NUTRITIONAL EVALUATION OF INSECT MEALS AS SUBSTITUTES FOR FISHMEAL IN *OREOCHROMIS MOSSAMBICUS* AND *CLARIAS GARIEPINUS* DIETS

By

LIVHUWANI EVA NEPHALE

THESIS

Submitted in fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

AQUACULTURE

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Agricultural and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO

SUPERVISOR: Prof. N.A.G. Moyo

CO-SUPERVISOR: Prof. M.M. Rapatsa-Malatji

2024

DECLARATION

I declare that the thesis hereby submitted to the University of Limpopo, for the degree of Doctor of Philosophy in Aquaculture has not previously been submitted by me for a degree at this or any other university; that this is my own work in design and in execution, and that all materials contained herein has been dully acknowledged.

_____ **02 /04/ 2024**

Livhuwani Eva Nephale (Ms.) Date

ACKNOWLEDGEMENTS

“Now unto the King eternal, immortal, invisible, the only wise God, be honour and glory for ever and ever, Amen”.

I thank God for granting me an opportunity to pursue my studies, for carrying me, and keeping me to complete this doctorate. *Yeshua* I thank you.

My main supervisor Prof N.A.G Moyo, I would like to thank you for your strong, competent, and fair supervisory skills. I acknowledge all the time you spent reviewing and improving the manuscript. It is an honour to be groomed under your supervision.

My co-supervisor Prof M.M. Rapatsa-Malatji, I acknowledge your commitment to co-supervise this study. Your input and time spent reviewing the manuscript is appreciated.

Mr Gavin Geldenhuys, thank you for the technical assistance offered to make this study possible.

Mr William J. Arries (Laboratory Analyst, GC-MS Division) of Central Analytical Facilities (CAF) of Stellenbosch University is appreciated for his assistance with amino acid and fatty acid profile analysis. Your competency and accuracy are outstanding.

I want to thank Mrs Manape Lekganyane (University of Limpopo, Biotechnology Unit) for always welcoming me to her laboratory for enzyme assay.

My parents: Mary Ramadimetja Nephale and Azwianewi Watson Nephale have been very supportive and encouraging toward my studies. Your love and prayers are appreciated.

My siblings: Phumudzo, Ndivhuweni and Akonaho, thank you for always rooting for me.

This study has been made possible through funding from the National Research Foundation and the University of Limpopo, Aquaculture Research Unit.

To my dear husband Lucky Justeen Mabidilala, thank you for your enormous support and encouragement to pursue my heart desires.

I am grateful to God for bringing Khathutshelo Cathrine into my life. Thank you for your immeasurable support and friendship.

I would like to thank my fellow colleagues: Selaelo, Fiona, Casswell, Maripa, and Mahuma for walking the academic journey with you.

My uncle Nephale Nndanduleni instilled in me a love for science at a young age. I am forever grateful for the beautiful childhood memories he granted me.

The Aquaculture Research Unit Staff: Eva, Clara, Dr. Raphalo, Dr. Mbokane, Dr. Mehrnoush, Jemina, Meggie, Molepo and KG are acknowledged.

SAPSE ACCREDITED PAPERS PUBLISHED FROM THE THESIS

1. Nephale, L.E., Moyo, N.A.G. and Rapatsa, M.M. (2023) Dietary full-fat Stinkbug (*Encosternum delegorguei*) meal effects on growth performance, blood chemistry, liver and intestinal histology of juvenile Mozambique tilapia (*Oreochromis mossambicus*). Cogen Food & Agriculture, 9(1), 2253717, https://doi.org/10.1080/23311932.2023.2253717 (**APPENDIX A**)

2. Nephale, L.E., Moyo, N.A.G. and Rapatsa-Malatji, M.M. (2024) Partial replacement of fish meal with soldier termite in juvenile Mozambique tilapia: Effects on growth performance, blood serum chemistry, and histomorphology. Journal of Animal and Feed Sciences, 33(2), 243-252, https://doi.org/10.22358/jafs/175919/2024 (**APPENDIX B**)

3. Nephale, L.E., Moyo, N.A.G. and Rapatsa-Malatji, M.M. (2024) Utilization of an insect-based diet by a herbivorous fish (*Oreochromis mossambicus*) and an opportunistic predator (*Clarias gariepinus*). Scientific African, 24, e02125, https://doi.org/10.1016/j.sciaf.2024.e02125 (**APPENDIX C**)

DEDICATION

Lucky Justeen

Gabriel Ronewa

ABSTRACT

The aim of this thesis was to investigate the potential of enhancing freshwater fish production through replacement of fishmeal with insect meals.

The effect of dietary full-fat Stinkbug (*Encosternum delegorguei*) meal on growth performance, blood serum chemistry, liver and intestinal histology, and digestive enzyme profile of *Oreochromis mossambicus* and *Clarias gariepinus* was evaluated. The Stinkbugs were purchased from the Thohoyandou Town open market. Five diets were formulated to partially substitute fishmeal at 0, 10, 30, 50, and 70%. The diets were formulated to be isonitrogenous (30% crude protein), isolipidic (12% fat content), and isocaloric (15 MJ/Kg). *Oreochromis mossambicus* juveniles (4.2 ± 0.3 g) were stocked randomly in triplicates at 15 fish per tank. *Clarias gariepinus* fingerlings (6.4 ± 1.02 g) were stocked randomly in triplicates at 5 fish per tank. The experimental system used for both species was the same. There was a decline in growth performance indices (SGR, TGC, FI, PER, APDC) with an increase in Stinkbug meal inclusion in both *O. mossambicus* and *C. gariepinus*. The decline in growth performance was more evident in inclusion levels above 30% in both species and it was attributed to the poor amino acid profile of the Stinkbug meal and its low (29%) protein content. The organosomatic indices (CF, VSI, HSI) were not influenced ($P>0.05$) by the inclusion of Stinkbug meal. The AST and ALT levels significantly increased at the highest inclusion level ($P<0.05$) in both *O. mossambicus* and *C. gariepinus*. This shows that an inclusion level above 50% may pose stress to these fish species. However, the liver and intestinal histology showed no significant alterations with higher inclusion levels in both *O. mossambicus* and *C. gariepinus*. This showed that the inclusion of the Stinkbug meal in the diets of these two species did not affect their liver and gastrointestinal tract. The digestive enzyme profile (amylase, protease, and lipase) showed that both *O. mossambicus* and *C. gariepinus* are probably equipped to utilise insect-based diets. Despite the decline in growth performance, cost-benefit analysis showed that the Stinkbug meal may be a sustainable alternative to fishmeal.

Due to poor amino acid, fatty acid profiles, and the low inclusion level of the Stinkbug meal, it was thus prudent to search for locally available alternative insects to potentially replace fishmeal in the diets of *O. mossambicus* and *C. gariepinus*. The Alates termites

have a better nutritional profile than the Stinkbug meal and are widely available. The utilisation of the Alates termite meal (*M. falciger*) based diets was compared between *O. mossambicus* and *C. gariepinus*. De-winged Alates termites were purchased from the Thohoyandou Town open market. Diet formulation and experimental set-up were adopted as in the previous chapter. The Alates termite meal replaced fishmeal up to 50% without compromising the growth performance of *O. mossambicus* and *C. gariepinus*. The higher inclusion level (50%) in Alates termite meal was partly attributed to its protein content (40.46%) which meets the requirements for *O. mossambicus* (30%) and *C. gariepinus* (40%). Moreover, the Alates termite meal recorded an Essential amino acid index (EAAI) (1.87) which was higher than the Stinkbug meal EAAI (0.11). However, at inclusion levels above 50%, SGR, WG, TGC, FI, PER, and APDC significantly declined ($P<0.05$) in both species. The organosomatic indices followed a similar trend. The ALT and AST levels were not influenced by the inclusion of Alates termite meal in *O. mossambicus*. On the other hand, these enzymes were significantly ($P<0.05$) elevated at inclusion levels above 50% in *C. gariepinus*. This shows that inclusion levels above 50% may impose stress on *C. gariepinus*. The liver and intestine of both fish species were not compromised even at the highest inclusion level. Normal histological micrographs of the liver and intestine were observed across experimental diets in both species. The cost-benefit analysis showed that substituting fishmeal with the Alates termite meal is economically viable. Defatting the Alates termite meal is recommended before incorporation into fish diets. The major limitation of Alates termites is their seasonal variation. It was thus important to search for an insect that could be harvested throughout the year within the geographical area.

The Soldier termite caste of *M. falciger* was also used as a potential insect to partially replace fishmeal in the diets of *O. mossambicus* and *C. gariepinus*. Unlike the Stinkbugs and the Alates termite, the Soldier termite is one of the insects that is available throughout the year. The Soldier termites were purchased from the Thohoyandou Town open Market. The same experimental diet formulation procedure was adopted as in the previous chapters. Juvenile *O. mossambicus* (9.70 ± 1.2 g) were randomly stocked in triplicates at a stocking density of 10 fish per tank. On the other hand, *C. gariepinus* fingerlings (5.3 ± 0.8 g) were randomly assigned their diets in triplicates at a stocking density of 5 fish per tank. The best growth performance

parameters (SGR, TGC, FI, PER, APDC) were recorded at the 50% inclusion level in *O. mossambicus*. On the contrary, a decline in growth performance with an increase in the Soldier termite meal was observed in *C. gariepinus*. Inclusion levels beyond 10% significantly reduced the growth performance and nutrient utilisation of *C. gariepinus*. The difference in growth performance of these two species was ascribed to their feeding habits. Herbivorous fish species have shown the potential to utilise diets with higher insect meal inclusion levels. The organosomatic indices were not ($P>0.05$) influenced by the inclusion of Solider termite meal in the diets of both *O. mossambicus* and *C. gariepinus*. The study showed that the health status of both fish species was not compromised. Histomorphology micrographs also confirmed the normal health status observed in both fish species. The study further showed that *O. mossambicus* and *C. gariepinus* have different digestive enzyme profiles and are differently equipped to digest insect-based diets. Substitution of fishmeal with Soldier termite meal may yield higher profit margins than using fishmeal-based diets in *O. mossambicus* and *C. gariepinus*.

The study showed that locally available insects have the potential to replace fishmeal in the diets of two commonly cultured warm freshwater species. The Alates termite meal is recommended to replace fishmeal in the diets of both species. The Soldier termite meal is recommended to replace fishmeal in the diet of *O. mossambicus*. Defattening of the Stinkbug meal is also recommended before incorporating into the fish diets. Digestion can also be improved through supplementation of Natuzyme50® in fish diets. This study showed that the use of locally available insects in the diets of commonly cultured freshwater species has the potential for higher profit margins in aquaculture enterprises. However, a detailed economic analysis is recommended. This may increase warm freshwater aquaculture production and improve the livelihood of local fish farmers.

TABLE OF CONTENT

CONTENT **Page**

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1:

GENERAL INTRODUCTION

CHAPTER 1: GENERAL INTRODUCTION

1.1 INTRODUCTION

1.1.1 Aquaculture Overview in South Africa

South Africa has been described as a country with a great potential for aquaculture production (Adeleke *et al*., 2021; DAFF, 2021). Aquaculture in South Africa was introduced in 1673 and 1676 with a culture of oysters (FAO, 2010). In the 1980s, trout, mussel, ornamental and catfish farming were established (Hecht and Britz, 1990). Aquaculture production in South Africa has been increasing in the past decade. However, an increase of only 4% was recorded from 2014 to 2015 (DAFF, 2017). This is significantly lower than the increasing rate of other African countries. South Africa's contribution to Africa's aquaculture production is negligible. In 2018, South Africa ranked the 10th country in the top 10 producing countries in Africa (Figure 1.1).

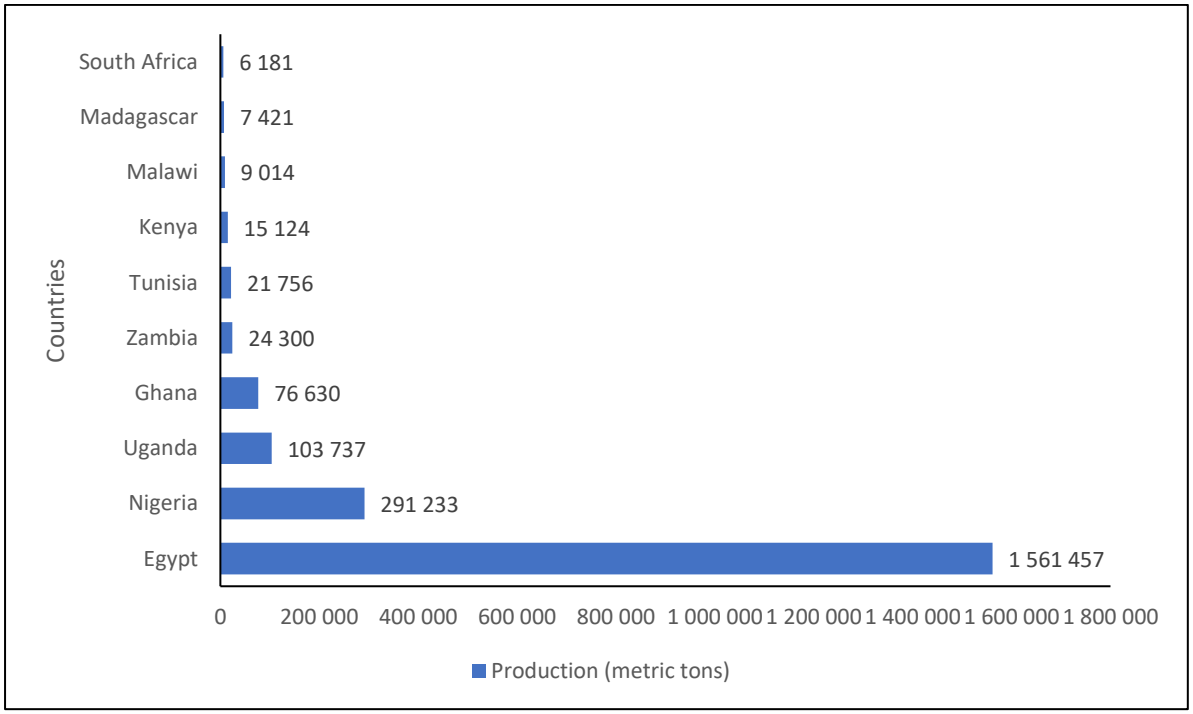

Figure 1.1: Top 10 aquaculture producers in Africa in 2018 (Adeleke *et al*., 2021).

Aquaculture in South Africa consists of freshwater and marine subsectors, with the marine subsector more developed than the freshwater. The marine subsector is dominated by molluscs, oysters and mussels and is mainly practised in the Western

Cape Province in Overberg (Adeleke *et al*., 2021). The main species farmed in marine aquaculture are abalone (*Haliotis midae*), Pacific oyster (*Crassostrea gigas*), mussels (*Mytilus galloprovincialis* and *Chromomytilus meridionalis*) and dusky kob (*Argyrosomus japonicus*) (DAFF, 2015). Abalone farming has been dubbed the powerhouse for South African marine aquaculture, contributing 76% of the total marine production (Britz and Venter, 2016). South Africa's marine aquaculture has a well-established policy, which was gazetted in 2007 (https://www.gov.za/sites/default/files/gcis_document/201409/30263.pdf)

Freshwater aquaculture in South Africa is mainly practised in Mpumalanga, Gauteng, and Limpopo Provinces (DAFF, 2016). Trout is a leading contributor to freshwater aquaculture and the two commonly farmed trout species are rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*). The Mozambique tilapia (*O. mossambicus*) is the second most produced freshwater species, after *O. niloticus* (DAFF, 2016). The African catfish (*C. gariepinus*), common carp (*Cyprinus carpio*) are also some of the farmed freshwater species in South Africa (DAFF, 2016).

1.1.2 Factors affecting aquaculture production.

The factors affecting aquaculture production in southern Africa were recently reviewed (Moyo and Rapatsa, 2021). These include physical, biological, and socio-economic factors. South Africa has limited suitable environmental conditions for aquaculture production (Britz and Venter 2016). The inland regions have water scarcity, due to the low annual rainfall. The main production system used in aquaculture production is earthen ponds, which require large volumes of water. South Africa has high summer temperature and low winter temperature which discourages either coldwater or warmwater freshwater species culture in open systems (Britz and Venter 2016). Furthermore, South Africa has a complex regulation regarding aquaculture production (Adeleke *et al*., 2021). The legislation is fragmented and involves different government departments such as the Department of Environment, Forestry and Fisheries (DAFF), Department of Environmental Affairs (DEA) and the Department of Water Affairs (DWA). These departments play different roles in the aquaculture sector. The complicated and ambiguous regulatory procedure delays permit issuing. Thus,

constraining aquaculture growth and development (Adeleke *et al*., 2021; Moyo and Rapatsa, 2021).

Among the biological factors, feed quality and high feed cost have been described as major hindrances to aquaculture production. Fish feed contributes between 60 and 70% of the total aquaculture production costs (Alfiko *et al*., 2022; FAO, 2016). In southern African, most fish feeds are imported, making them expensive. The principal source of protein in fish feed is the fishmeal. Fishmeal is considered a major protein source due to its high protein content, balanced amino acid profile, high digestibility, vitamins, and minerals that support fish development and health (Siddaiah *et al*., 2023; Shukla *et al*., 2019). Of the total annual global fish catch, about one-third is used in fishmeal production (Barlow, 2003). High demand for fishmeal in the animal feed industry has led to the overexploitation of wild fish, a decrease in human food security, and an escalation in fishmeal price (Liao *et al*., 2022). Global fishmeal price has been increasing for the past two decades and is currently selling at R 26 821.21 per metric ton (World Bank, 2022; Figure 1.2). It is predicted that in 2030, fishmeal price will rise by 30% compared to 2018 due to the increasing worldwide demand (Khieokhajonkhet *et al*., 2022). Moreover, high fishmeal price has also been prompted by the competition in the animal feed (poultry, livestock, fisheries) industry. Thus, fishmeal is no longer deemed sustainable to support the growth of aquaculture production.

