercial starter diet (Onura *et al*., 2018). The trend was due to protein-specific effects on intestinal growth and the proliferation of important intestinal macrobiotics in feed digestion (Estensoro *et al*., 2011; Khalila *et al*., 2018).

There is evidence of the starter diet's influence on fish larval digestive morphology and the physiological variations for feed-effective uptake (Jobling, 2016). Additionally, fish performance is affected by hatchery social factors, environmental conditions, a fish's physiological state, changes in the quality of its feed and feed nutritional composition, (Ali *et al*., 2003; Ramaswamy *et al*., 2013;

Al-Chokhachy *et al*., 2019; Biswal & Srivastava, 2021; Kumar, 2022). The impact of hatchery nutrition on later developmental stages has not been extensively studied (Py *et al*., 2022). This is possible because of limited comparative studies due to the use of variable protocols, different protein alternatives and ontogenetic shifts along the life cycle of a fish (Al-Chokhachy *et al*., 2019). This leaves missing links between hatchery nutrition and enhanced aquaculture production, aquaculture growth problems, resource use in crop cycles, environmental integrity, and enterprise cost-effectiveness (Das *et al*., 2016).

Past studies on how hatchery nutrition influences grow-out performance in fish are based on nutrition programming and feeding restrictions (Kumar *et al*., 2022). Larviculture's many challenges include poor feed quality and high stocking densities, which result in inherent fish stressors whose impacts are passed on to grow-out fish farmers. This is even worse considering that fingerlings and feed are variable costs to fish farmers and determine the farm's profitability through enhanced growth, survival, and the time taken to reach market size. Based on feed quality and individual fish larvae attributes, hatchery nutrition gives variable fingerling weights and lengths for stocking. It is therefore important to raise awareness about the value of weaning nutrition in the current aquaculture intensification. In light of the above, the study intends to provide insights linking starter diets and grow-out performance while contributing to the recognition of hatchery nutrition in aquaculture production. This study aimed to evaluate the effects of hatchery nutrition on grow-out nutrient utilisation, growth performance,

and survival of *C. gariepinus* fingerlings under a greenhouse. To achieve these, the study-specific objectives were:

1. To determine the effects of hatchery nutrition on nutrient utilisation of *Clarias gariepinus* fingerlings in grow-out tanks
2. To determine the effects of hatchery nutrition on the growth performance of *Clarias gariepinus* fingerlings in grow-out tanks
3. To evaluate the effects of hatchery nutrition on *Clarias gariepinus* fingerlings' survival in grow-out tanks
4. To assess water quality in the culture of *Clarias gariepinus* fingerlings in grow-out tanks

7.2 Materials and Methods

7.2.1 Study Setup

The study was conducted at Makindi fish farm, Kandara sub-county, Murang'a County, Kenya, 73 kilometres from Nairobi and 30 kilometres north of Thika town, at an elevation of 900 to 3,353 metres above sea level. The sub-county has a bimodal rainfall pattern with an average annual rainfall of >800 mm to the southeast and 2600mm to the northwest and a temperature range between 12 and 200 °C. Makindi Fish Farm was chosen because of its accessibility; aquaculture was already going and the farm management willingly allowed the use of their facilities for the study

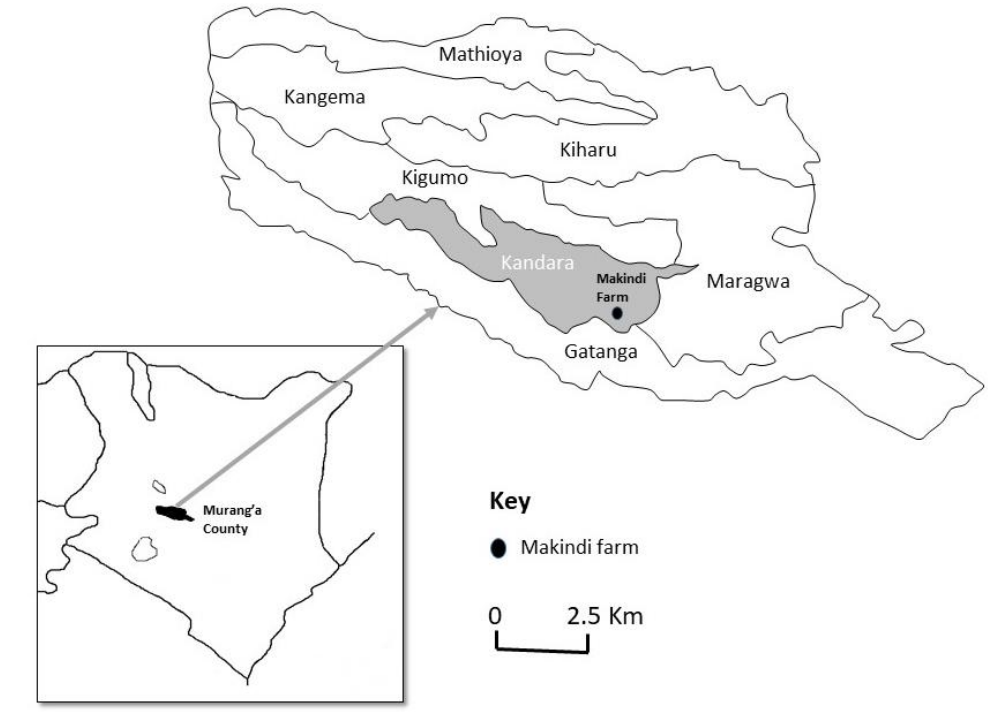

Figure 7.1: Makindi fish farm in Kandara sub-County, Murang'a County, Kenya (Drawn using Google Earth).

7.2.2 Hatchery nutrition

Eight-week-old *C. gariepinus* larvae with hatchery nutrition of *S. platensis*, *E. fetida* or a commercial (Gemma micro) diet were used for stocking in the grow-out tanks. Eight hatchery diets represented different nutrition histories/ hatchery nutrition for the fingerlings. These eight-hatchery nutrition were T_1=25%*S. platensis* + 75%*C. nilotica*, T_2=50%*S. platensis* + 50%*C. nilotica*, T_3=75%*S. platensis* + 25%*C. nilotica*, T_4=25%*E. fetida* + 75%*C. nilotica*, T_5=50%*E. fetida* + 50%*C. nilotica*, T_6=75%*E. fetida* + 25%*C. nilotica*, T_7=100%*C. nilotica* and T_0=a commercial diet (Gemma micro) are available to farmers.

7.2.3 Concrete Tank Preparation and Water Quality Measurement

Two concrete tanks measuring 3 m x 3 m x 1.5 m held 24 hapa nets of 0.25 m^3 under a greenhouse. The tanks were cleaned, disinfected with four kilogrammes of industrial salt (sodium chloride) per tank and dried for two weeks. The brown Makindi river water was sieved through clean cotton clothing to remove excess silt, then pumped into prepared tanks to fill up to two-thirds. There was no aeration in the tanks and the photoperiod was natural. A pH meter (MW106 having a temperature sensor (Milwaukee in Milwaukee, USA) was used to measure water pH temperature and respectively. Dissolved oxygen was measured using a dissolved oxygen probe connected to a metre (AZ8403, Bioevopeak Co., Ltd., Shandong, China). Ammonia was measured using a JBL ammonium test kit (JBL GmbH & Co., KG, Neuhofen, Germany).

7.2.4 Experimental Design

Fingerlings fed on hatchery nutrition above were obtained from the Department of Biology's Aquaculture Laboratory, University of Nairobi (UoN), Nairobi City County, Kenya. The fingerlings were pooled per treatment, and placed in open plastic buckets of 50 litres filled up to a third with culture water. These fingerlings were transported to the Makindi fish farm in Murang'a County, Kenya, for stocking. Ten-week-old *C. gariepinus* fingerlings with significantly different ($p<0.05$) body weights ranging from 0.60 - 1.51 g were stocked in the concrete tanks under a greenhouse. The different body weights at stocking were considered

effects of the eight different starter diets (hatchery nutrition) in *C. gariepinus* fingerlings from the controlled conditions (hatchery). One hundred and fifty C. *gariepinus* fingerlings per treatment were randomly but equally distributed into hapa nets in triplicate. fingerlings were stocked at 25 fingerlings/ hapa net two-thirds dipped in the tank water. This stocking density was lower than the farm stocking densities of 200 fingerlings/m^2. To eliminate tank effects, each tank was randomly assigned one of the two commercial diets (Raanan, Raanan Fish Feed Limited, I.Z. Milouod, Israel, or Skretting, Nutreco Feed Company, Netherlands) provided by the fish farm. The choice of the two diets for grow-out was based on their enhanced performance compared to other commercial diets on the market under similar culture conditions and their availability at the fish farm. The fingerlings were fed at an economically feasible rate of 5% of their body weight (Ikeogu *et al*., 2020), at a frequency of two times a day (10 a.m. and 3 p.m.), according to the farm's practices, for eight weeks. The fish farm provided information describing the two diets based on their analysis. All diets had a size of 800 and 1600 microns for the four weeks and weeks five to eight fingerlings, respectively. Raanan (A) diet had 44% crude protein and 12% crude fat, while Skretting (B) diet had 40% crude protein and 12% crude fat. Food rations were adjusted weekly based on fingerling wet body weight. Once a week, about a third of the bottom water in each concrete tank was disposed of through a valve on the lower side of each tank and replaced by clean and sieved river Makindi water of equal volume to ensure the water quality was preserved during the culture period.

7.2.5 Data Collection

To ensure high representation, 15 *C. gariepinus* fingerlings in each replicate and 45 fingerlings per treatment/tank were netted out of the hapa every two weeks (with replacement), counted and anaesthetised in ice-cold water for further analysis. On a wet measuring board, fingerlings were measured for total length to the nearest 0.1 cm, blot-dried, and weighed to the nearest 0.001g using a desktop weighing balance. At the start and the end of the experimental period, 15 fingerlings from each treatment were randomly removed (without replacement) weighed and oven dried at 104ºC for four hours to a constant weight. The difference between the start weight and weight after oven drying was used to calculate the Specific Growth Rate (SGR) evaluation. All food rations and adjustments were recorded for the analysis of nutritional efficiency. Data obtained was used to determine nutrient utilisation, survival, and growth of *C. gariepinus*. Weight gain, total length, specific growth rate and percentage survival were determined using the formulae 4.1, 4.2, 4.4 and 4.5 respectively.

7.3 Results

7.3.1 Growth in *Clsrias gariepinus* Fingerlings *with S. platensis* and *E. fetida* Hatchery Nutrition in Grow-out Tanks

Hatchery nutrition affected the growth of *C. gariepinus* fingerlings fed on Raanan or Skretting commercial diets in the grow-out tanks (Table 7.1). After eight weeks of feeding, fingerlings with a hatchery nutrition y of 75%*E. fetida* + 25%*C.*

nilotica (T_6) had the highest and significantly different ($p<0.05$) weight gains SGR and total body length) in the grow-out tanks. These fingerlings with a hatchery nutrition of diet T_6 recorded the highest weight gain (13g and 12.85 g for Ranaan and Skretting, respectively) in the grow-out ponds. This weight gain exceeded that of hatchery nutrition of commercial and T_5-fed larvae in the grow-out. However, fingerlings that had a hatchery nutrition of T_1 (25%*S. platensis* + 75%*C. nilotica*) gained the least weight when fed on Raanan or Skretting in the grow-out tanks, gaining only 7.11±0.15g and 6.56±0.13g, respectively. This fingerling also reported the lowest SGR and total body length for Raanan and Skretting.

The SGR for fingerlings with hatchery nutrition of diet T_6 was not different ($p =0.33$) from 4.33±0.02%/day posted by fingerlings with 100% C. *nilotica* hatchery nutrition for the Raanan diet. Notably, the nutrition histories of *E. fetida* enhanced their growth in the grow-out tanks compared to those of *S. platensis* under similar culture conditions. *Clarias gariepinus* fingerlings' total body length differences ($p = 0.54$) were not observed in the grow-out tanks when fed on either Raanan or Skretting. Generally, all growth parameters were higher in fingerlings fed on Raanan than in Skretting. However, no significant differences were discerned when compared using a student t-test.

Table 7.1: Growth performance (Mean ±SE, 45) of *C. gariepinus* fingerlings with *S. platensis* and *E. fetida* hatchery nutrition in grow-out tanks.