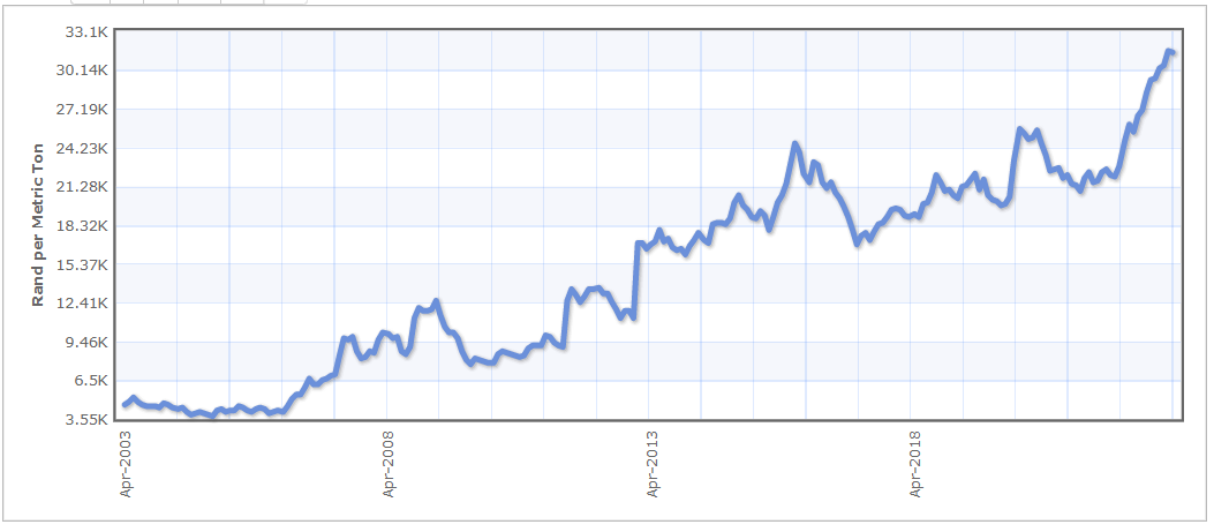

Figure 1.2: Global fishmeal price in Rand per metric ton from 2003 to 2023 (World Bank, 2022).

1.1.3 A search for an alternative protein source

Protein sources such as animal-based, and plant-based have been used as fishmeal replacements in aqua feed (Daniel, 2018). Animal protein sources such as fishery by-product (fish silage), terrestrial animal by-product (poultry, blood meal, meat and bone meal, feather meal) have also been employed as fishmeal replacement in the animal feed industry (El-Sayed, 2020). They have high protein content and good essential amino acid profile. However, some of these animal by-products are deficient in one or more essential amino acids such as lysine, isoleucine, and methionine.

Plant-based protein sources such as kikuyu grass (Hlophe-Ginindza *et al*., 2015; Hlophe *et al*., 2011), moringa leaf powder (Idowu *et al*., 2017; Hlophe and Moyo, 2014; Richter *et al*., 2003), soybean meal (Abdel-Warith *et al*., 2020; Liu *et al*., 2020) and cottonseed meal (Tiamiyu *et al*., 2013; Agbo *et al*., 2011; Soltan *et al*., 2011) have been widely explored in different fish species. The main challenge with kikuyu grass and moringa leaf powder is the presence of anti-nutritional factors that reduce growth performance, palatability, and digestibility. Moreover, these plant-based diets also showed a degraded liver and intestinal histology in the tested fish species (Hlophe and Moyo, 2014). In plant-based protein sources, soybean meal is deemed the most promising plant-based protein source in the animal feed industry (Moyo and Rapatsa -Malatji, 2023). This is attributed to its high protein content and a balanced amino acid

profile that meets the fish requirement and its wide availability (Liu *et al*., 2020; Shukla *et al*., 2019;). However, the limitations associated with soybean meal and other plant-based protein sources are their deficiency in sulphur-containing amino acids (methionine and cysteine), the presence of anti-nutrients such as protease inhibitors, lack of taurine and low palatability (El-Sayed, 2020, Novriadi, 2017). Moreover, an increase in demand for protein sources in the animal feed industry has resulted in an increase in soybean meal prices. Thus, plant-based protein sources are not a feasible solution in the aquaculture feed industry.

1.1.4 Insects as novel ingredient in aqua feed.

Insects have recently gained attention from researchers as novel ingredients for substituting fishmeal in aquafeeds. The European Union (EU) regulation has authorised the use of insect protein originating from seven insect species (Regulation 2017/893, Annex X) in animal feed production including aquaculture. These insects include black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*), and field cricket (*Gryllus assimilis*). The EU legislation further authorised silkworm (*Bombyx mori*) in 2021 November (European Union, 2021; European Union, 2017). The authorisation has also driven an increase in the number of trials using insect meals as fishmeal replacement. The major reason for the delay in authorising insect-derived protein sources in animal feed is the potential risk of mad cow disease (Bovine Spongiform Encephalopathy) (Renna *et al*., 2023). This is an incurable neurodegenerative disease of cattle transmissible to humans which occurred in the late ‘90s of the last century (Renna *et al*., 2022).

Insects form part of the natural diet of both marine and freshwater fish species (Makkar 2014; van Huis 2013) and different orders exhibit different nutritional compositions (Hua, 2021). They contain high quality protein content, fat content and a balanced amino acid and fatty acid profile (Alfiko *et al*., 2022; Henry *et al*., 2015). Insects also have a small ecological footprint and a high feed conversion efficiency (Makkar *et al*., 2014). Some of the insects that have been used in aquafeed include the black soldier fly larvae (*Hermetia illucens*) (Fischer *et al*. 2022; Mohan *et al*. 2022; Guerreiro *et al*.

2020), mealworm larvae (*Tenebrio molitor*) (Tran *et al*. 2022; Coutinho *et al*. 2021; Jeong *et al*. 2020;), housefly larvae (*Musca domestica*) (Akinwole *et al*., 2020; Saleh, 2020; Gbai *et al*. 2019), mopane worm (*Imbrasia belina*) (Nyuliwe *et al*., 2022; Rapatsa and Moyo 2019; Rapatsa and Moyo 2017), and superworm (*Zophobas morio*) (Henry *et al*. 2022; Prachom *et al*. 2021; Jabir *et al*. 2012). These insects have been used in different fish species (marine and freshwater) with varying degrees of success. However, in southern Africa, there are very few published articles where insects have been used as fishmeal replacements, especially in South Africa.

Southern Africa is a sub-tropical region that is endowed with a variety of insects that has the potential to replace fishmeal in aquafeed. However, there is very little that has been done in evaluating these insects as fishmeal substitutes. One of the insects that showed promising results is the Mopane worm (*Imbrasia belina*). Mopane worm contains 56.8% protein content and 12.9% fat content (Rapatsa and Moyo, 2017). The protein and fat content of Mopane worm is comparable to fishmeal (65.5% protein and 12% fat). This insect showed high growth rates when incorporated into the diets of *O. mossambicus* in South Africa (Rapatsa and Moyo, 2017). However, a decline in growth performance and nutrient utilisation have been observed in *C. gariepinus* when fed the same diet (Rapatsa and Moyo, 2019). The Mozambique tilapia (*O. mossambicus*) and *C. gariepinus* are warm freshwater species with different feeding habits. This explains their different response towards the Mopane worm-based diet. The limitation associated with the Mopane worm is its seasonal availability. It is bivoltine and the first generation is harvested between October and January whilst the second generation is harvested between February and May (Dube and Dube, 2010). Another limitation of Mopane worm is bioaccumulation of heavy metals. Greenfield *et al*. (2014) showed that metal concentrations in Mopane worms were higher than the European Union and United Kingdom recommended legal limits for human consumption. This is mainly because Mopane trees are found in mineral rich areas containing heavy metals. It is thus important to evaluate other insect species in southern Africa, especially in the rural communities where there is poor access to commercial feed. Some of the locally available insect species are the Stinkbugs (*E. delegorguei*), Soldier termites, and Alates termites (*M. falciger)*. The effect of these insects as potential substitute of fishmeal in *O. mossambicus* and *C. gariepinus* has not been investigated before.

Stinkbugs (*E. delegorguei*, Spinola, 1850) are edible insects from the Hemiptera Order. They are predominantly found in the northern part of South Africa (Limpopo and Mpumalanga Provinces), southern parts of Zimbabwe, Mozambique, Botswana, Eswatini, and Namibia (Makore *et al*. 2015, Dzerefos *et al*. 2013). Stinkbugs are phytophagous insects that mainly feed on sub-tropical plants such as *Dodonaea viscosa* and *Diospyros mespiliformis* (Dzerefos *et al*. 2009). Their protein content ranges between 35.2% and 37.7%, whilst the fat content ranges between 51% and 57.7% (Musundire *et al*., 2016; Makore *et al*., 2015; Teffo *et al*., 2007). Teffo *et al*. (2007) reported that Stinkbugs contain essential amino acids, fatty acids, vitamins, and minerals. Stinkbugs are harvested during the dry season (May, June, and July) and have a long shelf life. Although Stinkbugs have rich nutritional content, they have not been evaluated as feed in the animal feed industry.

Solder termites and Alates (winged) termites (*M. falciger*) are also some of the insects that are locally available. Both Soldier and Alates termites are also commonly eaten insects in southern Africa. They mainly feed on cellulose, which is obtainable from wood, grass, leaves and humus. Most termites of the family termitidae are humivores. These insects are rich in proteins, fats, essential amino acids, and vitamins (Netshifhefhe *et al*., 2018). They contain minerals such as phosphorus, calcium, iron, and magnesium (Mbah and Elekima, 2007). Sogbesan and Ugwumba (2008) reported that Alates termite meal contains 46.3% crude protein, 30% lipids, 7.3% fibre, 3.6% ash, and 2 457.61 KJ/100g gross energy. The literature on Soldier and Alates termites in the animal feed industry is also patchy. These insects have the potential to serve as a novel ingredient in substituting fishmeal in the diet of common cultured warmwater fish species in southern Africa.

The two commonly cultured warm freshwater fish species in southern Africa are the Mozambique tilapia (*O. mossambicus*) and the African catfish (*C. gariepinus*) (DAFF, 2019). *Oreochromis mossambicus* gained its preference due to its wide-ranging tolerance to ecological conditions, generalist dietary requirements, and rapid reproduction in captivity (Russell *et al*., 2012). *Clarias gariepinus* is one of the species of interest due to its high degree of hardiness, its rapid growth, and survival in low-oxygen water (Kari *et al*., 2021). *Clarias gariepinus* is an opportunistic predator species that feeds on various items such as fish, plankton, invertebrates, and insects

(Henry *et al*., 2015). Insects form part of the natural diet for both *O. mossambicus* and *C. gariepinus*. Thus, it is speculated that both species are pre-adapted to utilising insect-based diets. Moreover, *O. mossambicus* and *C. gariepinus* have different feeding habits. It is important to determine the response of fish with different feeding habits on insect-based diets.

1.1.5 Research Problem

Due to the escalating price, unreliable supply, and current fishmeal scarcity, insects have been deemed potential novel ingredient in replacing fishmeal in aqua feed. Some insects contain high protein content, balanced amino acid and fatty acid profile comparable to fishmeal. Insects such as *Hermetia illucens*, *Musca domestica*, *Tenebrio molitor*, *Acheta domesticus*, *Imbrasia bellina*, and *Zophobas morio* have been explored as substitutes for fishmeal in different fish species. Most insects showed promising results, depending on fish and insect species. It is of paramount importance to evaluate locally available insects as fishmeal substitutes in the diet of commonly cultured fish species of economic importance in southern Africa. The literature showed that fish with different feeding habits responds differently towards an insect-based diet. However, results from different studies are not comparable due to differences in diet formulations, dietary nutrient compositions, insect processing methods and rearing conditions. Thus, it is prudent to compare the response of two species with different feeding habits under the same experimental conditions for comparable results.

1.1.6 Aim, objectives and research questions

The aim of this study was to investigate the potential of enhancing freshwater fish production through replacement of fishmeal with insect meals.

Objectives of the study were:

I. To determine the effect of substituting fishmeal with Stinkbug meal, Alates termite meal and Soldier termite meal on the growth performance of *O. mossambicus* and *C. gariepinus*.

II. To determine serum chemistry of *O. mossambicus* and *C. gariepinus* fed diets with Stinkbug meal, Alates termite meal and Soldier termite meal as substitutes for fishmeal.
III. To determine the effect of Stinkbug meal, Alates termite meal and Soldier termite meal as fishmeal replacements in the liver and intestine histology of *O. mossambicus* and *C. gariepinus*.
IV. To determine enzyme profile of *O. mossambicus* and *C. gariepinus* fed diets with Stinkbug meal, Alates termite meal and Soldier termite meal as fishmeal replacements.
V. To determine the cost benefit analysis of replacing fishmeal with Stinkbug meal, Alates termite meal and Soldier termite meal in *O. mossambicus* and *C. gariepinus* diets.

The research questions to be addressed in this study were:

I. To what extent can Stinkbug meal, Alates termite meal and Soldier termite meal replace fishmeal in the diets of *O. mossambicus* and *C. gariepinus*?
II. How will insect-based diets (Stinkbug meal, Alates termite meal and Soldier termite meal) affect the blood serum chemistry of *O. mossambicus* and *C. gariepinus*?
III. Will insect-based diets affect the histomorphology of *O. mossambicus* and *C. gariepinus*?
IV. Does insect-based diet have an effect on the digestive enzyme profile of tested fish species?
V. Is it economically feasible to replace fishmeal with insect meals in the diets of *O. mossambicus* and *C. gariepinus*?

1.1.7 Thesis layout

The thesis is divided into seven chapters.

Chapter 1: Covers the general introduction and research problem.

Chapter 2: The literature of using insects as fishmeal replacement in fish diets has been reviewed in this chapter.

Chapter 3: The growth performance, blood chemistry, liver and intestinal histology and digestive enzyme activity of *Oreochromis mossambicus* and *Clarias gariepinus* fed diets with full-fat Stinkbug (*Encosternum delegorguei*) meal.

Chapter 4: A comparative study on the utilisation of an Alate (*Macrotermes falciger*) based diet by a herbivorous fish (*Oreochromis mossambicus*) and an opportunistic predator (*Clarias gariepinus*).

Chapter 5: Partial replacement of fishmeal with Soldier termite (*Macrotermes falciger*) in juvenile *Oreochromis mossambicus* and *Clarias gariepinus* fingerlings was evaluated. The effects on growth performance, blood serum chemistry, histomorphology and digestive enzymes were also assessed.

Chapter 6: The chapter covers the general summary of the findings and specific recommendations.

Chapter 7: The references of the study are outlined in this chapter.

CHAPTER 2:

LITERATURE REVIEW

CHAPTER 2: LITERATURE REVIEW

2.1 Insects as food

Insects have been utilised as both food and feed for millennia. Beetles, caterpillars, ants, locusts, crickets, bugs and termites are the most commonly eaten groups of insects (FAO, 2013). This is due to their nutritional value, rich protein, fats, and essential macronutrients (Rumpold and Schlüter, 2013). Insects are also consumed for health benefits, and economic viability (Tang *et al*., 2019; FAO, 2013). Most of these edible insects are harvested from the field. However, many countries have already begun innovative mass-rearing systems for these insects (FAO, 2013). Different countries have a varied number of edible insects (Figure 2.1). South Africa is one of the countries in Africa that has over 200 edible insects.

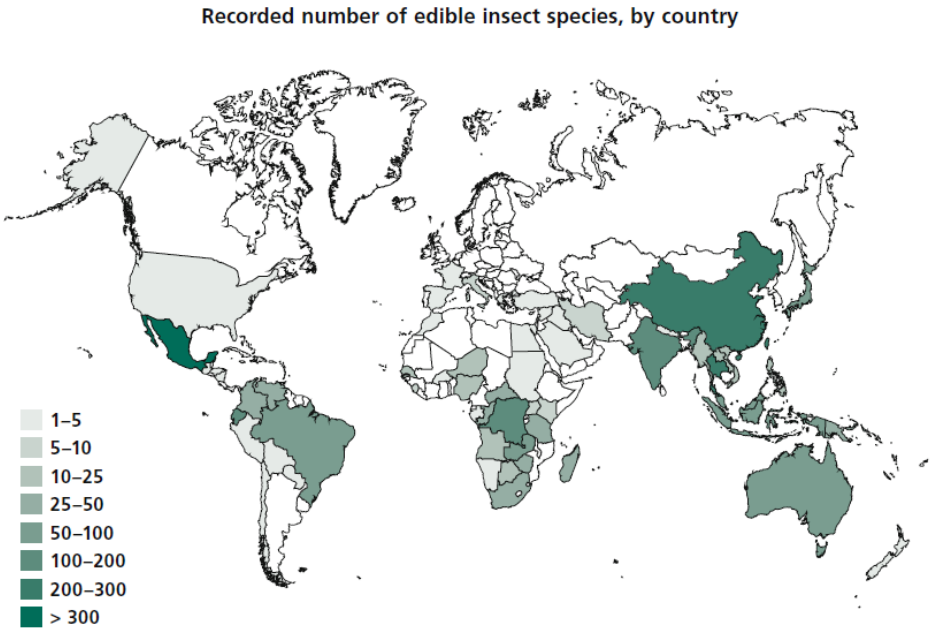

Figure 2.1: Recorded number of edible insect species in the world (FAO, 2013).

2.2 Use of insects in animal feed

Insects have been widely used as feed in poultry (Mutungi *et al*., 2017; Ssepuuya *et al*., 2017; Kenis *et al*., 2014), piggery (Hong and Kim, 2022; DiGiacomo and Leury, 2019) and fishery industries (Mastoraki *et al*., 2022; Shekarabi *et al*., 2021; Nogales-

Mérida *et al*., 2019 Stadtlander *et al*., 2017) and showed promising results. Insects are a part of the natural diet of both marine and freshwater fish species (Nogales-Mérida *et al*., 2019; Henry *et al*., 2015). Insects contain substantial amounts of high-quality protein of between 50 and 82% on a dry matter basis (Rumpold and Schlüter, 2013). Some insects have been reported to be rich in mono- and poly-unsaturated fatty acids such as oleic and linoleic acid. Insects of the Diptera order have an amino acid profile comparable to fishmeal (Tran *et al*., 2015). This shows that some insects have an amino acid profile that correlates with fish requirements (Alegbeleye *et al*., 2012; NRC, 2011). Insects are rich in minerals and vitamins (Arru *et al*., 2019; Henry *et al*., 2015). These includes iron, phosphorus, magnesium, calcium, selenium, and zinc (Mastoraki *et al*., 2022). Vitamins includes vitamin A, D, and B complex (Rumpold and Schlüter, 2013). Insects can be used as substitutes for fishmeal at either larvae, pupae, or adult stages. However, the larval stage is most preferred. Apart from these factors, most insects have specific physiological traits such as high reproduction rate, short life cycle, rapid growth and are easy to handle and manipulate (Rumbos and Athanassiou, 2021). Most insects used in fish feeds belong to the order Orthoptera, Coleoptera, Lepidoptera and Diptera (Henry *et al*., 2015).

Studies have also shown that several insects contain a valuable amount of taurine and hydroxyproline, which are essential for fish growth and health (Pinto *et al*., 2013). These are some of the aspects that make insects a promising protein source in replacing fishmeal in animal feeds. In aquaculture, some of the widely used insects in replacement of fishmeal in fish diets include the black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), mealworm (*Tenebrio molitor*), silkworm (*Bombyx mori*) and mopane worm (*Imbrasia belina*) and superworm (*Zophobas morio*). These insects are from the orders Diptera, Tenebrionidae and Lepidoptera.

The use of insect meals in aqua feed is associated with a number of limitations. These includes chitin content. Chitin is a polysaccharide of glucosamine and *N*-acetylglucosamine containing nitrogen atoms (Nogales-Mérida *et al*., 2019). It serves as a protective and structural component in arthropod exoskeletons such as insects and crustacean. Generally, chitin in insects is principally considered as fibre due to its similar structure to cellulose. Several studies have shown that a decline in growth performance and nutrient utilisation in fish fed an insect-based diet is attributed to

chitin levels in the insect meal (Azri *et al*., 2022; Adeoye *et al*., 2019). Kroeckel *et al*., (2012) also reported that chitin hinders diet digestibility. Caimi *et al*., (2020) showed that chitin could affect liver lipid accumulation and may induce intestinal inflammation. Finke (2007) estimated that chitin content in insects ranges between 11.6 and 137.2 mg/kg (DM), which is not high enough to reduce the growth performance and nutrient utilisation in fish species. Thus, an insect-based diet will only transfer a very small amount of chitin to the fish. There are studies that reported the benefits of chitin in fish diets and also elaborated that chitin content has no effects on fish growth and health (Priyadarshana *et al*., 2022; Abdel-Tawwab *et al*., 2020; Elia *et al*., 2018). Moreover, other studies reported that chitin promote growth of potentially beneficial gut microbes that *in vitro* inhibit the growth of common fish pathogens (Askarian *et al*., 2012). Chitin has also been reported as a beneficial factor regulating blood parameters such as plasma haemoglobin content, red blood cells, and white blood cells (Priyadarshana *et al*., 2022). The contradictory results and findings regarding chitin content may be due to the different insect species, their larval stage, and processing methods (Finke, 2007). The use of insects in animal feed has the potential to raise competition with human food. In western societies, insect consumption is not widely practiced, unlike in Africa where insects have long served as traditional foods (Sogari *et al*., 2023; DeFoliart, 1999). Thus, it is of paramount importance to consider using insects that serve no competition with human food, especially in African societies.

2.3 Insects used to substitute fishmeal and factors affecting their utilisation.

2.3.1 Black Soldier Fly (*Hermetia illucens*)

The black solider fly is one of the few insects approved for use in animal feed by the European Union (European Union, 2017). It belongs to the order Diptera of the Stratiomyidae family. It is one of the most promising insect species in replacing fishmeal in aquafeeds. This is mainly due to its nutritional profile which makes it the best candidate for use in animal feed. It is one of the insects where a 100% inclusion level has been achieved in fish species (Tippayadara *et al*., 2021). Its nutritional composition is highly dependent on the substrates it feeds on and the processing methods. The BSFL (black soldier fly larvae) is one of the insects that has the ability to assimilate nutrients from a variety of organic wastes.

The crude protein content in the BSFL has been reported by several authors. Barragan-Fonseca (2017) reported that its crude protein ranges from 37 to 63%, Nogales-Mérida *et al*. (2019) reported a range of 30 to 58%, whilst Makkar *et al*. (2014) reported a crude protein range of 40 to 44%. These protein content ranges are comparable to, and in some cases even higher than that of traditional protein sources such as soybean. The protein content of the BSFL may be improved by defatting (Alfiko *et al*., 2022). An increase in crude protein from 42.1% to 56.9% has been reported when BSFL was defatted (Alfiko *et al*., 2022).

The black soldier fly larvae is one of the insects that have an amino acid profile comparable to fishmeal (English *et al*., 2021; Fisher *et al*., 2020; Henry *et al*., 2015). Alfiko *et al*. (2022) showed that the BSFL had valine, isoleucine, leucine, phenylalanine, tyrosine, histidine, aspartic acid, and alanine amino acids higher than fishmeal. This insect contains both essential and non-essential amino acids. The essential amino acids that are most abundant in the BSFL meal are leucine, isoleucine, lysine, valine, and arginine, whereas the most abundant non-essential amino acids are alanine, aspartate, and glycine (Abdel-Tawwab *et al*., 2020).

The fat content of insect meals is affected by factors such as the stage of development and the substrate the insect is fed on (Barroso *et al*., 2019). However, the lipid content of insect meals can be improved by manipulating its substrate composition. The BSFL meal contains a lipid content between 10 and 30%, depending on the growth media (Diener *et al*., 2009). The major fatty acid constituents of BSFL are saturated fatty acids and monounsaturated fatty acids (Ewald *et al*., 2020). Its most dominant fatty acid is lauric acid, which contributes from 13% to 52% of the total fatty acid profile (Oonincx *et al*., 2019). The unsaturated fatty acid constitutes 19-37% (Gasco *et al*., 2021). There is dichotomy in fatty acid profile between terrestrial and aquatic insects. Several algal groups can synthesise polyunsaturated fatty acids. Most terrestrial insects lack PUFA compared to aquatic insects. The eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels of BSFL are less than fish oil (van Huis, 2022).

Chitin is a polymer of glucosamine in the exoskeleton of insects (Lindsay *et al*., 1984). Thus, insects contain a certain amount of chitin. The chitin content in insects is species-specific and varies with stages of development (Mousavi *et al*., 2020). Chitin has been described as the limiting factor in using insects as fishmeal substitutes in

aquafeed (Ng *et al*., 2001). Several studies reported a decline in growth performance and nutrient utilisation on fish-fed diets with higher BSFL inclusion levels (Fischer *et al*., 2022; Zarantoniello *et al*., 2021; Caimi *et al*., 2020). Insects are known to contain an estimated chitin content between 11.6 and 137.2 mg/kg (Finke, 2007). Abdel-Tawwab *et al*. (2020) recorded a chitin content of 5.11 g/kg in BSFL meal. The effect of chitin on the growth performance of fish species is species-specific. It is also based on the presence of chitinase enzyme activity in the gastrointestinal tract of fish species. Chitin content limits the incorporation of BSFL in fish diets at higher inclusion levels. Thus, the inclusion of this insect may not be suitable for fish species that lack chitin-digesting enzymes such as chitinase. However, studies have shown that microbes of the genus *Bacillus* are chitinase producers (Schallmey *et al*., 2004). They are known for their potential to secrete degradative enzymes such as chitinases. Thus, they may be a viable solution to inclusion of BSFL in fish diets.

The BSFL originated in the tropical, subtropical, and warm temperate zones of America (Makkar *et al*., 2014). However, this insect has now been introduced to various parts of the world including the tropical and warmer temperate regions. The BSFL is not native to southern Africa. Thus, it is not readily available in South Africa. The current level of adoption for producing BSFL is poor. Although this insect is one of the most promising protein sources in aquafeed, there has not been any commercial uptake in southern Africa. To the best of the authors' knowledge, there is only one commercial farm that produces the BSFL meal that is in the City of Cape Town, Western Cape Province. Its location is one major limitation for farmers who are in rural communities.

Black soldier fly larvae has been extensively used as a fishmeal replacement in the animal feed industry. Its potential to substitute fishmeal in aquafeed has been evaluated in different fish species with different feeding habits. Adeoye *et al*. (2019) showed that *Hermetia illucens* can replace fishmeal up to 50% in the diet of an opportunistic predator (*C. gariepinus*) fingerlings. The growth performance observed at a 50% inclusion level was attributed to the pattern of essential amino acids of BSFL, which is similar to fishmeal. The inclusion levels were 0, 25, 50, and 100%. The experimental trial was conducted in 30L capacity tanks in a flow-through system for 6 weeks and fish were fed to apparent satiation. The study showed that inclusion levels

beyond 50% showed a reduction in the growth, feed intake, and protein efficiency ratio of *C. gariepinus*. The reduced growth performance was associated with the low feed intake and poor acceptability of the diet. Thus, reducing the protein and energy intake the species requires. The hematological parameters were not influenced by the *H. illucens* inclusion across the diets. This showed that dietary replacement of fishmeal with BSFL did not have deleterious effects on the health and welfare of *C. gariepinus*.

Fawole *et al*. (2020) also showed that *H. illucens* can replace fishmeal up to 50% in the diet of *C. gariepinus* fingerlings without impairing growth, nutrient utilisation, and health status. A slight depression in growth performance was also observed at inclusion levels above 50%. The study attributed the decline to chitin content. Moreover, the blood parameters and differential leucocyte counts were not significantly different. The fish were reared in static tanks and fed diets with 25, 50, and 75% inclusion levels for 8 weeks. These studies showed that *C. gariepinus* grows best at 50% inclusion levels. *C. gariepinus* is an opportunistic predator and is known to feed on a variety of items. Both these studies show that this species can only utilise up to 50% inclusion level of BSFL. However, different observations were recorded when species of different feeding habits were fed a BSFL-based diet.

Black soldier fly larvae showed the potential to completely replace fishmeal in the diets of *O. niloticus*, which is an omnivore (Kishawy *et al*., 2022; Tippayadara *et al*., 2021). Tippayadara *et al*. (2021) assessed the replacement of fishmeal with black soldier fly larvae meal in the diet of sub-adult *O. niloticus*. Black soldier fly larvae meal inclusion levels used were 10, 20, 40, 60, 80 and 100%. Fish were reared in a glass tank. The study showed that specific growth rate, feed utilisation efficiency, feed intake, and survival rate was not significantly different between fish fed the control diet (0% black soldier fly larvae meal) and fish fed diets with BSFL meal. Furthermore, assessed blood parameters were not affected by the inclusion of black soldier fly larvae meal in the diets. The study further concluded that BSFL meal can substitute fishmeal up to 100% inclusion level without compromising the growth, feed efficiency, and health of *O. niloticus.* Kishawy *et al*., (2022) also showed that *H. illucens* can completely replace fishmeal in the diet of Nile tilapia (*O. niloticus*) without compromising the growth rate and feed efficiency. The inclusion levels used were 25, 50, and 100% and sub-adult *O. niloticus* were reared in a glass aquarium for 12 weeks. The study further showed

that the inclusion of *H. illucens* boosted the serum immune parameters and modulated cytokines expression.

Replacement of fishmeal with BSFL has also been evaluated in carnivorous species such as rainbow trout (*Oncorhynchus mykiss*). Biasato *et al*. (2022) recorded the best growth performance at 32% when *H. illucens* replaced fishmeal in the diet of *O. mykiss* without affecting the growth performance, nutrient digestibility, somatic indices, and histology. The inclusion levels used in the study were 25, 50, and 100%. In another study, *H. illucens* showed that it can replace fishmeal up to only 15% in the diet of *O. mykiss* without showing adverse effects on growth performance, fillet's physical characteristics, gut and liver health, and digestibility (Caimi *et al*., 2021).

These studies show that species with different feeding habits respond differently towards an insect-based diet. The BSFL was able to completely replace fishmeal in the diet of an omnivorous species (*O. niloticus*). In an opportunistic predator, BSFL could only replace up to 50% inclusion level. In carnivorous species, lower inclusion levels were observed. Due to the different inclusion levels used, different processing methods of the insect, larval stage and reading prospects, fish rearing systems, these studies are not directly comparable. Moreover, BSFL is not easily accessible in southern Africa, especially to fish farmers who are based in remote and rural communities. South Africa has one well-established black soldier fly larvae farm (Agriprotein) located in the City of Cape Town, Western Cape Province. Its location may be a huge hindrance to rural fish farmer's access. It is thus important to search for alternative insects, especially locally available insects that have the potential to substitute fishmeal in the diet of commonly cultured fish species of economic importance in southern Africa. It is also important to investigate the response of fish species with different feeding habits under the same experimental setup.

2.3.2 Common housefly (*Musca domestica*)

The common housefly (*Musca domestica*) has been extensively used in fish diets (Wang *et al*., 2017; Ezewudo *et al*., 2015; Ogunji *et al*, 2008). It is one of the species approved to produce feed in aquaculture under the European Union legislation (Alfiko *et al*., 2022). Like black soldier fly larvae, the common housefly maggot feeds on a

wide range of substrates. These include manure and any decaying organic matter. Poultry droppings have been listed as the major substrate to grow maggots. *Musca domestica* is regarded as one of the most promising insect ingredients in fish diets along with black soldier fly (Sogari *et al*., 2019).

Housefly maggots (*Musca domestica*) contain crude protein between 40 and 60% (Llagostera *et al.*, 2019). A crude protein range of 28 to 70% has also been reported (Nogales-Mérida *et al*., 2019). The crude protein range of maggot meal is comparable to fishmeal. However, the protein content also varies based on the substrate fed the insect.

Musca domestica contains a lipid content between 9 to 26% (Llagostera *et al*., 2019). The saturated fatty acid component of this insect is approximately 32.66%, which is higher than fish oil and soybean meal oil (Nogales-Mérida *et al*., 2019). Its monounsaturated fatty acid content ranges between 22.22% and 73.20%. With respect to polyunsaturated fatty acid, terrestrial insects generally contain lower levels of EPA and DHA than fish oil but higher than soybean oil. This is also the case with *M. domestica*.

Musca domestica belongs to the order Diptera. Insects from this order are known to contain amino acid profiles comparable to fishmeal (Hameed *et al*., 2022). *Musca domestica* contains all essential and non-essential amino acids. This insect is high in methionine and lysine, which are some of the most common limiting amino acids in fish. It has a methionine level of 2.2 g/100g and a cysteine level of 0.7 g/100g (Rapatsa and Moyo, 2022). Lysine levels in *M. domestica* are higher than in fishmeal. In a review and a meta-analysis of the effects of replacing fishmeal with insect meals on the growth of Tilapias and Sharptooth Catfish, *M. domestica* was reported to have the highest essential amino acid index (EAAI, 3.26) (Rapatsa and Moyo, 2022).

Musca domestica has a wide distribution, including faecal matter, and garbage heaps (Saleh, 2020). This makes it one of the most available insects used in aqua feed. It is also cheap and easy to rear and has a nutritional index that is comparable to fishmeal (Rapatsa and Moyo, 2022).

Replacement of fishmeal with maggot meal has been evaluated in species of different feeding habits. The most tested fish species are *C. gariepinus* (Okore et al., 2016; Adewolu *et al*., 2010; Aniebo *et al*., 2009; Fasakin *et al*., 2003; Idowu *et al.*, 2003) and *O. niloticus* (Gbai *et al*., 2019; Mustapha and Kolawole, 2019; Ezewudo *et al*., 2015; Ogunji *et al*., 2008).

Ezewudo *et al*. (2015) evaluated the substitution of fishmeal with maggot meal (MM) in the diet of omnivore (*O. niloticus*) fingerlings. The inclusion levels were 0, 20, 30, 40, 50, 60, 70, and 80% and fish were reared in 40 L plastic tanks for 10 weeks. The study recorded the highest mean weight gain, relative growth rate and specific growth rate in *O. niloticus* fingerlings fed a diet with a 50% MM inclusion level. The study concluded that fishmeal can be replaced with MM at 50 to 60% for optimal growth performance, nutrient utilisation, and survival of *O. niloticus* fingerlings. Wang *et al*. (2017) also assessed MM as a substitute for fishmeal in the diet of *O. niloticus*. The study reported that fish that have been fed diets with up to 75% inclusion level showed no effect on growth performance, feed utilisation, and survival. Feed intake and apparent digestibility coefficient were also not significantly different between inclusion levels. However, at the 100% inclusion level, significant retardation was observed in fish performance and survival. The study further suggested that the best inclusion level of MM in the diet of *O. niloticus* is 50%. In an omnivore species, the highest inclusion level of 50% was achieved (Wang *et al*., 2017; Ezewudo *et al*., 2015).

Substitution of fishmeal with maggot meal has also been evaluated in an opportunistic predator (*C. gariepinus*) juvenile (Idowu and Afolayan, 2013). The inclusion levels were 0, 10, 20, 30, 40, and 50%. The study showed that fish fed the 50% inclusion level diet recorded the highest mean weight gain, specific growth performance, and protein efficiency ratio. Thus, optimal growth and high nutrient utilisation of *C. gariepinus* juvenile were obtained at a 50% inclusion level. Another study that evaluated the substitution of fishmeal with maggot meal in the diet of *C. gariepinus* juveniles showed that the best growth performance and improved health conditions were observed in fish fed 45% inclusion level (Okore *et al*., 2016). Both these studies suggest that the best inclusion level of Maggot meal in the diet of an opportunistic predator is achieved at 50%. These studies suggest that higher inclusion levels of maggot meal may be achieved in omnivorous fish species than in carnivorous species.

However, different experimental conditions make it challenging to deduce the conclusion.

Maggot meal has also been widely used to feed fish species as whole, frozen, live or chopped (Henry *et al*., 2015; Makkar *et al*., 2014). Ebenso and Udo (2003) fed *O. niloticus* a mixture of wheat bran and 20% live maggots. The study showed that the best growth performance, specific growth performance, feed conversion ratio, and survival were recorded in fish fed the mixture than fish fed wheat bran only. This observation was also recorded in *C. gariepinus* fed 50% live maggots and 50% artificial diet with low (3.5%) fishmeal (Oyelese, 2007). The study showed that fish fed a combination diet (50% maggot meal and 50% artificial diet) recorded the best growth performance indices than fish fed an artificial diet only. Similar results were also reported in *C. gariepinus* (Achionye-Nzeh and Ngwudo, 2003).