	Stocking		Raanan (A)			Skretting (B)		
Diets	Length(cm)	Weight(g)	Length(cm)	Weight gain(g)	SGR(%/day)	Length(cm)	Weight gain(g)	SGR(%/day)
T_1	4.85±0.16^{c}	0.87±0.07^{cd}	18.03±0.21^{b}	7.11±0.15^{e}	3.44±0.04^{e}	17.95±0.21^{b}	6.56±0.13^{e}	3.22±0.03^{f}
T_2	4.3±0.15^{d}	0.60±0.03^{d}	17.88±0.15^{b}	7.37±0.08^{de}	3.57±0.02^{d}	16.95±0.14^{b}	7.04±0.15^{d}	3.30±0.06^{f}
T_3	4.59±0.11^{d}	0.69±0.06^{d}	17.84±0.13^{b}	7.76±0.13^{d}	3.77±0.03^{c}	17.19±0.16^{b}	8.48±0.13^{d}	3.44±0.02^{e}
T_4	5.31±0.12^{b}	1.10±0.09^{b}	17.82±0.18b	12.02±0.12^{c}	3.98±0.02^{b}	18±0.61^{ab}	11.30±0.24^{c}	3.74±0.01^{d}
T_5	5.47±0.17^{a}	1.32±0.13^{a}	18.90±0.15^{ab}	12.73±0.15^{ab}	4.03±0.02	18.69±0.17^{ab}	12.70±0.08^{ab}	3.77±0.11^{b}
T_6	4.94±0.14^{b}	0.81±0.12^{cd}	20.10±0.17^{ab}	13.01±0.11^{a}	4.45±0.01^{a}	20.12±0.22^{a}	12.85±0.09^{a}	4.17±0.04^{a}
T_7	5.00±0.19^{b}	0.83±0.11^{bcd}	19.53±0.10^{ab}	12.71±0.13^{ab}	4.33±0.02^{a}	19.62±0.19^{ab}	12.62±0.22^{ab}	3.86±0.18a^{b}
T_0	6.09±0.17^{a}	1.51±0.13^{a}	20.97±0.20^{ab}	12.40±0.15^{ab}	4.02±0.0.02^{b}	19.45±0.16^{a}	11.92±0.23^{b}	3.60±0.17^{b}
F	11.27	12.37	131.982	411.09	225.48	154.26	207.83	158.19
p	0.01	0.01	0.002	0.01	0.03	1.03E-56	1.60E-137	0

**SGR=specific growth rate, Different superscript values differ significantly different (p<0.05), hatchery Nutritional were: T_1=25%S. platensis + 75%C. nilotica, T_2=50%S. platensis+ 50%C. nilotica, T_3=75%S. platensis+ 25%C. nilotica, T_4=25%E. fetida + 75%C. nilotica, T_5=50%E. fetida + 50%C. nilotica, T_6=75%E. fetida + 25%C. nilotica, control (T_7) =100%C. nilotica, T_0 = a commercial diet (Gemma micro) available to farmers.*

7.3.2 Nutrient Utilization of *Clarias gariepinus* Fingerlings with *S. platensis* and *E. fetida* Hatchery Nutrition in Grow-out Tanks

All fingerlings with different hatchery nutrition and fed on Raanan or Skretting posted FCR in the ranges of 1.19±0.01 to 2.38±0.04 throughout the experiment (Table 7.2). The FCR progressively decreased until week six of the study before increasing in week eight. When fed Raanan or Skretting diets, fingerlings with a hatchery nutrition of T_6 (75%*E. fetida* and 25%*C. nilotica*) had significantly different ($p<0.05$) and lowest FCRs of 1.2 and 1.4, respectively, in the grow-out tanks. Though not significantly different ($p = 0.11$) from T_7 (100%*C. nilotica*) hatchery nutrition when fed on Raanan or SkrettingAt the end of this experimental period, the hatchery nutrition of 25%*S. platensis* and 75*%C. nilotica* (T_1) was least utilised by fingerlings to produce significantly different ($p<0.05$) and the highest FCRs of 1.65±0.01 and 1.79±0.01 for Raanan and Skretting respectively. An FCR of 1.60 was posted by fingerlings with hatchery nutrition of T_0 (Gemma micro) and fed on either Raanan or Skretting, in the grow-out. All experimental diets posted FCRs lower than 2 by the end of the study. Fingerlings with a hatchery nutrition of *S. platensis* had relatively higher and poorer FCRs than *E. fetida*.

Table 7.2: Feed conversion ratios of *C. gariepinus* fingerlings with *S. platensis* and *E. fetida* hatchery nutrition in grow-out tanks.

	Time (Weeks)							
	Raanan (A)				**Skretting (B)**			
Diets	**2**	**4**	**6**	**8**	**2**	**4**	**6**	**8**
T_1	1.94±0.05^{bc}	1.59±0.01^{a}	1.36±0.02^{a}	1.65±0.01^{a}	2.02±0.04^{b}	1.74±0.01^{c}	1.43±0.01^{a}	1.79±0.01^{a}
T_2	2.12±0.03^{a}	1.35±0.01^{a}	1.27±0.03^{c}	1.38±0.01^{d}	2.39±0.04^{a}	1.96±0.02^{a}	1.28±0.02^{c}	1.46±0.00^{d}
T_3	2.01±0.03^{a}	1.38±0.02^{a}	1.19±0.01^{d}	1.36±0.01^{d}	2.27±0.05^{a}	1.96±0.02^{a}	1.15±0.00^{d}	1.46±0.00^{d}
T_4	1.80±0.05^{b}	1.39±0.02^{d}	1.22±0.01^{d}	1.36±0.00^{d}	2.05±0.04^{b}	1.60±0.00^{d}	1.14±0.02^{d}	1.47±0.00^{d}
T_5	1.76±0.01^{c}	1.38±0.01^{b}	1.27±0.01^{d}	1.48±0.01^{d}	1.79±0.01^{c}	1.89±0.01^{b}	1.17±0.01^{d}	1.56±0.00^{d}
T_6	1.68±0.02^{b}	1.29±0.02^{d}	1.14±0.01^{e}	1.19±0.01^{e}	2.10±0.03^{b}	1.6±0.01^{d}	1.09±0.00^{e}	1.39±0.00^{e}
T_7	1.79±0.02^{b}	1.32±0.01^{d}	1.140.01^{e}	1.24±0.03^{e}	2.09±0.03^{b}	1.64±0.01^{d}	1.07±0.01^{e}	1.40±0.00^{e}
T_0	1.67±0.02^{c}	1.46±0.02^{ab}	1.30±0.01^{b}	1.60±0.01^{a}	1.73±0.02^{c}	1.90±0.02^{ab}	1.34±0.00^{b}	1.60±0.00^{b}
F	39.0	116.	1665	213	31.6	43.6	33.6	398
P	0.02	0.01	0.00	0.00	0.01	0.01	0.01	0 .00

Different superscript values differ significantly different ($p<0.05$), and hatchery Nutrition were: T_1=25%S. platensis + 75%C. nilotica, T_2=50%S. platensis+ 50%C. nilotica, T_3=75%S. platensis+ 25%C. nilotica, T_4=25%E. fetida + 75%C. nilotica, T_5=50%E. fetida + 50%C. nilotica, T_6=75%E. fetida + 25%C. nilotica, control (T_7) =100%C. nilotica), T_0=a commercial diet (Gemma micro)

7.3.3 Survival of *Clarias gariepinus* Fingerlings with *S. platensis* and *E. fetida* Hatchery Nutrition in Grow-out Tanks