Although *M. domestica* is deemed as the potential insect in aquafeed, there are factors that may pose as limitation to its use. *Musca domestica* is regarded as a nuisance since it is associated with rotting materials. It is a major transmitter of diseases and its use in aquafeed may raise health and fish consumers' acceptability concerns. Thus, a consumer survey study should be carried out before any commercial uptake is implemented.

2.3.3 Mopane worm (*Imbrasia belina*)

Mopane worm is one of the few locally available insects that has been extensively investigated as a potential replacement of fishmeal. It is a larval stage of the mopane worm moth and belongs to the Lepidoptera order and Saturniidae family. The worm is found in its host plant, mopane (*Colophospermum mopane*), which is found in the southern region of Africa (Moyo *et al*., 2019; Moreki *et al*., 2012). The southern African countries in which Mopane worm is found include South Africa, Botswana, Namibia, Zambia, Angola, Mozambique, Zimbabwe, and Malawi (Makhado *et al*., 2012). The Mopane worm is known to be bivoltine, consisting of two generations per annum (Kwiri *et al*., 2014). The first generation occurs during the rainy season between November and January, whilst the second occurs between March and May.

The nutritional content of the Mopane worm (*Imbrasia bellina*), like any other insect varies based on the stage of metamorphosis, insect origin, and its diet (Finke and Oonincx, 2014). Mopane worms are a good source of high-quality protein. The protein content of *I. bellina* ranges between 54-59% (Manyeula *et al*., 2013; Moreki *et al*., 2012; Dube and Dube, 2010; Madibela *et al*., 2009). However, a lower protein content of 48.3% has also been reported (Glew *et al*., 1999). The protein content of *I. bellina* is higher than soybean meal but lower than fishmeal (Moyo *et al*., 2019).

Mopane worm meal was reported to contain an amino acid profile comparable to fishmeal (Rapatsa and Moyo, 2017; Nobo *et al*., 2012). Mojeremane and Lumbile (2005) reported a higher concentration of threonine, valine, tryptophan, and phenylalanine in the Mopane worm meal than in fishmeal and soybean meal. Moreover, lysine and methionine amino acid content are comparable to fishmeal (Nobo *et al*., 2012). Rapatsa and Moyo (2022) reported that the Mopane worm has an essential amino acid index (EAAI) of 2.26, a nutritional index of 6.57, and a feed value of 14.12.

The fat content of the Mopane worm ranges between 12.9% to 16.7% (Manyeula et al., 2013; Moreki et al., 2012; Dube and Dube, 2010; Madibela *et al*., 2009). Rapatsa and Moyo (2019) recorded the same value of fat content (12%) as fishmeal. Its fatty acid profile is dominated by polyunsaturated fatty acids and α-linolenic acid constitutes the highest percentage (Rapatsa and Moyo, 2019; Yeboah and Mitei, 2009). The saturated fatty acid is dominated by the palmitic acid. Mopane worm has a 54:49 ratio of total unsaturated fatty acid to total saturated fatty acid (Moyo *et al*., 2019). However, this insect lacks EPA (C20:5n3) and DHA (C22:6n3), which are the essential fatty acids for fish species.

Mopane worm is known to be bivoltine, consisting of two generations per annum (Kwiri *et al*., 2014). The first generation occurs during the rainy season between November and January, whilst the second occurs between March and May. This shows that the Mopane worm availability is seasonal. Its prevalence is highly influenced by prevailing environmental conditions such as high temperatures and the amount of rain (Makhado *et al*., 2012). Thus, the Mopane worm availability cannot be guaranteed. Mopane worm is also a delicacy to some of the tribes in southern Africa. This makes it one of the

most expensive insects on the market. Furthermore, the Mopane worm has been described as the most expensive insect whose price is similar to that of fishmeal (1.49 $/kg) (Rapatsa and Moyo, 2022).

Mopane worm has recently gained attention as a substitute for fishmeal in animal feed such as poultry (Marareni and Mnisi, 2020; Chiripasi et al., 2013; Moreki *et al*., 2012; Mareko *et al*., 2010). Several studies have attempted substituting fishmeal with the Mopane worm meal in both freshwater and marine fish species with different feeding habits (Nyuliwe *et al*., 2022; Rapatsa and Moyo, 2019; Rapatsa and Moyo, 2017; Mwimanzi and Musuka, 2014;). Rapatsa and Moyo (2019) substituted fishmeal with Mopane worm meal at 0, 10, 20, 40, and 60% inclusion levels in the diets of an opportunistic predator (*C. gariepinus*). The study reported that an increase in Mopane worm meal inclusion levels resulted in a decrease in *C. gariepinus* growth performance indices. The inclusion of Mopane worm meal in fish diets showed negative effects on the liver of *C. gariepinus*, which was confirmed by the liver degradation score and histological alterations. The study concluded that although the Mopane worm meal has high nutritional value, it is not easily digested by *C. gariepinus*. However, positive findings were observed when the same diet was fed herbivorous *O. mossambicus* (Rapatsa and Moyo, 2017). The study showed that increasing Mopane worm meal resulted in an increase in specific growth rate, thermal-unit growth coefficient, protein efficiency ratio, and apparent digestibility coefficient in *O. mossambicus* (Rapatsa and Moyo, 2017). The study reported no histological alterations in the liver and intestines of *O. mossambicus*. The growth performance observed was attributed to the nutritional composition of the Mopane worm and that *O. mossambicus* possesses pre-requisite enzymes for efficient digestibility. However, the different responses of the two species (*O. mossambicus* and *C. gariepinus*) towards the Mopane worm meal may also be due to their feeding habits. Although the Mopane worm has a high nutritional value, one of its major limitations is its seasonal availability and high feed value. Moreover, a review and a meta-analysis showed that the Mopane worm is the most expensive insect (1.49 $/kg) and is the most poorly available among the five assessed insects of southern Africa (Rapatsa and Moyo, 2022). Moreover, the Mopane worm meal contains high levels of chitin (27%), which is known to affect feed digestibility and impede protein utilisation (Kwiri *et al*., 2014). Bioaccumulation of heavy metals is also one of the limitations of Mopane worm inclusion in fish diets (Greenfield *et al*., 2014).

2.3.4 Mealworm (*Tenebrio molitor*)

Mealworms (*Tenebrio molitor*) are darkling beetles of the Tenebrionidae family. They are known as pests of grain and flour and are native to Europe and are now found worldwide. Mealworms are usually commercially grown for feeding animals including chickens and fish. They are one of the seven insect species that have been authorized for use in fish feeds by the European Union in 2017 (Basto *et al*., 2020; European Union, 2017).

Tenebrio molitor protein content ranges from 47-60 (Llagostera *et al*., 2019; Makkar *et al*., 2014). Yellow mealworm is one of the insects that have amino acids comparable to fishmeal (Gasco *et al*., 2016). Its valine, isoleucine, leucine, phenylalanine, tyrosine, histidine, serine, proline, and alanine are higher than fishmeal (Alfiko *et al*., 2022). This makes *T. molitor* a suitable candidate to use in animal feed as a replacement for fishmeal in both poultry and fishery.

The lipid content range of *T. molitor* is between 31 and 43% (Llagostera *et al*., 2019). The monounsaturated fatty acid constitutes the highest percentage of fatty acid in this insect, dominated by oleic acid (Alfiko et al., 2022). Moreover, the *T. molitor* also contains polyunsaturated fatty acid, which is dominated by linoleic acid (C18:2n-6). The main challenge with this insect is its fatty acid profile (Llagostera *et al*., 2019). The fatty acid profile is much richer in omega 6 and poor in omega 3. This often results in fish species adapting to fatty acid profile of the supplied diet (Renna *et al*., 2017). This makes the fish fillets contain n-3/n-6 ratio that is significantly lower than the desired one and low polyunsaturated fatty acid levels. This ratio depreciates the fish quality for human consumption.

Mealworms are widely available worldwide. Unlike terrestrial insects that are harvested by traditional methods, the mealworm is easy to breed and does not require a large area for production (Selaledi *et al*., 2020). Moreover, mealworm produces much less greenhouse gas compared to other animals (Oonincx and De Broer, 2012). The mealworm is one of the insects that are already produced on a large scale in countries like China. Although some countries have shown willingness to accept the use of insects in animal feed, the willingness of fish farmers to incorporate this insect

in their aquafeed in South Africa is not clear. Gasco *et al*., (2016) showed that mealworm contains a chitin content of 5.90%, which is one of the limiting factors of incorporating insect meals in aquafeed.

In fish feed, mealworm meal has been widely used as a potential substitution for fishmeal in fish species with different feeding habits (Chemello *et al*., 2020; Gasco et al., 2016; Ng *et al*., 2001). However, contradictory results have been observed. Jeong *et al*., (2020) substituted fishmeal with mealworm in the diets of carnivorous species (*Oncorhynchus mykiss*). Isonitrogenous and isocaloric diets consisting of 0, 7, 14, 21, and 28% inclusion levels were formulated. Fish fed the 28% inclusion level diet showed the highest specific growth rate and weight gain. Moreover, fish-fed diets with mealworm as an alternate protein source recorded a higher protein efficiency ratio and lower feed conversion ratio compared to fish fed the control diet (0% MWM). The study suggested that a 28% inclusion level in *O. mykiss* diets does not compromise the growth performance, nutrient composition, and health status of fish.

In another carnivore species (*Argyrosomus regius*), *T. molitor* could only replace fishmeal up to 10% inclusion level without affecting the growth performance and nutrient utilisation (Coutinho *et al*., 2021). Furthermore, the inclusion of *T. molitor* had no influence on the mortality, hepatosomatic indices, AA catabolism enzyme activity. Another carnivore species that showed low inclusion levels is the largemouth bass (*Micropterus salmoides*) (Gu *et al*., 2022). The study replaced fishmeal at 0, 11.1, 22.2, 33.3, 44.4, 55.5 and 66.6% *T. molitor* inclusion levels. Fish were fed isonitrogenous (47%), and isocaloric (19 MJ/Kg) diets for 8 weeks. Fish fed diet with 11.1% inclusion level showed no significant difference in the weight gain rate and specific growth rate with fish fed the control diet. Higher inclusion levels (55.5% and 66.6%) significantly decreased the plasma total protein, triglyceride, and albumin contents. The study concluded that the *T. molitor* inclusion levels in the diet of *M. salmoides* should not exceed 11.1%. The low inclusion levels have been attributed to high chitin content in the *T. molitor* meal, which is one of the major limiting factors in substituting fishmeal with insect. These studies shows that in carnivore species, *T. molitor* can replace fishmeal at relatively low inclusion levels. However, these studies are not comparable.

In African catfish (*C. gariepinus*) diets, *T. molitor* was also used as a novel ingredient (Ng *et al*., 2001). The study showed that *T. molitor* may replace fishmeal up to 40% inclusion level without any significant reduction in growth performance and feed efficiency ratio. These studies showed that the inclusion of *T. molitor* in fish diets highly depends on the feeding habits of fish species. There is no account of studies that compared the growth performance and nutrient utilisation of *T. molitor* in fish species with different feeding habits.

2.3.5 Locust/grasshoppers

Locusts are major crop pests that belong to the order Orthoptera. They belong to the grasshopper family of Acrididae, which contains 6, 787 known species (Cullen *et al*., 2017). Locusts are known to transform between a cryptic solitarious phase and a swarming gregarious phase, which can swarm for long distances (Egonyu *et al*., 2021). The four most common locust species found in southern Africa are the desert locust (*Schistocerca gregaria*), the migratory locust (*Locusta migratoria*), the red locust (*Nomadacris septemfasciata*), and the brown locust (*Locustana pardalina*) (van Huis *et al*., 2013). They have been described as the major agricultural pests and their main outbreak areas are the semi-arid Karoo region (Todd *et al*., 2002). In South Africa, locust outbreaks have been recorded in the Eastern Cape, Northern Cape, Western Cape, and Free State provinces in 2021. Unlike plant protein-based meals, locusts contain a negligible amount of anti-nutritional factors. These attributes have made them novel ingredients in the animal feed industry.

Locusts are highly nutritious and contain crude protein between 50 and 76% on a dry matter basis (Tran *et al*., 2015; Makkar *et al*., 2014). The protein content of locusts varies based on the developmental stage and adults are known to contain higher protein content than pupa and larvae (Mariod, 2020). The protein content of locusts is comparable to fishmeal (Henry *et al*., 2015).

Locusts also contain an acceptable essential amino acid profile (Alegbeleye *et al*., 2012). Although they contain low levels of lysine, locusts have high levels of cysteine and methionine (Alegbeleye *et al*., 2012). A meta-analysis study showed that *Schistocerca gregaria* contains cystine, histidine, isoleucine, phenylalanine, threonine, and valine levels higher than fishmeal (Rapatsa and Moyo, 2022). The study further

showed that its essential amino acid index (EAAI) was also higher (2.95) than fishmeal (2.68).

The fat content of locusts has been reported to range between 6.1 to 32.3% (Egonyu *et al*., 2021). *Schistocerca gregaria* contains 13% total fat, which is slightly higher than fishmeal (12%). This is one of the species that contain high levels of linolenic acid, which is a polyunsaturated fatty acid that most insects lack. The dominant fatty acid in *S. gregaria* is oleic acid, palmitic acid, linoleic, and α-linolenic acid, respectively (Rapatsa and Moyo, 2022). The *Oxya hyla hyla* species is dominated by linoleic acid, oleic acid, and α-linolenic acid (Das and Mandal, 2014). Its amino acid profile is equivalently comparable to fishmeal. Moreover, several amino acids were higher than in fishmeal.

Locusts are some of the insects that are already produced at a commercial scale in southeast Asia (Makkar *et al*., 2014). In India, mass-rearing of grasshopper species such as *Oxya fuscovittata*, *O. hyla*, and *Spathosternum prasiniferumis* is already taking place. However, South Africa has not achieved a commercial up-take of producing locusts. Their availability in this region is determined by outbreaks, which are not guaranteed. Moreover, locusts are prone to bioaccumulate heavy meals from the surrounding environment (Handley *et al*., 2007).

There is poor documentation of the use of the four common locust species as fishmeal substitutes in the animal feed industry. However, several species have been used to replace fishmeal in the diet of different fish species. Mohammed *et al*. (2020) assessed the effect of substituting fishmeal with different levels of locust meal in *O. niloticus* fry. The Nile tilapia (*O. niloticus*) is an omnivore species that has become the most-reared tilapia species globally. The study replaced fishmeal at 0, 25, 50, and 100% inclusion levels. The fish fed diet containing 50% locust meal showed the highest level of absolute weight gain and a low feed conversion ratio. The study concluded that *O. niloticus* fry grows best when fed a diet with a 50% locust meal. Ghosh and Mandal (2019) also evaluated the inclusion of grasshopper meal in the diet of an omnivore species (*Labeo rohita*). The inclusion levels of the study were 0, 17, 33, 50, 67, 83, 100% and the trial was conducted for 100 days. The study also recorded the best growth performance, apparent protein digestibility, nitrogen metabolism, feed conversion ratio (FCR), and protein efficiency ratio (PER) on fish-fed diet with a 50%

grasshopper meal inclusion level. Furthermore, replacement levels above 50% showed poor FCR and PER.

When *Zonocerus variegatus* L. replaced fishmeal in the diet of an opportunistic predator (*C. gariepinus*), the best growth performance was reported at 25% inclusion level, which did not differ significantly with fish fed the control diet. The study showed that the essential amino acid profile of *Z. variegatus* was close to the *C. gariepinus* requirement (Alegbeleye *et al*., 2012). Lower inclusion levels of locusts in aqua feed have also been reported. In other studies, grasshopper meal could only replace fishmeal with 10% in the diet of *C. gariepinus* without adverse effects on growth performance and nutrient utilisation (Tsado *et al*., 2021) The findings in these studies are inconclusive. This is due to the different insect species of grasshopper/locust and the different feeding habits of tested fish species. However, locusts in southern Africa are not viable fishmeal replacement due to their poor availability and lack of mass-production. It is thus important to search for locally available insects that have the potential to substitute fishmeal in aquafeed without posing health concerns.

2.4 The potential of locally available insects in aqua feed

Several insect species are consumed in Africa. However, species consumed vary with regions, countries and geographical areas (Hlongwane *et al*., 2022). This is mainly due to consumer preferences, socio-economic status and insect availability and acceptability. In the northern part of South Africa, commonly available and edible insects include *Imbrasia bellina*, *Gynanisa maja*, *Carebata vidua*, *Sternocera orissa*, *Cirina forda* and various species of locusts and grasshoppers (Nemadodzi *et al*., 2023). Most of these insects have the potential to replace fishmeal in aqua feed due to their nutritional composition. Insects such as *Imbrasia bellina*, *Cirina forda*, and locusts have been evaluated as potential fishmeal substitutes (Rapatsa and Moyo, 2019; Oyegoke *et al*., 2013; Alegbeleye *et al*., 2012). *Encosternum delegorguei*, and Soldier and Alates termites (*M. falciger*) are also some of the locally available insects that have the potential to substitute fishmeal in animal feed. Furthermore, the potential for these insects has not been explored.

2.4.1 Stinkbug (*E. delegorguei*)

Stinkbugs (*E. delegorguei*) are edible insects of the order Hemiptera and Tessaratomidae family. The name Stinkbug was derived from a smell that the insect secretes as a defence mechanism (Aldrich, 1988). Stinkbugs are known to inhabit subtropical, open woodland and bushveld (Teffo *et al*., 2007). They are found in southern parts of Zimbabwe and northern parts of South Africa and Mozambique (Makore *et al*., 2015). In Limpopo Province, the Stinkbugs are mainly found in Thohoyandou (Vhembe District), Ga-Modjadji (Mopani District), and Bushbuckridge (Ehlanzeni District) (Dzerefos *et al*., 2009). They are considered a delicacy in the northern part of Limpopo Province mainly by the Venda tribe and are commonly known as *Thongolifha* in vernacular.