All fingerlings fed on either Raanan or Skretting posted survival percentages above 86% at the end of the grow-out growth period. Fingerlings with hatchery nutrition of T_2 (50%*S. platensis* + 50%C. *nilotica)* and T_3 (75%*S. platensis* + 25%*C. nilotica*) had respectively high survival rates of 92–93% for Raanan and Skretting (Figure 7.2). *Clarias gariepinus* fingerlings fed on Raanan or Skretting after a hatchery nutrition of 100% *C. nilotica* recorded a survival range of 90–92%. However, fingerlings with a hatchery nutrition of T_6 (75% E. *fetida* + 25%*C. nilotica*) had the lowest survival when they fed on Raanan or Skretting in the grow-out tanks, followed by the survival of fingerlings with a hatchery nutrition of T_1 (25%*S. platensis* + 75%*C. nilotica*). Fingerlings with *E. fetida* hatchery nutrition had lower survival though, without significant differences (p=0.10) from those having *S. platensis* diets and 100% *C. nilotica* hatchery nutrition**.**

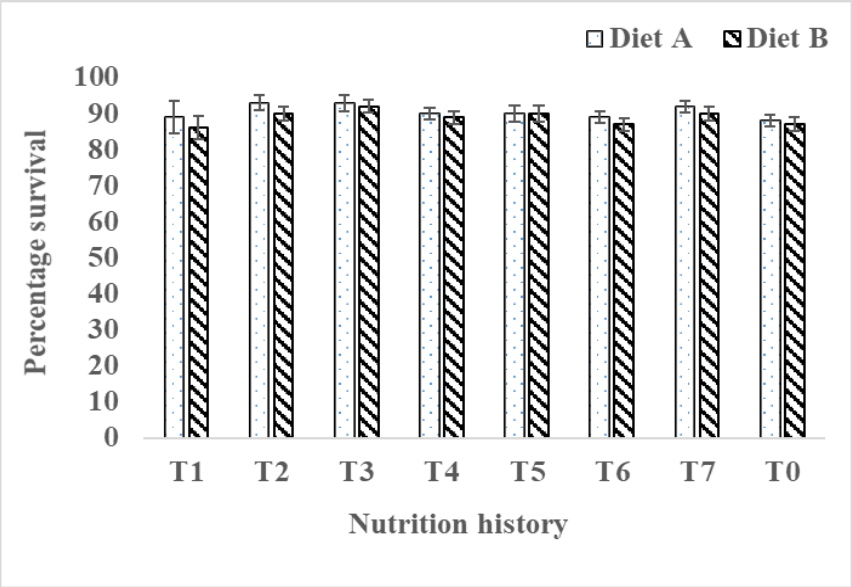

Figure 7.2: Percentage survival (% mean ± SE) of *C. gariepinus* fingerlings with *S. platensis* and *E. fetida* hatchery nutrition fed on commercial diets in grow-out tanks.
(T_1=25%S. platensis + 75%C. nilotica, T_2=50%S. platensis+ 50%C. nilotica, T_3=75%S. platensis+ 25%C. nilotica, T_4=25%E. fetida + 75%C. nilotica, T_5=50%E. fetida + 50%C. nilotica, T_6=75%E. fetida + 25%C. nilotica, control (T_7) =100%C. nilotica), T_0=a commercial diet (Gemma micro)

7.3.4 Water Quality Parameters Monitored during the Study

The feeding schedule for *C. gariepinus* fingerlings fed on the commercial diets, Raanan or Skretting, did not significantly differ (p = 0.08) affect water quality parameters (Table 7.3). Water temperature ranged between 25 and 26◦C, a pH of 7, a dissolved oxygen level of 4.6–5.1 mg/l, and ammonia of 0.40±0.00-0.49±0.02 mg/l were similar for both Raanan and Skretting and were within the standard requirements for *C. gariepinus* (Boyd, 2017).

Table 7.3: Water quality (Mean ± SE, n=3) parameters monitored in the grow-out tanks for eight weeks.

	Raanan (A)				Skretting (B)			
Weeks	**Temp (◦C)**	**DO (mg/l)**	**pH**	**NH_3(mg/l)**	**Temp (°C)**	**DO (mg/l)**	**pH**	**NH_4(mg/l)**
0	24.8±0.29	4.81±0.29	7.04±0.04	0.49±0.01	25.1±0.34	5.06±0.23	7.07±0.03	0.49±0.01
2	26.2±0.39	4.95±0.41	7.03±0.03	0.47±0.00	26.3±0.38	5.09±0.33	7.05±0.03	0.48±0.00
4	25.7±0.37	4.64±0.34	7.03±0.04	0.48±0.01	25.7±0.36	4.93±0.32	7.04±0.04	0.40±0.01
6	25.8±0.37	4.90±0.32	7.02±0.04	0.47±0.02	25.8±0.38	5.08±0.30	7.04±0.04	0.40±0.02
8	25.8±0.40	4.71±0.31	7.00±0.03	0.40±0.02	25.7±0.40	4.85±0.29	7.03±0.03	0.48±0.00
F	2.04	0.12	0.15	0.05	1.34	0.1	0.1	0.03
P	0.09	0.98	0.94	0.217	0.27	0.98	0.94	0.17

Temp= temperature, DO= dissolved oxygen, NH_4 =total ammonia

7.4 Discussion

Starter diets for *C. gariepinus* have been formulated with different protein sources, and their influence on larval performance has been evaluated using larval biological attributes and water quality parameters. Of great importance are the growth and survival of the larvae. However, it is necessary to determine the effects of hatchery nutrition on grow-out nutrient utilisation, survival and growth. This provides an understanding of the dependence of sustainable aquaculture and later fish development on larval stage performance. Hatchery management affects the well-being of larvae, and suboptimal care at the hatchery results in increased fish larvae mortalities, stunted growth, and compromised well-being, all of which are transferable to fish farmers. Numerous factors, such as feed quality, feeding schedule, aggression, handling, or size- or age-dependent possibilities, can result in stunted growth or compromised well-being in fish larvae. These challenges are

not obvious to the farmers when they are supplied with fingerlings for grow-out. This study evaluates the effects of eight hatchery nutrition with *S. platen*sis, *E. fetida* protein or fishmeal on nutrient utilisation, survival and growth of *C. gariepinus* fingerlings in grow-out tanks. The goal was to determine whether similar conditions in grow-out tanks could eliminate the differences from hatchery management effects.