Their distribution is highly dependent on a number of environmental factors. During the dry season (winter), they congregate at high altitudes and lower altitudes during the wet season (Summer). Stinkbugs are harvested in the dry season (May to August) and are found aggregating on their host plant (Dzerefos *et al*., 2009). They play a major role in the livelihood of local communities as a source of nutrition and income for harvesters. Makuku (1993) reported that Stinkbugs are a valuable source of income in the Norumedzo community in Zimbabwe. Stinkbugs mainly feed on plants and grass. Their plant hosts are mainly *Dodonaea viscosa* Jacq. var. *Angustifolia* (L.f.) Benth. and *Diospyros mespiliformis* (Teffo *et al*., 2007). Moreover, plants such as *Combretum imberbe*, *Combretum molle*, *Peltophorum africanum* and Pennisetum grass have been reported to host Stinkbugs (Mawanza, 1999).

The nutritional composition of the Stinkbug is not adequately documented. The Stinkbug contains protein content between 33.2% and 35.34% (Musundire *et al*., 2016; Makore *et al*., 2015; Teffo *et al*., 2007). The fat content ranges from 50.5% to 62.4%. Stinkbug has been reported to contain minerals such as iron, calcium, potassium, magnesium and phosphorous and vitamins. The amino acid profile of the Stinkbug meal is poorly documented. However, Musundire *et al*., (2016) reported that it contains essential amino acids, including the most limiting (lysine and methionine) amino acids. Teffo *et al*., (2007) showed that amino acids that dominated this insect were valine, leucine, and lysine. The fatty acid profile of the Stinkbug is poorly understood.

However, this insect contains linoleic acid, which is a vital polyunsaturated fatty acid for growth (Musundire *et al*., 2016). Of the ten fatty acids identified, seven were unsaturated fatty acid derivatives while three were saturated fatty acid derivatives. The Stinkbug has been a delicacy for most tribes in the northern provinces of South Africa for ages. However, its nutritional profile is still patchy in the published literature. Although Stinkbug has a good nutritional composition, there are no studies that documented its use as a feed in the animal feed industry. Moreover, their use as fishmeal substitution in fish diets has also not been evaluated. Thus, the Stinkbug meal will be used as a fishmeal substitution in the diet of *O. mossambicus* and *C. gariepinus*. Moreover, the distribution of the Stinkbug in most cases coincides with the distribution of *O. mossambicus* and *C. gariepinus*. The response of these two species towards the diet will also be evaluated and compared.

2.4.2 Alates termite meal (*M. falciger*)

The Alates termites are the winged queens and kings that emerge at the onset of the rainy season (van Huis, 2017). They are the reproductive caste and after shedding their wings, they mate and start new colonies. Alates termite feeds on cellulose which is mainly obtained from wood, grass, leaves and manure of herbivorous animals. Alates termites are collected using lamps placed above buckets with water (Netshifhefhe *et al.*, 2018). The most commonly found Alates termite belong to the genus *Macrotermes*, Termitidae family, and Blattodea order (Netshifhefhe and Duncan, 2022). These include *Macrotermes falciger*, *Macrotermes bellicosus*, *Macrotermes nigeriensis*, *Macrotermes natalensis,* and *Macrotermes subhyalinus*. The nutritional composition of these species is well documented in west Africa. Akullo *et al*., (2018), Adepoju and Omotayo (2014), Kinyuru *et al*., (2013), Ekpo and Onigbinde (2007) and Banjo *et al*., (2006) have evaluated the nutritional composition of Alates of the *Macrotermes bellicosus*. These studies reported a crude protein range of 21.1% and 40.74%. The nutritional profile of Alates of *M. falciger* showed that their protein content ranges from 23% to 43.26 (Chulu, 2015; Siulapwa *et al*., 2012; Phelps *et al*., 1975).

The Alates termites are known for their high fat content which was reported to range from 43.0% to 46.03%. The *M. falciger* fatty acid profile comprises mainly of

unsaturated fatty acid, which accounts for 60.3% (Chulu, 2015). The fatty acid is dominated by oleic acid, palmitic acid, and stearic, respectively (Chulu, 2015). This species has been described as one of the commonly consumed in the Vhembe District, Limpopo Province, South Africa (Netshifhefhe and Duncan 2022). It is also one of the main insect species that are found at the Thohoyandou Town open market. However, there is little information available on its nutritional profile and its potential to use in animal feed industry, especially aquafeed.

The *Macrotermes subhyalinus* is one of the Alate species that has been used as fishmeal substitute in the diet of African catfish (*C. gariepinus*) (Olaniyi *et al*., 2016). It contains protein content ranging from 39.34% to 47% (Olaniyi *et al*., 2016; Kinyuru *et al*., 2013). The best growth performance was observed in fish fed the diet with 40% *M. subhyalinus*. A similar study was also conducted, where *M. subhyalinus* meal substituted fishmeal in the diet of a mud catfish (*Heterobrunchus longifilis*) (Sogbesan and Ugwumba, 2008). Fish fed diet with 50% Alates meal showed the highest weight gain, relative growth rate, and specific growth rate. Fish fed this diet also recorded the lowest feed conversion ratio and highest protein efficiency ratio. The diet with a 50% inclusion level proved to be economically viable since it recorded the lowest incidence of cost, the highest profit index, and the best benefit-cost ratio. These studies suggest that Alates termites of *Macrotermes* species have the potential to replace fishmeal in the diet of *C. gariepinus*. The current study will use Alates of the *M. falciger* to replace fishmeal in the diets of *O. mossambicus* and *C. gariepinus*. There is no literature on the use of *M. falciger* as a fishmeal replacement in animal feed, especially in aquafeed formulations. The response of a herbivorous and an opportunistic predator fish species towards an Alate-based diet will be evaluated.

2.4.3. Soldier termite (*M. Falciger*)

Termites belong to the order Blattodea. They are consumed in many parts of Africa and are classified as social insects. Africa has over 1000 termite species (van Huis, 2017). They are mainly found in tropical and sub-tropical regions. In sub-Saharan Africa, over 14 species of the Macrotermitidae family are edible (Netshifhefhe *et al*., 2018). Soldier termites are the second most consumed insects in South Africa, especially in Limpopo and Kwazulu-Natal Provinces (Egan *et al*., 2021). *Macrotermes falciger*, *Macrotermes natalensis,* and *Macrotermes michaelseni* are the three

commonly consumed termite species in South Africa (Netshifhefhe and Duncan, 2022). They are collected from the mounds by inserting succulent grass into holes or shafts of a termite mound where Soldier termites attack the grass with their mandibles and get entrapped (Musundire *et al*., 2021).

The Soldier termite's nutritional composition of the *Macrotermes* species is poorly documented. The protein content recorded was 54.69% (Ntukuyoh *et al*., 2012), 19.3% and 14.4% (Netshifhefhe and Duncan, 2022), and 69.75% (Hlongwane et al., 2022). The *Macrotermes* species have relatively low-fat content ranging between 2.03 and 7.97% (Hlongwane *et al*., 2022; Netshifhefhe and Duncan, 2022). There is no study that determined the fatty acid profile of Soldier termites of the *Macrotermes* species Hlongwane *et al*., (2022) reported that Soldier termites of the *Macrotermes* species contain essential amino acids.

Unlike most terrestrial insects that are seasonal, the Soldier termites are harvested throughout the year. This makes them the most locally available insect. Soldier termites are also one the insects that can be found in the local open market. Moreover, nutritional information on Soldier termites of *M. falciger* species is scarce in the literature. It is thus important to determine the nutritional profile of this species and evaluate its potential to replace fishmeal in the diet of commonly cultured fish species.

Solder termites are used as feed in the poultry sector, especially in rural communities (Dao *et al*., 2022; Dao *et al*., 2020; Boafo *et al*., 2019). This shows that their use in animal feed is well accepted. Although the Soldier termite meal has good protein and a balanced amino acid profile, there are no studies that evaluated this insect as a novel ingredient in replacing fishmeal in aquafeed. Their year-round availability makes them an insect of interest. The current study will use Soldier termite meal (*M. falciger*) as a fishmeal replacement in the diets of two commonly cultured warm freshwater species in southern Africa with different feeding habits.

2.5 Commonly cultured freshwater fish species in South Africa

Freshwater aquaculture species that are mainly cultured in South Africa include trout (*Oncorhynchus mykiss* and *Salmo trutta*), *Cyprinus carpio*, *O. mossambicus*, *C. gariepinus*, *O. niloticus* and *Micropterus salmoides* (Adeleke *et al*., 2021; DAFF 2017).

These fish species are currently operating at a commercial scale apart from *O. mossambicus* and *C. gariepinus.* Moreover, these two species are the commonly cultured species in the northern part of South Africa. Assessing their potential to utilise an insect-based diet may increase their production and improve their operational scale.

2.5.1 The African catfish (*C. gariepinus*)

Clarias gariepinus (Burchell 1822) is one of the commonly cultured freshwater species globally (Figure 2.2; Skelton, 2001). In South Africa, its natural distribution is northwards of the Orange river system in the west and northwards of the Umtamvuna river in the east (Figure 2.3).

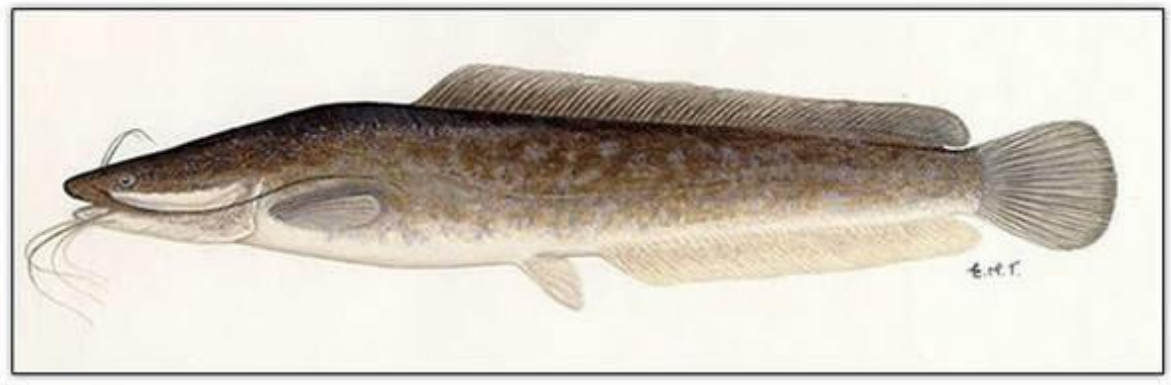

Figure 2.2: The African catfish, *C. gariepinus* (Skelton, 2001).

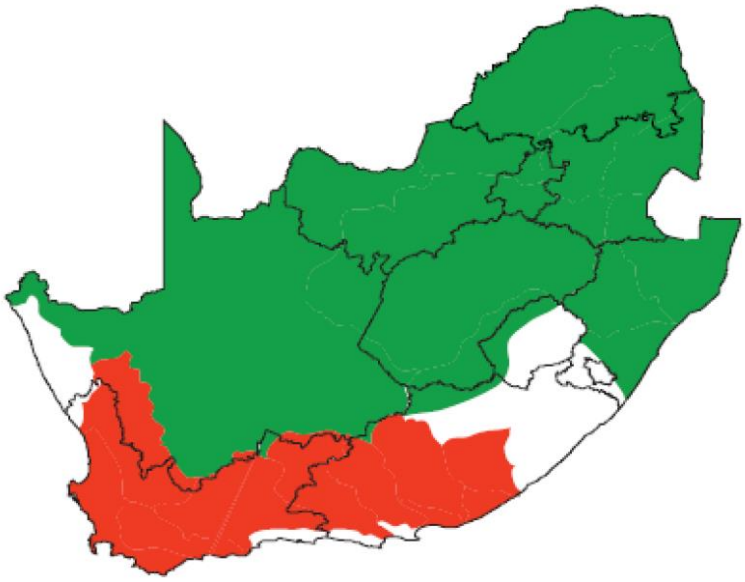

Figure 2.3: The map of the distribution of *C. gariepinus* in South Africa. The red colour shows where it has been introduced while the green shows where it is native (Picker and Griffiths, 2011).

Clarias gariepinus is known as an opportunistic and omnivorous feeder, feeding on items such as zooplankton, fish, detritus, macrophytes, insects and detritus (Tesfahun, 2018). A study conducted in Lake Babogaya, Ethiopia showed that insects had the highest frequency of occurrence in *C. gariepinus* stomach (Admassu *et al*., 2015). Adewumi *et al*. (2014) found that *C. gariepinus* from the Egbe Reservoir fed mostly on phytoplankton (*Cyanophyceae*, *Chlorophyceae*, *Euglenophyceae*) which constituted 97.10%. Insects, zooplankton, detritus, and crustaceans were also found in the stomach. In other studies, fish constituted a major part of the food components in *C. gariepinus* (Adeyemi *et al*., 2009). In South Africa, Kadye and Booth (2012) showed that insects constituted 87% of the total diet composition in *C. gariepinus* from the Great Fish River in the Eastern Cape Province. The literature affirms that insects form a huge part in the diet of *C. gariepinus*. Thus, it has the potential to utilise insect-based protein sources as fishmeal replacements in its diet (Anvo *et al*., 2017).

It has been reported that feed composition of *C. gariepinus* changes as the fish grows (Munro, 1967). Smaller fish tends to feed on insects of the Diptera order, particularly chironomid pupae and zooplankton as the fish grows. Moreover, the diet composition of *C. gariepinus* also depends on the habitat, photoperiod, and season change

(Tesfahun and Alebachew, 2023; Houlihan *et al*., 2001). In Lake Sibaya, South Africa, *C. gariepinus* ignored preying on fish during daylight and feed mainly on invertebrates, which are easy to catch and in abundance. At night, it preys on fish since they were vulnerable (Bruton, 1979). For *C. gariepinus* to feed on wide variety of food organisms, it is equipped with anatomical adaptations which includes a wide mouth that is capable of considerable vertical displacement for engulfing large prey (Tekle-Giorgis *et al*., 2016; FAO, 1996). A broad band of recurved teeth on the jaws and pharyngeal teeth that keep prey from escaping. *Clarias gariepinus* also has several sensory organs on the body, head, lips and barbels. The digestive system of the African catfish comprises of the oropharyngeal cavity, oesophagus, stomach, intestine, rectum, and anus. All these organs are involved in the breaking down of ingested food into absorbable micromolecules which plays a role in maintenance, growth, and energy needs of its body.

Clarias gariepinus has been widely tested on a variety of insect-based diets, with different degrees of success (Adeoye *et al*., 2019; Anvo *et al*., 2017; Alegbeleye *et al*., 2012; Aniebo *et al*., 2009; Fasakin *et al*., 2003). Most studies showed that *C. gariepinus* can utilise an insect-based diet up to 50% inclusion levels without affecting its growth, nutrient utilisation, and health status. Moreover, most studies were carried out in west Africa and in some studies the authors' mixed-up replacement levels with inclusion levels (Akinwole *et al*., 2020; Aniebo *et al*., 2009). The current study will compare the effects of an insect-based diet on *C. gariepinus* (opportunistic predator) with *O. mossambicus* (herbivore) on their growth performance, nutrient utilisation, blood serum chemistry, liver and intestine histology and their digestive enzyme profile.

2.5.2 The Mozambique tilapia (*O. mossambicus*)

The Mozambique tilapia (*O. mossambicus*) (Peters,1852) is also one of the freshwater species of economic importance in southern Africa (Figure 2.4). This fish species is mainly distributed in the east coastal rivers from the lower Zambezi system south to the Bushmans system in South Africa (Figure 2.5). In South Africa, *O. mossambicus* is mainly farmed in tropical regions of the country where high temperatures are prominent throughout the year. These include Limpopo, Mpumalanga, and Kwazulu Natal Provinces (FAO, 2016). The Mozambique tilapia is classified as vulnerable on

the International Union for Conservation in Nature (IUCN) red list (Waiyamitra *et al*., 2021)

Figure 2.4: The Mozambique tilapia, *O. mossambicus*.

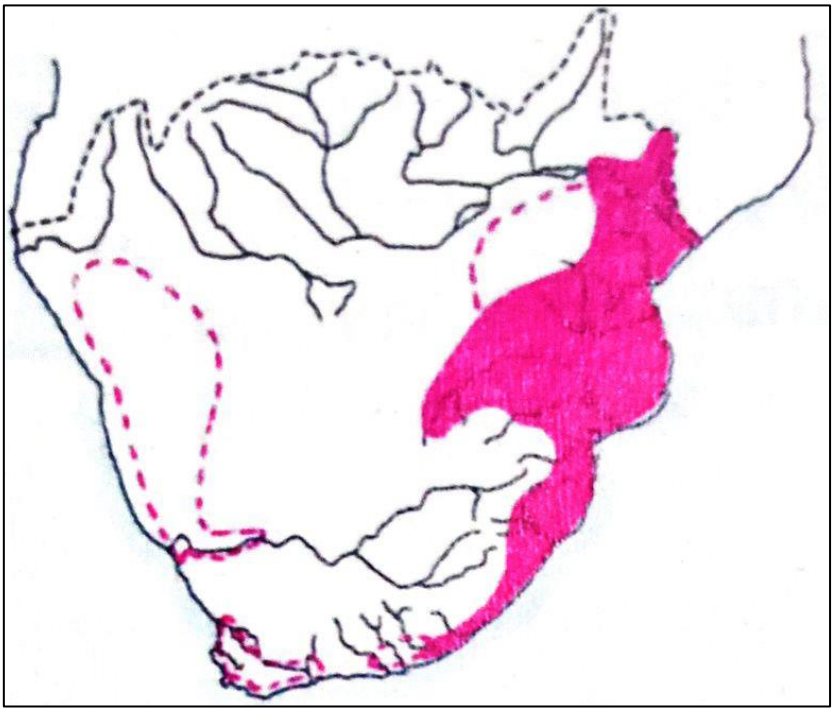

Figure 2.5: Distribution of *O. mossambicus* in South Africa (Skelton, 2001).

Oreochromis mossambicus is a herbivore and primarily feeds on phytoplankton, periphyton, zooplankton, macrophytes, detritus, insects and aquatic vegetation,

(Russell *et al*., 2012). A study conducted in Borna Reservoir, Maharashtra showed that rotifers (35%) constituted the main food item in juvenile *O. mossambicus*, followed by copepods (30%) (Sakhare and Jetithor, 2016). Morphological features such as jaw structure, mouth size and shape, dentition, and gill raker's size and number allow this species to occupy a wider range of feeding niches (El-Sayed, 2020). Furthermore, their diet preference depends on species size, time of the day, photoperiod, and geographical locations (El-Sayed, 2020).