Fingerlings with a hatchery nutrition of T_6 (75% *E. fetida* + 25% *C. nilotica*) and T_7 (100% *C. nilotica*) had stocking weights of 0.81g and 0.83 g, respectively and 12g by the eighth week of the growth. Their weight gain surpassed what was reported for *C. gariepinus* fingerlings with hatchery nutrition of T_5 (50% *E. fetida* + 50% *C. nilotica*) and T_0 (Gemma micro), which had the highest weights of 1.32g and 1.51 g, respectively, at the time of stocking in grow-out tanks. Faster growth of *C. gariepinu*s fingerlings with T_6 and T_7 nutrition histories in grow-out was hypothesised to be a reaction of recovery from sub-optimal nutritional content or culture conditions during larval feeding. During hatchery rearing, many inherent stressors like feed quality cause growth arrests due to partial starvation or sub-optimal water quality (Das *et al*., 2016). Fingerlings with T_6 and T_7 nutrition histories had reduced growth in the hatchery, suggesting growth arrest. This was attributed to less supply of nutrients for optimal growth due to the increased content of non-digestible chitin and garlic smell in high *E. fetida* diets (Tacon *et al*., 1983; Mugo-Bundi *et al*., 2013; Musyoka *et al*., 2019). Chitin and garlic smell in a diet increase as the worm proportions increase leading to reduced diet

digestibility and palatability respectively. This results in a limited supply of nutrients for optimal larval growth and is one of the triggers for growth arrest. A similar trend of converging growth has been reported with the provision of relatable culture conditions and changes in the quality and quantity of feed (Ali *et al*., 2003).

Feeding fingerlings with nutrition histories of T_6 and T_7 on commercial diets, on Raanan or Skretting, in the grow-out tanks provided quality nutrients that were efficiently utilised to compensate for the growth arrest experienced in the hatchery. As an adaptation to nutrient insufficiency in the larval stage, *C. gariepinis* fingerlings possibly increased their appetite and ingested more to allow them to store the excess in readiness for any unfavourable conditions in the future. There are reports of enhanced growth when diets were changed and optimal conditions returned in *Leiocassis longirostris* (Zhu *et al*., 2004), *L. rohita* and *L. longirostris* (Py *et al.,* 2022), and Nile Tilapia (Passinato *et al*., 2015). The similarities are attributed to enhanced protein synthesis for improved tissue biomass accumulation. These findings of enhanced growth in fingerlings with a hatchery nutrition of 75%*E. fetida* deviated from those reported for African catfish fed to satiation after partial starvation (Ali & Jauncey, 2004) and Tilapia fingerlings stocked with variable sizes (Wainaina *et al*., 2022). This was probably because of the differences in diets used, age variations of the experimental fish, and duration of exposure during hatchery practices.

Clarias gariepinus fingerlings with a hatchery nutrition of 25%*S. platensis* + 75%*C. nilotica* (T_1) had the lowest weight gain of 7.11g and 6.56g for Raanan and Skretting diets, respectively, in the grow-out tanks. This was not expected since fingerlings with a hatchery nutrition of diet T_1 had a growth performance similar (P = 0.35) to that of T_6 (75%*E. fetida* + 25%*C. nilotica*) and T_7 (100%*C. nilotica*) at the start of the experimental period. Further, the fingerlings with hatchery nutrition of T_1 had the lowest and significantly different ($p<0.05$) survival (Figure 4.3), though they were robust (Table 6.2). Their robustness did not support their failure to accelerate growth in the grow-out tanks. It is assumed that these fingerlings (hatchery nutrition T_1) did not experience growth depression in earlier life stages. An indication of enough nutrient supply for optimal growth or no competition for space since they were few (lowest survival in the aquarium). Additionally, T_1 hatchery nutrition fingerlings had slow growth in the grow-out suggesting, severe effects from hatchery feeding. Changing from hatchery nutrition to Raanan or Skretting might have failed to provide enough nutrients or energy to counteract the negative hatchery effects that were transferred to grow-out tanks. This is because stressor metabolic adjustments are proportional to stressor severity (Kumkhong *et al*., 2020). These study findings agreed with those reported for Tilapia subjected to nutrition interventions (Kumkhong *et al*., 2020), Siberian sturgeon (*Acipenser baerii),* and Atlantic salmon (*Salmo salar*) in the return of favourable conditions (Hvas *et al*., 2022; Py *et al*., 2022). However, these studies' findings did not agree with the catch-up growth for Rohu, *L. rohita,*

and Chinese long-snout catfish, *L. longirostris* (Wu *et al*., 2001; Py *et al*., 2022). This implies that T_1 fingerlings were not feasible for grow-out farming because they were likely to extend crop cycle duration. Therefore, their rearing could increase the operating cost further due to the extra time and resources required to maintain fingerlings until they reach market size (Ali *et al*., 2016).

Nutrient utilisation efficiency was assessed using FCR and was well within a range of 1.1–2.4 for both diets, Raanan and Skretting, throughout the experiment (Table 7.2). The highest FCR values at the end of week two in the grow-out were attributed to the time it took fingerlings to adapt to new environmental conditions and diets. This is because the digestive system takes time to recover from the effects of hatchery nutrition (Py *et al*., 2022). At the end of eight weeks, the best FCR was 1.2 and 1.4, respectively, for Raanan or Skretting-fed fingerlings with hatchery nutrition of T_6 (75%*E. fetida* + 25%*C. nilotica*) and T_7 (100%*C. nilotica)* when fed on Raanan and Skretting diets, a signal of efficient nutrient utilisation. Provision of Raanan or Skretting diet to fingerlings with nutrition histories of T_6 and T_7 possibly provided favourable conditions that enhanced digestive morphology and physiological functionality for efficient conversion of nutrients into body biomass (Gabriel *et al*., 2018).

All FCRs had a decreasing trend up to the sixth week and were consistent with earlier reports of decreasing FCR when the diet for *C. gariepinus* fingerlings was changed (Tiamiyu *et al*., 2018; Ikeogu *et al*., 2020). The similarity was attributed to a feeding rate of 5% in both studies since it influences feed utilisation.

Nevertheless, all FCR for fingerlings fed on Raanan or Skretting diets were below 2 at the end of the eight weeks and within the recommended level for enhanced feed efficiency and profitability (Craig *et al*., 2017).

At the end of the eight weeks, fingerlings with a hatchery nutrition of 25% *S. platensis* and 75*%C. nilotica* (T_1) had FCRs of 1.7 and 1.8, compared to 1.6 posted by larvae fed on a commercial diet (T_0) for the Ranaan and Skretting diets respectively. These were significantly different ($p<0.05$) and higher than the FCR reported in other hatchery nutrition. This meant low nutrient assimilation for body mass buildup and a possible explanation for their low growth in the grow-out. Reduced growth means reduced profits for the farmer stocking fingerlings with hatchery nutrition of T_1 and T_0 due to the lengthened culture time.

Growth determines survival; however, organisms will not always grow to their maximum, resulting in the survival of the healthy ones only. Fingerlings with a hatchery nutrition of T_1 (25%*S. platensis*) had the lowest survival rate when fed on Raanan or Skretting in the grow-out. The low survival rate and poor FCR for *C. gariepinus* fingerlings with a T_1 hatchery nutrition presented them as low-quality fingerlings for stocking. Nevertheless, slightly low survival in *C. gariepinus* fingerlings with T_6 hatchery nutrition was recorded despite their fastest growth in the grow-out tanks. This low survival was not expected because energy reserves for survival are normally quickly replenished on the return of favourable conditions. Therefore, the decreased survival of *C. gariepinus* fingerlings with hatchery nutrition of T_6 was probably due to high physiological impacts. This is

because increased growth influences an equal amount of mortality in an organism. Fingerlings with T6hatchery nutrition had a higher growth rate during grow-out, resulting in cells damaged.

Clarias gariepinus fingerlings fed on Raanan diet with a hatchery nutrition of 50%*S. platensis* + 50%*C. nilotica* (T_2), 75%*S. platensis* + 25%*C. nilotica* (T_3), and 100%*C. nilotica* (T_7) recorded the highest survival during grow-out despite their growth variations at the start of the study. This suggests uniform physiological adjustments and the ability to acclimatise to prevailing conditions after feeding on Raanan or Skretting diets. These findings were similar to the 90% survival reported for Olive Flounder (Cho *et al*., 2005). However, the highest survival of 90–92% in the current study was not supported by the 100% survival for *C. gariepinus* fingerlings reported in another study (Ikeogu *et al*., 2020). This was probably because of differences in the nutrient composition or stored energy reserves (Ali *et al*., 2003).

Water quality influences the biological performance of cultured organisms and the economic performance of fish farmers. Water quality negatively influences fish by diverting the species' optimal functioning. Dissolved oxygen, pH, temperature and un-ionised ammonia measured in the current study remained within the acceptable limits for *C. gariepinus* fingerlings with no significant differences discerned. Therefore, water quality was not responsible for growth, nutrient utilisation or survival differences in the treatments in the grow-out tanks. At the end of the study period, the acceptable water quality levels in the parameters

evaluated contributed to improved nutrient utilisation (all FCRs were less than 2). The temperature and pH results were not similar to those found in tanks with young *C. gariepinus* fish fed Skretting at a 5% feeding rate (Temitayo et al., 2021). This difference is attributed to additional aeration in the earlier study compared to the use of static water in the current study.

7.5 Conclusion and Recommendations

Hatchery nutrition influenced *C. gariepinus* growth, nutrient utilisation, and survival in the grow-out tanks when fed on Raanan or Skretting commercial diets. *Clarias gariepinus* fingerlings with nutrition histories of T_6 and T_7 had the highest growth in grow-out tanks to overtake the growth of fingerlings with hatchery nutrition of diet T_5 and commercial diets. This translates into more aquaculture production for enhanced food security. However, there was reduced survival in fingerlings with hatchery nutrition of diet T_6 due to physiological cost trade-offs. All diets with an *S. platensis* hatchery nutrition and fed on Raanan or Skretting had the least ability to offset inherent stressors for improved growth performance in the grow-out. This means the utilisation of *S. platensis* nutrients was not optimal in providing the energy necessary to recover from growth arrests or inherent stressor effects transferred from the hatchery to the grow-out.

Consequently, fingerlings with a hatchery nutrition of T_1 were the most expensive to rear in the grow-out with reduced economic returns to the farmer due to the extra resources required to sustain them in tanks until they reached market size.

The study finds *C. gariepinus* fingerlings with hatchery nutrition of T_6 viable for aquaculture development due to feasible economic returns about the culture period. The study recommends feeding *C. gariepinus* larvae with diets of different protein levels (*S. platensis* or *E. fetida)* and feeding them with similar protein content in grow-out to evaluate time-to-market size attainment and profitability.

CHAPTER EIGHT

8.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1. Introduction

Spirulina platensis and *E. fetida* are natural feeds for fish in nature that can be cultured under controlled conditions to produce high biomass. They have a high protein content and can be manipulated nutritionally to fit different fish species and age-specific diets in aquaculture. Therefore, *S. platensis* or *E. fetida* have the potential to replace aquatic animal protein in the aquaculture feed industry, now that the world is under the threat of climate change. The skyrocketing plant protein and aquatic animal protein prices pose a challenge to the economic sustainability of the aquaculture feed industry. Based on the above, *S. platensis* and *E. fetida* partially replaced freshwater shrimp (*C. nilotica*) in *C. gariepinus* larvae dry starter diets. Experiments were designed under laboratory-controlled conditions (hatchery) to investigate the digestive capacity, growth, stress tolerance, nutrient utilisation and survival of *C. gariepinus* larvae fed on the formulated diets 48 hours post-hatching. These larvae were transitioned to fingerlings and their growout performance was evaluated at Makindi fish farm in hapa nets.

8.2: Growth Performance, Nutrient Utilisation and Survival of *Clarias gariepinus* Larvae under Controlled Conditions

Formulated diets variably influenced the growth performance and nutrient utilisation in *C. gariepinus* larvae. Growth performance and nutrient utilisation were low in larvae fed on 70%*E. fetida* and 50 -75%*S. platensis* diets. Low feed utilisation efficiency at high inclusion of *S. platensis* or *E. fetida* suggested feed wastage, compromised environmental integrity and economic losses due to low survival and high feed formulation cost. The water quality parameters evaluated did not indicate any threat to the environment or larvae in the culture water. Observation attributed to a higher water dilution rate. Every morning before feeding, aquaria were cleaned and about 50% of the bottom water was removed and replaced with fresh water before feeding. Therefore, minimal time was available for excess feed decomposition hence, low nitrogenous waste. However, this study sees a possibility of water pollution in intensive hatchery farms using *S. platensis* diets but a low frequency of water dilution.

Eisenia fetida diets were economically feasible because of high returns on investment (ROI >1) compared to ROI of < 1 in larvae fed on *S. platensis* and commercial diets. This suggests a need for increased efforts in producing *E. fetida* biomass for fish feed industries. Such efforts could drastically reduce the reliance of fish feed industries on shrimp meal or forage fish species protein. Thus, Enhanced availability of quality larval feed at a low cost compared to *S. platensis* and commercial diets. To the farmers, the availability of quality larval feed is one

of the main drivers of increasing larvae survival for supply and increased returns. This is important as hatchery returns are based on the total number of individual fish larvae available for sale and their selling price compared to their weight gain. Though growth may not be important in hatchery economics, it remains key in determining larval survival and optimal functionality and quality. The result will be increased fingerlings for stocking and a reduction in fish farmers' apathy.

The specific growth rate in larvae fed on 50% *E. fetida* diets was similar to those fed the commercial diet. Therefore, the use of *E. fetida* in aquafeed has the potential to maintain a balanced aquatic biodiversity while increasing aquaculture production and sustainability. Despite the outstanding growth performance of *E. fetida* diets over that of 100%*C. nilotica,* they have an opportunity cost of reduced returns on investment and price index. However, the study findings did deviate from earlier reports of the optimal performance of African butter catfish (*Schilbe mystus*) at 75% of *E. fetida* meal in a diet (Chakraborty *et al*., 2021). This variation was due to differences in catfish species, culture conditions, diet composition and protein content.