The digestive system of *O. mossambicus* is simple, consisting of a short oesophagus which is connected to a small, saclike stomach and a long-coiled intestine which can reach up to 7-13 times the total fish length (El-Sayed, 2020; Balarin and Hatton, 1979). Based on its long intestine, *O. mossambicus* is regarded as herbivorous. This fish species also has the potential to utilise diets with insects-based protein sources as fishmeal replacement (Rapatsa and Moyo, 2017).

2.6 Literature Review Summary

Insects have been utilised as both food and feed for millennia. Beetles, caterpillars, ants, locusts, crickets, bugs and termites are the most commonly eaten groups of insects. They are mainly eaten for their nutritional value, health benefits and economic viability. Insects have gained attention as potential fishmeal replacements in the animal feed industry. This is due to their high protein content, balanced amino acid and fatty acid profile, lipids, vitamins, and minerals comparable to fishmeal. Insects also form part of the natural diet for both freshwater and marine fish species. The most widely used insects in fishmeal replacement studies are the black soldier fly, common housefly, mopane worm, mealworm, locusts and have been reviewed in this study. These insects have been evaluated in different fish species. These studies have shown that insects can partially or completely replace fishmeal in fish diets. However, there are factors that affect the utilisation of insect-based diets in fish species. These includes their crude protein content, fat content, amino acid and fatty acid profile, the developmental stage of the insect and its feeding substrate. Studies have shown that fish with different feeding habits response differently towards an insect-based diet. Herbivorous species showed that they can utilise insect-based diets at higher inclusion levels than predatory species. Complete replacement of fishmeal with insect-based

diets has also been observed in herbivorous species. However, these findings are not comparable. Mainly because researchers used different inclusion ration, insects were processed differently; affecting the proximate composition, and fish were reared in different systems. Thus, it is important to compare the effects of an insect-based diet in fish species of different feeding habits under the same rearing conditions. Some of the locally available insect species in southern Africa are Stinkbugs, Soldier termites, and Alates termites. These insects have the potential to replace fishmeal in the diets of commonly cultured fish species. These insects will be tested in *O. mossambicus* and *C. gariepinus* diets. These are the two commonly cultured warm freshwater species of economic importance in southern Africa. They are widely distributed and have different feeding habits. The Mozambique tilapia is herbivorous, whilst the African catfish is an opportunistic predator. Studies have shown that these two fish species have the potential to utilise insect-based diets. This study will thus increase aquaculture production, alleviate poverty, increase food security, and improve the livelihood of fish farmers in the rural Limpopo Province and other regions.

Chapter 3:

Dietary full-fat Stinkbug (*Encosternum delegorguei*) meal effects on growth performance, blood chemistry, liver and intestinal histology, and digestive enzyme profile of juvenile Mozambique tilapia (*Oreochromis mossambicus*) and African catfish (*Clarias gariepinus*).

CHAPTER 3: Dietary full-fat Stinkbug (*Encosternum delegorguei*) meal effects on growth performance, blood chemistry, liver and intestinal histology, and digestive enzyme profile of juvenile Mozambique tilapia (*Oreochromis mossambicus*) and African catfish (*Clarias gariepinus*).

3.1 INTRODUCTION

The use of insect meal in aquafeed has recently become a research priority in the aquaculture sector (Abdel-Tawwab *et al*., 2020; Llagostera *et al*., 2019). Several insects have partially and completely replaced fishmeal in different fish species. It is thus important to search for insects within our geographical area with the potential to substitute fishmeal in fish species that are commonly cultured. In southern Africa, one of the insects that can be used to replace fishmeal is the Stinkbug (*E. delegorguei*). The Stinkbugs are one of the three groups frequently consumed in the order Hemiptera (Feng *et al*., 2018). Some of the Stinkbugs in the Hemiptera that have been evaluated for nutrition include *Aspongopus chinensis*, *Cyclopelta parva*, *Dolycoris baccarum*, *E. delegorguei*, *Eurostus validus*, *Eusthenes saevus*, *Lethocerus indicus*, *Mictis tenebrosa*, *Monosteria unicostata*, *Halyomorpha picus*, *Sphaerodema rustica*, *Tessaratoma papillosa*, *Urochela luteovaria* (Gao *et al*., 2022). Among these Stinkbugs, *E. delegorguei* is one of the most popular edible Stinkbug that several studies attempted to document its nutritional profile (Teffo *et al*., 2017; Musundire *et al*., 2016; Dzerefos *et al*., 2009). Stinkbugs of the order Hemiptera such as *Bathycoelia distincta*, *Nezara viridula*, and *Chinavia pallidoconspersa* are known as pests on Macadamia trees in South Africa (Sonnekus *et al*., 2022). However, *E. delegorguei* is not considered an agricultural pest (Athey *et al*., 2019; Koch *et al*., 2017). It is widely distributed in subtropical, open wood and bushveld of southern regions of Zimbabwe and northern provinces of South Africa (Makore *et al*., 2015). In South Africa, this insect is mostly harvested in Thohoyandou, Ga-Modjadji and Bushbuckridge (Figure 3.1).

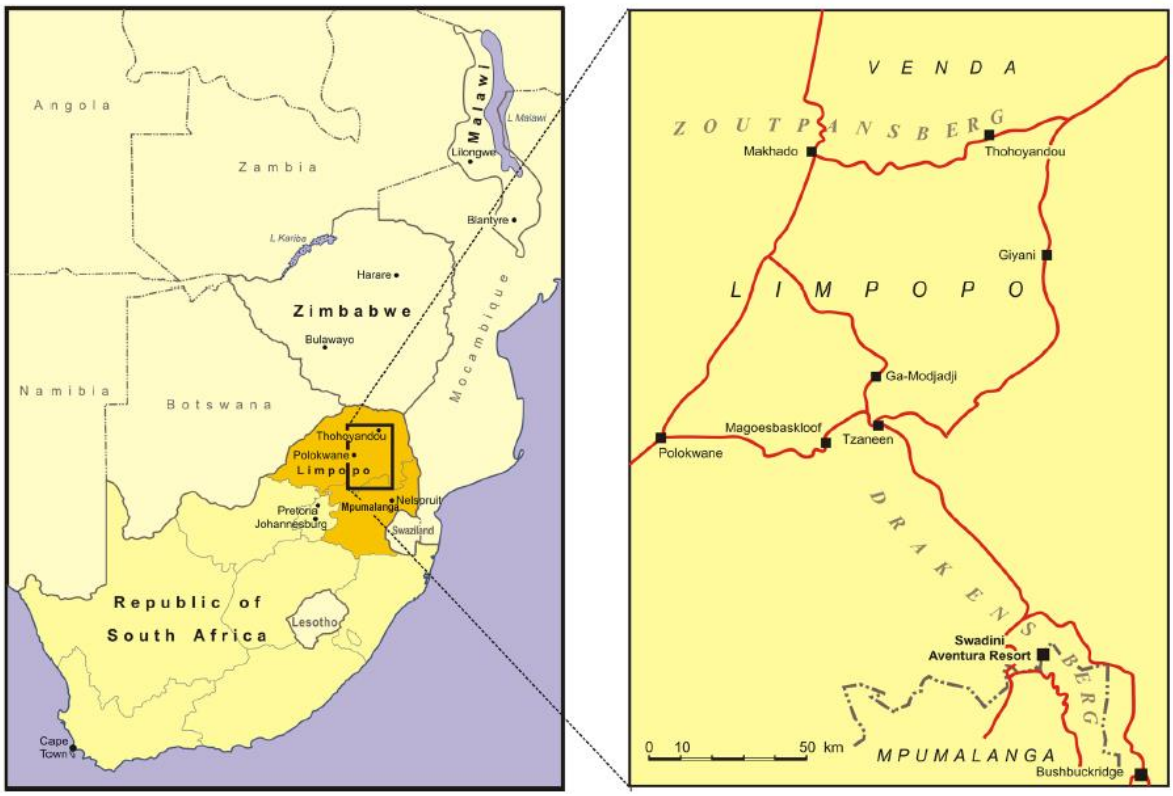

Figure 3.1: The map showing harvesting sites of *E. delegorguei* in South Africa: Thohoyandou, Ga-Modjadji and Bushbuckridge (Dzerefos *et al*., 2013).

Seasonal availability is one of the challenges associated with the replacement of fishmeal with insects (van Huis, 2022). Most insects are available during the rainy season (October to March). However, *E. delegorguei* is one of the insects that is harvested during the dry season (April to September), when other insects are scarce. The lifecycle of the Stinkbug comprises the eggs, nymphs, and adults (Mapendembe and Mujere, 2014) (Figure 3.2). The hard-shelled eggs are laid on stems and twigs of host plants at the beginning of the Spring season and hatch in Summer. The nymphs develop into five instars before moulting into winged adults. The fully grown insect is ready and harvested in winter. The Stinkbug is known to contain a high-fat content (Teffo *et al*., 2007). However, its protein content, fatty acid profile and amino acid profiles are poorly understood. Its nutritional composition is influenced by factors such as sex, reproduction stage, diet, season, and habitat (Dandadzi *et al*., 2023). The Stinkbug is a phytophagous terrestrial insect that feeds on sub-tropical savannah plants. The Stinkbug is also one of the insects sold at the open market in Thohoyandou

Town. Unlike the Mopane worm, the Stinkbug is cheap. It plays a role in the income generation of local harvesters and a source of protein for local communities.

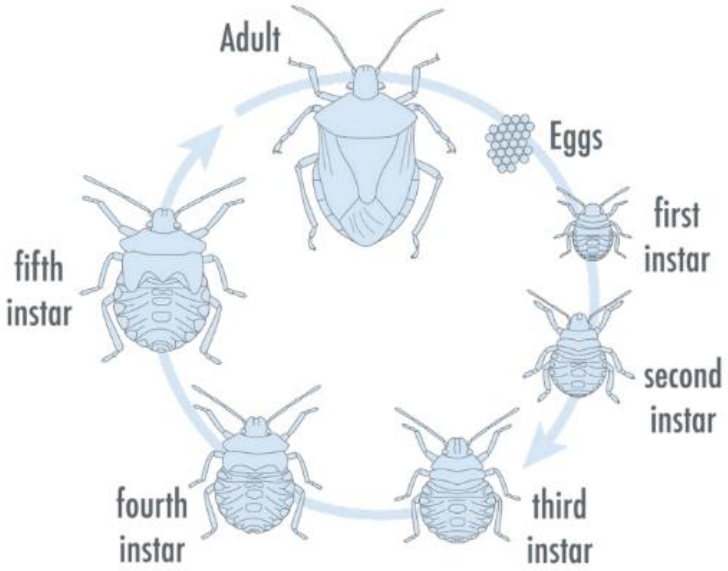

Figure 3.2: The life cycle of *E. delegorguei* showing developmental stages (Kobayashi, 1967).

The potential for Stinkbug as a fishmeal substitute in animal feed has not been investigated. Reviews on the use of insects in animal feed have focused on orders such as Coleoptera, Diptera, Orthoptera, and Lepidoptera, (Henry *et al*., 2015; Barroso *et al*., 2014), However, the order Hemiptera has been largely ignored. In a recent meta-analysis, *E. delegorguei* was not reviewed due to a lack of information on published literature (Rapatsa and Moyo, 2022). Most insects are used in their larvae/pupae developmental stage. However, the Stinkbug is utilised at its adult stage like most insects in the Orthoptera order. It is therefore imperative to carry out a detailed study on the effect of dietary full-fat Stinkbug (*E. delegorguei*) meal as a fishmeal replacement on some aspects of the physiology of *O. mossambicus* and *C. gariepinus*. The Mozambique tilapia and the African catfish are native species of economic importance in southern Africa. They both have shown the potential to utilise insect-based diets and higher inclusion levels have been recorded (Adeoye *et al*., 2019; Rapatsa and Moyo, 2017). These species have different feeding habits and their response towards an insect-based diet will promote and improve feed formulation.

Moreover, the response of these species when fed an insect-based diet has not been compared before.

3.2 OBJECTIVES AND NULL HYPOTHESES

The objectives of this chapter were:

I. To determine the effect of replacing fishmeal with Stinkbug meal on the growth performance of *O. mossambicus* and *C. gariepinus*.
II. To determine the effect of replacing fishmeal with Stinkbug meal on the blood serum chemistry of *O. mossambicus* and *C. gariepinus.*
III. To evaluate the liver and intestinal histology of *O. mossambicus* and *C. gariepinus* fed diets with an insect-based diet.
IV. To determine the effect of digestive enzyme profile on the distal intestine of *O. mossambicus* and *C. gariepinus* fed diets with Stinkbug meal as a substitute for fishmeal.
V. To determine cost benefit analysis of replacing fishmeal with Stinkbug meal on the diets of *O. mossambicus* and *C. gariepinus*.

Null Hypotheses:

I. There is no effect on the growth performance of *O. mossambicus* and *C. gariepinus* fed diets with Stinkbug meal as a fishmeal replacement.
II. There is no effect on the blood serum chemistry in *O. mossambicus* and *C. gariepinus* fed an insect-based diet.
III. There is no effect on the liver and intestine histology of *O. mossambicus* and *C. gariepinus* fed diets with Stinkbug meal as a fishmeal replacement.
IV. There is no effect on the digestive enzyme profile on the distal intestine of *O. mossambicus* and *C. gariepinus* fed diets with Stinkbug as a fishmeal replacement.
V. There is no effect on the cost benefit analysis of replacing fishmeal with Stinkbug meal on the diets of *O. mossambicus* and *C. gariepinus.*

3.3 MATERIALS AND METHODS

3.3.1 Diet formulation

Stinkbugs were purchased from the Thohoyandou Town open market, in the Vhembe District of Limpopo Province, South Africa. Stinkbugs were ground using a miller. Five diets were formulated to partially substitute fishmeal at 0, 10, 30, 50, and 70% (Table 3.1). The 0% diet contained 30% fishmeal and served as a control, with no Stinkbug meal. The diets were named D1, D2, D3, D4, and D5, denoting 0, 10, 30, 50, and 70% replacement levels, respectively (Table 3.1). The diets were formulated to be isonitrogenous (30% crude protein), isocaloric (15 MJ/Kg), and isolipidic (12% fat). The Winfeed 3, EFG (Natal) program was used to formulate diets. The yellow maize, wheat bran, sunflower meal, and sunflower oil were purchased from a local market. Methionine and lysine were purchased from Nutroteq Pty Ltd. Vitamin/Mineral premix, fishmeal, and binder from Irvines Africa (Pty) Ltd. Chromic oxide was purchased from Sigma Aldrich. All the ingredients were ground and screened through a sieve and mixed thoroughly using a planetary mixer (Hobart, Troy, OH, USA) until they were well mixed. Chromic oxide (Cr_2O_3) was used as an inert marker in each diet at (10 g/kg). During mixing, water was added (10-20% v/w) occasionally until the desired dough thickness was reached. The dough was pelleted through a meat grinder (SLP-45 Shang Hai Taiyi, China) and pushed through to produce extruded strands (2 mm diameter). Pelleted strands were sun-dried and then stored in polyethylene buckets with lids separately at 4°C. The proximate composition for experimental diets is illustrated in Table 3.1

Table 3.1: Ingredients (g/kg, DM) used to formulate experimental diets where Stinkbug meal replaced fishmeal at different replacement levels and proximate composition.

Ingredients	Experimental diets				
	D1	D2	D3	D4	D5
Fishmeal[a]	300	270	210	150	90
Stinkbug	0	30	90	150	210
Yellow Maize	221	222	233	230	228
Wheat bran	219	218	207	210	212
Sunflower meal	100	100	100	100	100
Sunflower oil	60	60	60	60	60
Methionine	20	20	20	20	20
Lysine	20	20	20	20	20
Vitamin/Mineral premix	20	20	20	20	20
Binder	30	30	30	30	30
Chromic oxide	10	10	10	10	10
Total	1000	1000	1000	1000	1000
Proximate composition					
Dry matter (%)	92.24	92.40	92.97	92.78	92.10
Crude protein (%DM)	30.18	30.02	30.33	30.49	30.21
Fat (%DM)	12.06	12.91	12.25	12.11	12.65
Ash (%DM)	10.59	10.60	10.83	10.25	10.20
Gross Energy (MJ/Kg of DM)	15.78	15.85	15.11	15.52	15.22

[a]Fishmeal (crude protein: 65.5%, fat: 12.0%).

[b]Vitamin/Mineral premix: Vit. A, 12 000 IU; Vit. D3, 1 200 IU; Vit. E, 120 IU; Vit. B4, 10000g; Vit. C, 120g; Vit. B3, 25g; Vit. B5, 15g; Vit. B2, 6g; Vit. B6, 5g; Vit. B1, 4g; Vit. K3, 2g; Vit. B9, 1g; Vit. H, 0.25g; Vit. B12, 0.04g. ZnO, 200g; $FeSO_4$; $CuSO_4$, 7g; MnO, 5g; KI, 2g; Na_2SeO_3, 0.15g; $CoSO_4$, 0.05g. D1 (0%), D2 (10%), D3 (30%), D4 (50%), D5 (70%).

3.3.2 Proximate composition

Stinkbug meal was analysed for dry matter by drying samples using an oven at 105°C until a constant weight was reached. Moisture content was determined by obtaining the difference between the initial weight and the weight lost after samples were dried

in the oven at 105°C for 24 h. Crude protein (AOAC #984.13) was determined using the Micro-Kjeldahl method, and the content was estimated by multiplying nitrogen with 6.25 (Foss 2400 Kjeltec analyser unit, Denmark) (AOAC, 2003). Fat content (AOAC #954.02) was determined using solvent extraction with petroleum ether (Soxtec system, Foss Tecator lipid analyser (AOAC, 2003). Ash content (AOAC #942.05) was determined by incinerating samples at 550°C for 6 hours in a muffle furnace (Heraeus K1252, Germany) (AOAC, 2003). The crude fibre was determined by digesting dried lipid-free residues with 1.25% H_2SO_4 and 1.25% NaOH. The loss on ignition was recorded as crude fibre (Hlophe-Ginindza *et al*., 2015). Gross energy was analysed using a DDS Isothermal CP 500 bomb calorimeter. Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) were determined by boiling samples with a neutral detergent solution and acid detergent solution, respectively (van Soest *et al*., 1991).