Clarias gariepinus larvae fed on *S. platensis* had lower survival, growth, poor nutrient and ROI of < 1 in all inclusion levels utilisation compared to those fed on *E. fetida.* Consequently, using *S. platensis* in hatchery feeding may decrease the number of fingerlings supplied for stocking and increase their financial returns. In addition, poor nutrient utilisation posted by larvae fed on *S. platensis* means increased water pollution and reduced contribution of aquaculture to the global

food supply. Higher performance of *E. fetida* diets compared to those fed on *S. platensis* compared to earlier reports of low survival in *Chitala chitala* fed on *S. platensis* compared to worm-based (*Tubifex tubifex)* diets (Sarkar *et al.,* 2006).

8. 3: Digestive Capacity of *Clarias gariepinus* Larvae

The digestive capacity of *C. gariepinus* larvae fed on formulated diets was determined using the activities of total protease, trypsin, alpha-amylase, and lipase. The aim was to contribute to understanding *C. gariepinus* larvae's ability to utilise *S. platensis* or *E. fetida* in formulated diets. A general progressive increase in digestive enzyme activities with age in all formulated diets signified enhanced complexity of *C. gariepinus* larvae's competencies in utilising different diets over time. However, the digestive capacity in *C. gariepinus* larvae was variably influenced by *E. fetida* and *S. platensis* diets.

Larvae were sampled in the morning before feeding and enzyme activity was expected to be highest assuming a conditioned response to hunger (García-Ortega *et al*., 2000). Nevertheless, larvae fed on diets T_2 and T_3 postest the lowest and significantly different ($p<0.05$) activities in all the enzymes evaluated compared to all other formulated diets at 28 DPH. The observation pointed to low nutritional status or possible starvation in larvae fed diets T_2 and T_3. Earlier reports that enzyme activities are stimulated in the presence of food support this observation Pradhan *et al*., (2024). Low enzyme activities in larvae fed on diets T_2 and T_3

were a possible reason for their failure to accelerate growth in the grow-out due to compromised digestive gland integrity for effective nutrient utilisation.

Clarias gariepinus larvae fed on diet T_5 (50%*E. fetida* replacing *C. nilotica)* had the highest and significantly different ($p<0.05$) digestive enzyme activity compared to all other formulated diets. This explains their enhanced growth, nutrient utilisation and survival. An indication of T_5 high nutritional quality and larvae fed on this diet possibly did not starve. Diet T_5 (50%*E. fetida* and 50%*C. nilotica*) stimulated digestive enzymes better to attain high nutrient utilisation. Therefore, this diet had less wastage and was environmentally friendly.

Trypsin plays key roles in activating other digestive enzymes and its activity levels indicate the status of digestive physiology. A drop-in trypsin activity is a signal of enhanced acid digestion, the presence of a functional stomach, or the exhaustion of substrate (Hamre *et al*., 2013). In the current study, trypsin activity dropped twice at 2 and 28 DPH. The second drop at 28 DPH came much later than the expected age of 14 DPH (Rønnestad *et al*., 2013). Generally, *C. gariepinus* larvae are transferred to nursery ponds at 14 DPH as most organs are developed and the body is pigmented for survival in various environments. However, the delayed drop of trypsin at 28 DPH was evidence of the slow development of digestive physiology under the influence of formulated dry diets. This implies a long time before larvae are ready for stocking and there is an inherent risk of mortalities in cases of transfer to nursery ponds before acid digestion is fully functional. In addition, delays in trypsin drops come with an

opportunity cost in the growth and rearing period before the sale fingerlings. This serves as a warning about the fingerling supply challenges. However, the survival of 60-81% posted in the current study could increase the number of fingerlings available for supply and stocking. However, comparing the results of this study with past research was a challenge due to differences in the enzyme assay protocols and variations between laboratory conditions

8.4 Stress Tolerance of *Clarias gariepinus* Larvae

The stress tolerance of *C. gariepinus* larvae was assessed to provide information on how formulated diets influenced larval resilience in various potential nursery or culture conditions after hatchery rearing. Formulated diets in the current study variably influenced larval quality. This was based on differences in survival time during exposure, total mortality and stress indices when *C. gariepinus* larvae were exposed to different unionised ammonia levels. *Clarias gariepinus* larvae fed *E. fetida* diets were less sensitive to unionised ammonia than those fed on *S. platensis* and therefore, of high quality. The improved quality of larvae fed on *E. fetida* was attributed to their low total mortality, longer survival and small stress index on exposure to different unionised ammonia levels compared to other formulated diets. This finding suggests enhanced development of un-ionised ammonia excretory sites and sufficient energy to support its excretion in larvae fed on *E. fetida*. An assumption supported by earlier reports on responses elicited by an organism towards a stressor is proportional to the intensity of stress effects.

Therefore, stocking larvae fed on *E. fetida* in ponds with some un-ionised ammonia have a higher chance of survival than those fed on *S. platensis* diets. Further, a longer survival time before the death of 50% of the exposed larvae allowed time for them to apply remedies to reverse un-ionised ammonia effects. Increased un-ionised ammonia tolerance in *E. fetida*-fed larvae was similar to those reported for Caspian roach (*Rutilus caspicus*) larvae by Rufchaei *et al*. (2019) and mirror carp where *E. fetida* replaced herring meal (Rawling *et al*., 2014).

Larvae fed on *S. platensis* had low nutrient utilisation (high FCR values) and growth performance was attributed to low digestive enzyme activities. This implies that low energy reserves were available to sustain stressful encounters. This might have left the larvae weak and vulnerable to stress. This study finding presents larvae fed on S. *platensis* as less robust for transfer to nursery ponds with unionised ammonia. Poor growth and nutrient utilisation are indicators of low energy reserves for enhanced stress-coping mechanisms. Thus, stocking larvae fed on *S. platensis* in ponds could lead to economic losses due to increased operation costs or enhanced mortalities. The low sensitivity to un-ionized ammonia stress posted by larvae fed on *E. fetida* was attributed to high lipid content hence more energy reserves for greater physiological efficiency in handling stress.

8.5: Grow-out Growth Performance, Nutrient Utilisation, and Survival of *Clarias gariepinus* Fingerlings in Tanks

The study aimed to provide information on how larviculture nutrition influenced fish performance in the grow-out phase. Most fish farmers face the challenge of stunted growth in the grow-out, resulting in high economic losses. As a result, most fish farmers lose their investments and are forced out of aquaculture. Therefore, it is important to relate pond performance to hatchery nutrition and feeding management.

Hatchery nutrition variably influenced nutrient utilisation, survival and growth of *C. gariepinus* fingerlings in grow-out tanks. *Eisenia fetida and S. platensis* hatchery nutrition had different effects on the weight increase, survival, and nutrient utilisation of *C. gariepinus* fingerlings fed with either Raanan or Skretting in the grow-out tanks. Fingerlings with *S. platensis* hatchery nutrition posted poor nutrient utilisation and low growth (compared to *E. fetida* diets). This suggests low ability in such fingerlings to offset hatchery nutrition effects in the grow-out. Failure of larvae fed on these *S. platensis* diets to accelerate growth in the grow-out was evidence of starvation during hatchery rearing and compromised digestive glands functionality. Despite higher survival in the grow-out, such larvae require a lengthy management period and extra resources to attain table size. The outcome is fish farmers' apathy and reduced contribution of aquaculture to food security

Unfortunately, *C. gariepinus* fingerlings with a hatchery nutrition of 25% *S. platensis* and 75%*C. nilotica* recorded an unexpectedly low survival rate and the lowest weight gain in the grow-out tanks. This was observed despite these fingerlings' respective larvae being ammonia stress-tolerant with moderate growth performance. This indicates compromised culture conditions in the grow-out or the inability of the larvae to offset hatchery nutrition or culture conditions' effects transferred from the hatchery to the grow-out tanks. Fish larvae' variable growth performance in the earlier stages is expected to be offset once larvae are subjected to similar culture conditions and management. slow growth posted by larvae with the hatchery nutrition of T_1 fingerlings suggested the absence of competition. However, stocking these larvae in hapa nets 25/ hapa restricted movement and possibly induced stress in the grow-out.

8.6 Conclusions

1. It is possible to replace *C. nilotica* with *S. platensis* or *E. fetida* in formulated diets up to 25% or 75% though, with reduced survival. It is also possible to replace fishmeal in commercial diet(T_0) up to 50%*E. fetida* in a starter diet without negative effects on growth. This will reduce reliance on fishmeal and enhance the availability of cheaper feed for hatchery feeding. Feed availability as may be demanded will encourage more farmers to invest in hatchery management and large quantities of fingerling will be produced and supplied for stocking.

2. The digestive capacity of *C. gariepinus* can be enhanced by replacing fishmeal up to 50%*E. fetida*. Fingerlings fed on diet T_5 had the highest competence in utilising diet macronutrients as indicated by the high enzyme activities in all enzymes estimated. This resulted in improved nutrient utilisation in larvae fed on T_5 translating to economic returns to hatchery managers and environmental sustainability. The enhanced digestive capacity in larvae fed on T_5 will encourage hatchery nutritionists and manufacturers to increase its use in larval feed.
3. *Clarias gariepinus* larvae fed on *E. fetida* diets were more robust with low mortalities, smaller stress indices and longest survival time in un-ionised ammonia. Farmers will benefit economically from the higher survival of stocking larvae fed on cheaper *E. fetida* in ponds with lower amounts of un-ionized ammonia. This will make aquaculture more resilient and sustainable in feed resources while increasing its contribution to the global food supply.
4. The study demonstrated that hatchery nutrition affected growth, survival and nutrient utilisation in grow-out. *Clarias gariepinus* fingerlings fed on 75% *E. fetida* (T_6) and 100% *C. nilotica* accelerated growth, improved nutrient utilisation and survival in the grow-out. This will guarantee food resilience and environmental integrity.
5. it is economically beneficial to invest in *E. fetida* as a feed input compared to *S. platensis* for use in the diets of *C. gariepinus* larvae

8.7 Recommendations

1. Encourage capacity-building in earthworm and *Spirulina* cultures to enhance production and improve nutritional quality to become alternatives to aquatic animal protein resources. Strengthening skills and abilities in earthworm and *Spirulina* production and processing these ingredients into quality fish feed will increase their supply and eventually reduce their market prices. Capacity building in the cultures of earthworms and *Spirulina* will also attract new entrants into the market, resulting in competition and competitive prices for quality products. This will push aquaculture towards a better future and ensure the adoption of climate-smart aquaculture.
2. Civic education should be conducted to increase community awareness on earthworms and *Spirulina* uses to produce quality organic fish products for human consumption. This will recruit more communities to accept fish raised on earthworms for improved consumption.
3. A comparative economic analysis of table-size *gariepinus* fed *E. fetida* and *S. platensis* diets. This will aid in making proper decisions about feed formulations for improved income and aquaculture sustainability.
4. Evaluation of heavy metal content in *S. platensis* and *E. fetida* ingredients and their resultant catfish larvae diets. This is important in ensuring safety standards for use in fish feed. Also, publishing heavy metal levels in *E. fetida* will boost confidence in using it as a feed ingredient or provide an

incentive to search for alternative methods to improve its safety for human consumption.

8.8 Proposed Further Research

1. Investigate the potential of improving the inclusion level of *E. fetida* in fish larval nutrition by reducing the chitin content in earthworm meal.
2. A study was conducted to determine the performance of combined *E. fetida and S. platensis.* This will exploit the synergy of the ingredients' nutritional content to the advantage of the larvae.
3. A study assessing compensatory growth after restrictive feeding of *E. fetida* and *S. platensis* diets to establish optimal performance at a minimum feed input.
4. A study to evaluate anti-nutritional factors and non-digestible proteins in *E. fetida* and *S. platensis* feed ingredients
5. Investigate the effects of amino acids like histidine on *C. gariepinus* larval survival.

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APPENDICES

Appendix 1: Approval of proposal by Faculty Biosafety, Animal Use and Ethics Committee

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
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Direct Line. 4448648

REF: FVM BAUEC/2021/382

Callen Onura,
Dept. of Biology
University of Nairobi
07/09/2021

Dear Onura,

RE: Approval of proposal by Faculty Biosafety, Animal use and Ethics committee

"An investigation on the growth, digestive capacity and stress tolerance of African cat fish (*Clarias gariepinus*) laevae fed on *Arthrospira platensis* and sludge worm as a partial replacement of fish meal (*Caridina nilotica*)'.
Callen Onura I80/51911/2017

We refer to your PhD. proposal submitted to our committee for review and your application letter dated 22nd September 2021. We have reviewed your application for ethical clearance for the study.

The protocols used to assess the growth, stress and digestive capacity for *C. gariepinus larvae* performance including Grow-out *C. gariepinus* growth performance through laboratory analysis meets the minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, Ph.D
Chairperson, Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi

Appendix 2: Protein Ingredients (*E. fetida, C. nilotica* and *S. platensis*) used in the formulation of the fish feed

a) E. fetida **b) *C. nilotica*** **c) *S. platensis. ineach of the diagram***

Appendix 3: Feed Formulation Process a). Mixing of measured ingredient proportions b). Blending of the worm c). Dough kneading and d). Pelleting.

A. B C D

Appendix 4: Formulated Diets Packed in Polythene Bags