3.3.3 Mineral composition

Selected minerals (iron, potassium, and sodium) were analysed in the Stinkbug meal. Samples were hydrolysed with 65% concentrated nitric acid and 37% hydrochloric acid. Hydrogen peroxide was used to remove nitrous vapours. The mineral content was then determined using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Manditsera *et al*., 2019). The analysis was performed using standard solutions of known concentrations. All chemicals used were analytical reagent grade.

3.3.4 Amino acid profile analysis

Stinkbug meal was analysed for amino acids at the Central Analytical Facilities (CAF) of Stellenbosch University (South Africa). Samples (20 mg) were hydrolysed with 6M HCI at 110°C for 4 hours in sealed tubes (Ishida *et al*., 1981). About 130 µL was transferred into a 2 ml tube and dried under a gentle stream of nitrogen. The samples were reconstituted and derivatized with 30 µL MTBSTFA and 100 µL acetonitrile at 100°C for 1 hour. The samples were allowed to cool down to room temperature and injected into the gas chromatograph (6890N, Agilent technologies network). Separation was performed on a gas chromatograph coupled to Agilent technologies inert XL EI/CI Mass Selective Detector (MSD) (5975, Agilent Technologies Inc., Palo Alto, CA).

The Essential amino acid Index (EAAI) of the Stinkbug meal was calculated using the Peńaflorida (1989) formula:

$$EAAI = \sqrt[n]{\frac{aa1}{AA1} \times \frac{aa2}{AA2} \times \cdots \cdots \frac{aan}{AAn}},$$

Where aa is the amount of the amino acid in the Stinkbug meal, AA is the amino acid requirement of the fish, and *n* is the total number of amino acids used in the calculation.

3.3.5 Fatty acid profile

The fatty acid methyl esters (FAMEs) for Stinkbug meal were also analysed at the Central Analytical Facilities (CAF) of Stellenbosch University (South Africa). This was done by adding 2 ml of 2:1 chloroform: methanol solution to 100 mg of the sample (Folch *et al*., 1957). The sample was vortexed and sonicated at room temperature for 30 min. The sample was then centrifuged at 3000 rpm for 1 min. Chloroform (200 µl) was dried completely with a gentle stream of nitrogen, reconstituted, and vortexed with 170 µl of methyl tert-butyl ether (MTBE) and 30 µl of trimethylsulfonium hydroxide (TMSH). One µl of the derivatized sample was injected in a 5:1 split ratio onto the GC-FID. Separation was performed on a gas chromatograph (6890N, Agilent Technologies) coupled to a flame ionization detector (FID). Separation of the FAMEs was performed on a polar RT-2560 (100 m, 0.25 mm ID, 0.20 µm film thickness) (Restek, USA) capillary column. Hydrogen was used as the carrier gas at a flow rate of 1.2 ml/min. The injector temperature was maintained at 240°C. One µl of the sample was injected in a 5:1 split ratio. The oven temperature was programmed as follows: 100°C for 4 min, ramped to 240°C at a rate of 3 °C/min for 10 min.

3.3.6 Experimental design

Feeding trials were conducted at the Aquaculture Research Unit (ARU), University of Limpopo, South Africa. Mozambique tilapia (*O. mossambicus*) juveniles were bred at the Aquaculture Research Unit and kept in the hatchery tanks until use. African catfish (*C. gariepinus*) fingerlings were also obtained from the Aquaculture Research Unit.

Before the experiment commenced, fish were collected from the hatchery tanks and separately placed in a 100L container with water that was mixed with 2 phenoxyethanol (1 ml/ L) as an anaesthetic. For *O. mossambicus*, fish size of 4.2±0.3 g/fish were used. Fish were weighed in bulk (initial body weight) and stocked (15 fish per tank) in experimental tanks in triplicates. The average initial body weight of fish per diet ranged between 64.04±5.07g and 69.29±4.07g. A completely randomised design was used to run the experiment using 15 tanks. Each diet (D1-D5) was randomly allocated to tanks in triplicates into 500 L capacity fiberglass experimental tanks in a recirculating system (Filled to 400 L mark).

For *C. gariepinus*, fish size used was 6.4±1.02 g/fish. *Clarias gariepinus* fish were stocked at 5 fish per tank in triplicates. The average initial body weight of fish per diet ranged between 28.15±4.25g and 34.48±4.20g. The tanks were connected to a sump and pump that supplied the tanks with water at 10 L/min. The tanks were individually heated with a submersible aquarium heater (ViaAqua Glass heater, 200 W) and aerated with air stones. Fish were left to acclimatise in experimental tanks for 2 weeks while feeding on commercial pellets (Aqua-plus, Avi Products (Pty) Ltd). After the acclimatisation period, fish were anaesthetised, and the initial body weight was recorded for each tank. Fish were then randomly allocated to tanks in triplicates and allocated their diets.

3.3.7 Feeding procedure

Fish were fed their allocated experimental diets to apparent satiation for 8 weeks twice a day (09:00 h and 15:00 h). Fish were considered satiated when 2 pellets were left uneaten for 5 min in a tank. Feed intake for each tank was recorded daily. Fish tanks were fitted with the Guelph system, with mild modifications (Cho *et al*., 1982). Faecal matter samples were collected two weeks after the commencement of the experiment from the collection tube. The faecal matter samples were used for the apparent digestibility coefficient of a protein.

3.3.8 Water quality maintenance

Water temperature was kept between 27 and 29°C, dissolved oxygen (6.5 - 8.0 mg/l), ammonia (<1 mg/l), and pH (6.8 - 8.0). These parameters were monitored on a weekly

basis using a handheld multiparameter meter (Professional plus YSI 605000). The experiment was conducted under a natural photoperiod.

3.3.9 Growth performance indices.

Fish were weighed at the commencement of the trial, after every two weeks, and at the end of the feeding trial. At the end of the experiment, fish were starved for 24 hours before growth performance indices were measured. Three fish from each replica (9 fish per treatment) were randomly selected for measurement of growth parameter indices.

For growth performance, the following parameters were calculated:
Specific growth rate (SGR):

$$\text{SGR (g/day)} = \left[\frac{\text{In } W_t - \text{In} W_0}{t}\right] \times 100\%$$

Where In, W_t, W_0, and t are the natural logarithm, the final body weight (g), initial body weight (g), and time feeding period (days), respectively.

Thermal-unit growth coefficient (TGC) :

$$\text{TGC} = 1000 \times \frac{\text{Final weight (g)}^{\frac{1}{3}} - \text{Initial weight (g)}^{\frac{1}{3}}}{\text{Temperature (°C)} \times \text{Days}}$$

Feed conversation ratio (FCR): $\text{FCR} = \frac{\text{feed consumed (g)}}{\text{weight gained (g)}}$

Protein efficiency ratio (PER): $\text{PER} = \frac{\text{Increase in body mass (g)}}{\text{Protein consumed (g)}}$

$$\text{Fish survival} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

Weight gain (g) = final weight (g) - initial weight (g)

Feed intake (g/fish/day) = weight of eaten feed (g) / number of fish / feeding period (day).

Apparent protein digestibility coefficient (APDC) was determined according to Cho *et al*., (1982). Chromic oxide in diets and faeces was determined by adding concentrated nitric acid (HNO_3) into the samples and then 70% perchloric acid ($HClO_4$) was added to oxidize chromic oxide until the colour orange appeared. The samples were then read for absorbance at 350 nm, using ultrapure water as a blank. Chromic oxide was then calculated as follows:

$$\text{Weight of chromic oxide in sample} = \frac{\text{Absorbance} - 0.0032}{0.2089}$$

Chromic oxide (%) = (weight of chromic oxide / sample weight (mg)) x100

$$\text{APDC (\%)} = 100\left[1 - \left(\frac{Cr_2O_3 \text{ in diet}}{Cr_2O_3 \text{ in faeces}}\right) \times \left(\frac{\text{protein in faeces}}{\text{protein in diet}}\right)\right]$$

3.3.10 Organosomatic indices

Fish were collected from experimental tanks and sedated with 2-phenoxyethanol (1 ml/L). Three fish from each dietary replicate (9 fish/diet) were used for organosomatic indices. The condition factor, hepato-somatic index, and viscero-somatic index were calculated.

$$\text{CF} = \frac{\text{Body weight (g)}}{(\text{Fish length})^3(\text{cm})} \times 100$$

$$\text{HSI} = \frac{\text{Liver weight (g)}}{\text{Fish weight (g)}} \times 100$$

$$\text{VSI} = \frac{\text{viscera weight (g)}}{\text{Fish weight (g)}} \times 100$$

3.3.11 Blood serum chemistry

Three fish samples from each dietary replicate (9 fish/diet) were randomly selected and sedated with 2-phenoxyethanol (1 ml/L). Blood samples were drawn from the caudal vasculature into vials using heparinised syringes and centrifuged for 10 min at 3500 rpm. The serum was used for the analysis of aspartate aminotransferase (AST),

alanine aminotransferase (ALT), cholesterol, triglyceride, and glucose. The analyses were conducted using a commercial automatic biochemical kit (Sigma-Aldrich, USA).

3.3.12 Liver and intestinal histological analysis

Three fish from each dietary replicate (9 fish/diet) were used for liver and intestinal histomorphology. The liver samples from each fish were separately fixed in a 10% buffered formalin solution in sampling bottles. The distal intestine of each fish was also separately preserved in 10% formalin solution in sampling bottles. During analysis, liver samples were cut into 1 cm^3 blocks, whilst intestine samples were cut into 1 cm lengths. All samples were washed for 2 h in distilled water. Samples were then dehydrated in ethanol solution of 70, 80, 96, and 100% levels concentrations. Liver and intestine samples were then made transparent using xylene. Samples were then infiltrated using Tissue-Tek® III wax in an oven at 60°C. Samples were further embedded in Tissue-Tek® III wax blocks, sectioned at 5 µm thickness (Leica: RM 2155, Madrid, Spain). The sectioned samples were placed in microscope slides and left to dry. After drying, slides were stained with haematoxylin and eosin (H&E) and covered with coverslips as described by van Dyk and Pieterse (2008).

3.3.13 Liver and intestinal histological assessment.

Liver and intestine slides were assessed using a light microscope (Leica Microsystems model DM750, Leica, Bannockburn, IL, USA) which was equipped with a digital camera (Digital Video Camera Company, Austin, TX, USA). Slides micrographs, intestinal villi height, and villi thickness were captured using the digital image analysis software ANALYSIS™ (Soft Imaging Systems GmbH, Münster, Germany). The intestine histology was quantified using a modified scoring system according to Baeza-Ariño *et al*. (2014). For villi length and villi thickness, six measurements per slide were taken to establish mean values (n = 6). Goblet cells were quantified by counting the number present in each villus. Six villi per slide were quantified to establish mean values (n = 6).

Liver histology slides were quantified by analysing the liver hepatocyte condition according to McFadzen *et al*. (1997). The grading was from 1-3, where 1 describes a healthy liver, 2 (intermediate), and 3 (degraded). Dietary replicates were allocated an

average grade value based on the dominant histological alterations observed (Table 3.2).

Table 3.2: Liver histological scoring criteria used in *O. mossambicus* and *C. gariepinus* (McFadzen *et al*., 1997).

	Grade		
Tissue	1 (Healthy)	2 (Intermediate)	3 (Degraded)
Liver nuclei	Nuclei lightly granular, small, and distinct	Nuclei with abundant dark granules, nucleoli enlarged or indistinct.	Nuclei small dark and pyknotic
Liver hepatocyte cytoplasm	Structured: Varied texture, scattered granules with eosin-positive patches	Homogenous: Granular, slight variability in staining property	Hyaline: Lacking texture, dark small and often separated from the cell boundary
Hepatic vacuolation	Extensive: large cytoplasmic vacuoles following cell boundary and encroaching on the nucleus, PAS positive vacuoles	Slight: Occasional small PAS positive vacuoles, often in patches	None: cell devoid of PAS positive vacuoles, often large sinusoidal spaces

3.3.14 Gastrointestinal enzyme profile

Three fish from each dietary replicate were sedated in a 2-phenoxyethanol solution and sacrificed. Fish samples were dissected, distal intestines were removed and stored in sampling bottles at -80°C until analysis. During analysis, samples were homogenised (1:9 tissue/buffer) in ice using a 0.01 M Tris buffer (pH 7.4), and the samples were then centrifuged at 3000 x g for 10 min at 4°C. The supernatant was used for enzyme activity analysis.

Amylase activity was determined as described by Bernfeld (2006). The method uses 3,5-dinitrosalicylic acid (DNS) and starch as a substrate, which was prepared in 0.1 M phosphate buffer (pH 7). Substrate solution (33 µL), enzyme extract (3 µL), and buffer solution (27 µL) were incubated at 30°C for 10 minutes. The reaction was stopped by adding 100 µL of DNS solution. The solution was then boiled for 5 minutes and read at 540 nm using a Multimode analysis software version 3.3.0.9. These samples were prepared concurrently with blanks (without enzyme extract).

Lipase activity was determined according to Markweg *et al*. (1995). The substrate used in this method was 0.01 M para-nitrophenyl palmitate (pNNP). The substrate (10 µL), enzyme extract (12.5 µL), and buffer (0.1 M phosphate buffer, pH 7) were added together. The solution absorbance was read at 410 nm. These samples were also prepared concurrently with a blank.

Protease enzyme activity was determined by homogenising 40 mg of each sample in a 0.9% (w/v) NaCl using a Teflon pestle and glass mortar. The samples were centrifuged at 10 000 x g for 10 min at 4°C. The supernatant was used to determine protein content (Bezerra *et al*., 2005). Azocasein (50 µm; Sigma Aldrich) was used as a substrate and Trichloroacetic Acid (TCA) was used to stop the reaction. The absorbance of the solution was read on a microtiter plate reader (Bio-rad 550). The analyses were conducted along the blank. The enzyme activity was defined as one unit (U) of an enzyme capable of hydrolysing Azocasein to produce a 0.001 change in absorbance per minute. The analyses were performed for both *O. mossambicus* and *C. gariepinus* experiments.

3.3.15 Cost benefit analysis

The cost benefit analysis was determined according to Bahnasawy *et al*. (2003).

$$\text{Incidence cost} = \frac{\text{Cost of feed}}{\text{Quantity of fish produced (kg)}}$$

$$\text{Profit index} = \frac{\text{Local market value of fish}}{\text{Cost of feed}}$$

The underlying assumption is that all operating costs are constant, and the cost of ingredients was the only variable cost. The prices (USD) were set according to the local market retail prices (at the current time) of diets. The prices were as follows (USD/kg): Fishmeal, 1.81; insects, 0.43; yellow maize, 0.40; wheat bran, 0.20; sunflower meal, 0.91; sunflower oil, 0.71; methionine, 0.15; lysine, 0.15; vitamin/mineral premix, 0.35; binder, 0.19; chromic oxide, 0.12. The market price for fish in the study area was 2.63 USD.

3.4 STATISTICAL ANALYSIS

Shapiro-Wilk and Barlett's tests were used to test data for normality of distribution and homogeneity of variance, respectively. One-way analysis of variance (ANOVA) was used to test significant differences in growth parameter indices, organosomatic indices, blood serum chemistry, and histology. The data was significantly different when $P<0.05$. Tukey's HSD post hoc test was used to determine mean differences. The data was analysed using the SPSS version 28.0 software package (Statistical Package and Service Solutions, IBM, Chicago, IL, USA). Linear regression model was used to test the relationship between SGR, FCR and APDC and inclusion levels of insect meals (Stinkbug, Alates termite & Soldier termite) using SPSS.

3.5 RESULTS

3.5.1 Proximate composition

Stinkbug meal contained 29.53% protein and 48.59% fat content (Table 3.3). The ash content was 3.14%. Fibre content was 8.70%. The highest mineral recorded in the Stinkbug meal was sodium (69.2 mg/l).

The Stinkbug meal comprised high levels (7.46 g/kg) of saturated fatty acids (SFA) and monounsaturated fatty acid (MUFA) (7.45 g/kg), respectively (Table 3.3). Palmitic fatty acid dominated the SFA, whilst oleic fatty acid dominated the MUFA. Furthermore, Stinkbug meal contained polyunsaturated fatty acid (PUFA), linoleic acid which is one of the most essential omega-6 fatty acids.

Table 3.3: Proximate composition, mineral content, and fatty acid profile of the Stinkbug meal

Proximate composition	**%DM**	**Fatty acids**	**g/kg of TFA**
Dry matter (%)	88.75	C8:0	0.01
Moisture	11.25	C12:0	0.02
Protein	29.53	C13:0	0.02
Fat	48.59	C14:0	0.39
Ash	3.14	C16:0	6.62
Fibre	8.70	C18:0	0.40
NDF	27.11	C20:0	0.03
ADF	50.84	C14:1	0.32
Carbohydrates	7.76	C16:1	2.82
		C18:1n-9	3.76
Minerals	**mg/l**	C20:1	0.55
Iron (Fe)	0.91	C18:2n-6	1.17
Potassium (K)	21.7	ΣSFA	7.46
Sodium (Na)	69.2	ΣMUFA	7.45
		ΣPUFA	1.17

NDF: neutral detergent fibre; ADF: acid detergent fibre; DM: dry matter; SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acid. TFA: total fatty acids.

The experimental diets showed that the fatty acid profile was dominated by C18:2 cis (linoleic acid), C18:1 cis (oleic acid), C16 (palmitic acid), and C16:1 (palmitoleic acid) (Table 3.4). Palmitic acid, oleic acid, and palmitoleic acid were the fatty acids that increased in experimental diets with an increase in Stinkbug meal.

Table 3.4: The fatty acid profile of Stinkbug-based experimental diets (g/kg).

Fatty acid	**D1**	**D2**	**D3**	**D4**	**D5**
C12	0.02	0.02	0.03	0.02	0.03
C13	0.02	0.02	0.02	0.02	0.03
C14	1.26	1.58	1.79	1.37	1.58
C14:1	0.02	0.11	0.39	0.48	0.76
C15	0.13	0.15	0.14	0.09	0.07
C16	12.84	18.07	32.46	33.30	48.47
C16:1	2.11	7.87	22.36	26.55	41.79
C17	0.16	0.17	0.13	0.08	0.07
C17:1	0.11	0.16	0.11	0.06	0.05
C18	4.57	5.57	8.73	8.32	11.48
C18:1 (cis)	17.77	21.22	38.27	40.56	59.11
C18:2 (cis)	38.15	41.38	68.85	67.44	94.92
C20	0.30	0.29	0.60	0.64	0.81
C20:1 & C18:3n3	2.25	1.81	1.60	1.20	1.17
C21	0.01	0.02	0.02	0.01	0.01
C20:2	0.05	0.05	0.05	0.02	0.02
C22	0.52	0.60	1.04	0.97	1.42
C20:3n6	0.01	0.01	0.01	0.01	0.03
C22:1	0.67	0.76	0.68	0.34	0.23
C20:4n6	0.05	0.06	0.05	0.02	0.01
C23	0.03	0.01	0.03	0.03	0.04
C20:5n3	2.73	2.95	2.67	1.36	0.88
C24	0.15	0.16	0.24	0.20	0.30
C24:1	0.16	0.17	0.15	0.07	0.04
C22:6n3	4.36	4.57	4.14	2.01	1.31

The essential amino acids (EAA) composition of the Stinkbug meal showed that lysine (6.85 g/kg), leucine (4.77 g/kg), and valine (4.07 g/kg), were the most abundant (Table 3.5). On the other hand, cystine (15.31 g/kg), glutamic acid (10.19 g/kg), and aspartic acid (6.70 g/kg) showed the highest concentration of non-essential amino acids, respectively (Table 3.5). Moreover, the amino acid profile of the Stinkbug meal was relatively lower than fishmeal. The amino acid profile of the Stinkbug meal did not meet the requirements for *O. mossambicus* and *C. gariepinus* except tryptophan (Table 3.5). The Stinkbug meal recorded the EAAI of 0.11.

Table 3.5 Amino acid profile of the Stinkbug meal, fishmeal, amino acid requirements for *O. mossambicus*, and *C. gariepinus* (g/kg).

Essential AA	**g/kg (DM)**	**Fishmeal***	**Requirements for *O. mossambicus*** **	**Requirements for *C. gariepinus*** ***
Lysine	6.85	42.2	37.8	12-14.3
Methionine	1.76	19.4	09.9	6-6.4
Phenylalanine	4.07	37.4	25.0	12-14
Valine	4.77	27.7	22.0	7.1-8.4
Tryptophan	3.56	5.7	0.43	1.2-14
Threonine	2.50	23.1	29.3	5-5.6
Isoleucine	2.87	24.5	20.1	6-7.3
Histidine	0.08	17.5	10.5	4-4.2
Leucine	2.70	37.9	34.0	8-9.8
EAAI	0.1165			
Non-essential AA				
Tyrosine	4.04			
Glycine	4.76			
Aspartic acid	6.70			
Serine	3.82			
Proline	3.80			
Glutamic acid	10.19			
Alanine	4.80			
Cystine	15.31			

*Djissou *et al*. (2016); **Santiago and Lovell (1988); ***NRC (1993)

3.5.2 Growth performance parameters

In *O. mossambicus*, there was a decrease in growth performance with increasing Stinkbug meal inclusion (Table 3.6). The highest WG (167.37 g), SGR (2.16), TGC (1.30), and PER (2.95) were recorded in fish fed the control diet (D1). The fish fed the control diet (D1) also had the lowest FCR (1.42). A significant decline in feed intake has been observed with an increase in Stinkbug meal inclusion across diets ($P<0.05$). The APDC of fish fed the control diet (79.67%) was higher ($P<0.05$) than the rest of the diets. However, diets D2 to D5 showed no significant difference in APDC ($P>0.05$). Above 30% inclusion level, growth performance and nutrient utilisation indices declined (Table 3.6). Organosomatic indices (CF, VSI, HSI) were not influenced by Stinkbug meal inclusion ($P>0.05$).

In *C. gariepinus*, the control diet and diet D3 recorded the best growth performance, respectively (Table 3.7). The control diet recorded the highest WG (167.37 g), SGR (2.16), TGC (1.30), FI (0.74 g/fish/day), and PER (2.95). The WG, TGC, FI, and APDC recorded on the control diet were not significantly different to diet D3 ($P>0.05$). At the inclusion level beyond 30%, growth performance declined significantly ($P<0.05$). The organosomatic indices showed no discernible pattern and were not influenced by the inclusion of Stinkbug meal ($P<0.05$).

Table 3.6: Growth parameters and organosomatic indices (Mean ± SD) of *O. mossambicus* fed diets with Stinkbug meal as a fishmeal replacement at increasing substitution levels for 8 weeks. *n*=9.

Dietary groups	D1 (0%)	D2 (10%)	D3(30%)	D4(50%)	D5 (70%)
Indices					
IBW (g)	68.74±4.73	75.47±3.81	76.68±6.29	64.04±5.07	69.29±4.07
FBW (g)	236.11±6.08	218.01±2.03	205.79±3.90	166.16±5.05	145.38±2.30
WG (g)	167.37 ± 4.32^{a}	143.53 ± 3.93^{b}	128.32 ± 2.35^{c}	103.12 ± 2.33^{d}	75.09 ± 1.52^{e}
SGR (%)	2.16 ± 0.07^{a}	1.86 ± 0.17^{b}	1.73 ± 0.25^{c}	1.68 ± 0.33^{c}	1.28 ± 0.10^{d}
TGC	1.30 ± 0.03^{a}	1.12 ± 0.11^{b}	1.04 ± 0.18^{c}	0.95 ± 0.19^{d}	0.72 ± 0.06^{e}
FCR	1.42 ± 0.15^{a}	1.56 ± 0.30^{b}	1.72 ± 1.25^{c}	$1.77\pm0.0.54^{c}$	2.15 ± 0.43^{d}
FI (g/fish/day)	0.74 ± 0.05^{a}	0.55 ± 0.09^{b}	0.42 ± 0.04^{c}	0.39 ± 0.11^{c}	0.36 ± 0.15^{c}
PER	2.95 ± 0.09^{a}	2.87 ± 0.46^{ab}	2.82 ± 0.65^{b}	2.28 ± 0.47^{c}	1.66 ± 0.14^{d}
APDC (%)	79.67 ± 2.55^{a}	70.89 ± 2.10^{b}	70.21 ± 2.25^{b}	69.14 ± 1.93^{b}	65.57 ± 1.48^{b}
Organosomatic Indices					
CF (g/cm^3)	2.30 ± 0.13^{a}	2.02 ± 0.19^{a}	1.82 ± 0.38^{a}	1.74 ± 0.06^{a}	1.54 ± 0.25^{a}
VSI (%)	12.85 ± 0.79^{a}	12.33 ± 4.53^{a}	12.97 ± 2.01^{a}	12.85 ± 1.15^{a}	11.51 ± 5.00^{a}
HSI (%)	4.03 ± 0.24^{a}	3.64 ± 0.49^{a}	3.54 ± 1.82^{a}	3.36 ± 3.81^{a}	3.35 ± 0.41^{a}
Survival (%)	100	100	100	100	100

Means in a row with different superscripts were significantly different ($P<0.05$). IBW: Initial body weight, FBW: Final body weight, WG: Weight gain, SGR: Specific growth rate, TGC: Thermal-unit growth coefficient, FCR: Food conversion ratio, FI: Feed intake, PER: Protein efficiency ratio, APDC: Apparent protein digestibility coefficient, CF: Condition factor, VSI: Viscero-somatic index, HSI: Hepatosomatic index.

Table 3.7: Growth performance, protein utilisation, and organosomatic indices of *C. gariepinus* fed diets with Stinkbug meal as fishmeal replacement at different inclusion levels. Values expressed as Mean ± SD (*n*=9). The Means with different superscripts are significantly different (P<0.05, ANOVA).

Dietary groups	D1 (0%)	D2 (10%)	D3(30%)	D4(50%)	D5 (70%)
Indices					
IBW (g)	30.65±5.96	30.78±3.35	34.48±4.20	29.76±1.08	28.15±4.25
FBW (g)	65.34±12.84	57.37±5.77	67.76±6.22	53.77±1.97	50.41±6.21
WG (g)	35.68±6.87^{a}	26.59±2.41^{b}	33.28±2.02^{a}	24.01±0.89^{b}	22.26±1.96^{b}
SGR (%/day)	1.35±0.07^{a}	1.11±0.02^{c}	1.20±0.04^{b}	1.06±0.00^{c}	1.04±0.04^{c}
FCR	1.12±0.05^{a}	1.61±0.03^{c}	1.43±0.03^{b}	1.86±0.01^{d}	1.93±0.01^{d}
TGC	0.57±0.06^{a}	0.46±0.00^{b}	0.52±0.04^{a}	0.43±0.02^{b}	0.41±0.01^{b}
FI (g/fish/day)	0.80±0.08^{a}	0.71±0.16^{a}	0.75±0.09^{a}	0.57±0.04^{b}	0.53±0.06^{b}
PER	1.95±0.04^{a}	1.64±0.0^{b}	1.75±0.01^{c}	1.28±0.00^{d}	1.26±0.01^{d}
APDC (%)	71.25±2.11^{a}	57.25±2.00^{b}	67.41±1.63^{a}	52.41±2.36^{bc}	49.52±1.01^{bc}
			Organosomatic Indices		
CF	0.59±0.01^{a}	0.60±0.01^{a}	0.51±0.08^{a}	0.66±0.06^{a}	0.64±0.01^{a}
VSI (%)	8.62±2.46^{a}	9.43±2.45^{a}	10.03±2.61^{a}	10.22±0.77^{a}	6.10±0.36^{a}
HSI (%)	1.25±0.16^{a}	1.11±0.11^{a}	1.44±0.54^{a}	1.30±0.17^{a}	1.15±0.28^{a}
Survival (%)	100	100	100	100	100

3.5.3 Regression analysis of SGR, FCR and APDC of *O. mossambicus* and *C. gariepinus*.

The SGR was highest in the control diet for both *O. mossambicus* and *C. gariepinus* (Figure 3.3 A & B). A decline in SGR with an increase in Stinkbug meal inclusion was observed in both fish species. However, higher SGR was recorded in *O. mossambicus* than *C. gariepinu*s. The FCR increased with an increase in Stinkbug meal inclusion in both species (Figure 3.3 C & D). The APDC showed a negative linear relationship with Stinkbug meal inclusion levels in both species (Figure 3.3 E & F). Furthermore, *O. mossambicus* was able to digest Stinkbug-based diets better than *C. gariepinus*.

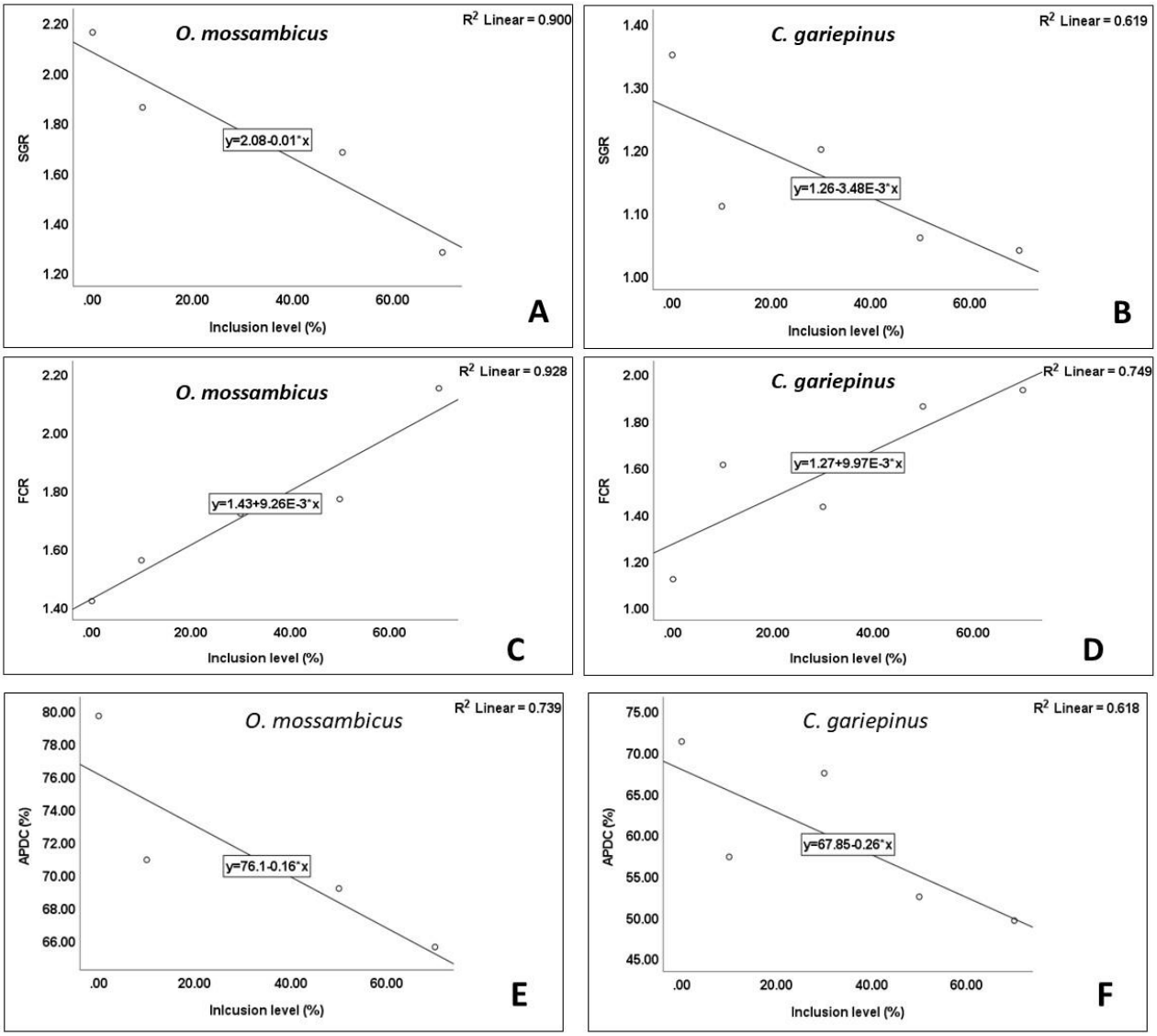

Figure 3.3: Linear regression analysis of SGR, FCR and APDC of *O. mossambicus* and *C. gariepinus*.

3.5.4 Blood serum chemistry

In *O. mossambicus*, the ALT value of fish fed the control diet (22.66 U/L) did not differ (P>0.05) from diets D2, D3, and D4 (Table 3.8). The diet with the highest inclusion level (D5) recorded the highest ALT level (32.66 U/L), which differed significantly (P<0.05) from the rest of the diets. The AST values showed no discernible pattern. However, the highest AST level was recorded in diet D5 (318.33 U/L) and differed (P<0.05) from other diets. Stinkbug meal did not influence (P>0.05) the cholesterol, triglyceride, and glucose levels in *O. mossambicus*.

In *C. gariepinus*, the control diet recorded an ALT level (21.25 U/L) not significantly (P>0.05) different from diets D3, and D4 (Table 3.8). At the highest inclusion level, the ALT level increased significantly (43.32 U/L). The AST level followed a similar trend (Table 3.8). Cholesterol, triglyceride, and glucose were not influenced by Stinkbug meal inclusion.

Table 3.8: Blood serum chemistry (Mean ± SD) of *O. mossambicus* and *C. gariepinus* fed diets with Stinkbug meal at different replacement levels, *n*=9.

Diets	ALT (U/L)	AST (U/L)	Chol (mmol/l)	Trig (mmol/l)	Glucose (mmol/l)
	O. mossambicus				
D1	22.66±3.51^{a}	221.33±3.21^{a}	0.35±0.00^{a}	0.02±0.00^{a}	3.11±0.12^{a}
D2	20.63±4.50^{a}	224.33±3.51^{a}	0.32±0.02^{a}	0.03±0.00^{a}	4.20±0.33^{a}
D3	24.33±4.93^{a}	213.00±3.00^{b}	0.33±0.01^{a}	0.02±0.00^{a}	6.31±0.41^{a}
D4	23.33±5.68^{a}	225.33±2.30^{a}	0.31±0.04^{a}	0.03±0.00^{a}	5.41±0.24^{a}
D5	32.66±2.51^{b}	318.33±2.08^{c}	0.30±0.01^{a}	0.02±0.00^{a}	5.31±0.36^{a}
	C. gariepinus				
D1	21.25±0.6^{a}	78.11±2.00^{a}	3.37±0.04^{a}	2.04±0.02^{a}	3.86±1.30^{a}
D2	39.91±0.07^{b}	115.4±3.50^{b}	3.03±0.05^{a}	2.03±0.04^{a}	4.51±0.60^{a}
D3	22.63±1.20^{a}	81.5±2.50^{a}	3.27±0.02^{a}	2.07±0.01^{a}	4.85±0.95^{a}
D4	26.25±1.50^{a}	84.5±1.50^{a}	3.14±0.02^{a}	2.12±0.02^{a}	4.12±0.80^{a}
D5	43.32±0.50^{b}	120.4±1.50^{b}	3.48±0.06^{a}	2.08±0.07^{a}	4.80±0.40^{a}

ALT: alanine aminotransferase; AST: aspartate aminotransferase; Chol: cholesterol; Trig: triglyceride; Different superscripts on each column are significantly different (P<0.05, ANOVA).

3.5.5 Liver and intestinal histology.

Oreochromis mossambicus showed no significant difference in the intestine villi height across the diets (P>0.05) (Table 3.9). However, a numerically higher villi height was recorded in diet D1 (201 µm). Villi thickness also did not differ significantly (P>0.05) across diets.

The villi height and thickness in *C. gariepinus* showed no discernible pattern (Table 3.9).

Substituting fishmeal with Stinkbug meal had no influence on the number of goblet cells in the intestine of both *O. mossambicus* and *C. gariepinus* across diets (P>0.05). Moreover, the goblet cell number in *C. gariepinus* was numerically higher than in *O. mossambicus*. The liver hepatocyte scored 1 across all diets in both *O. mossambicus* and *C. gariepinus* (Table 3.9).

Table 3.9: The score for the distal intestine of *O. mossambicus* and *C. gariepinus* fed diets with stinkbug meal at different replacement levels. Villi height and thickness are expressed as Mean ± SD. *n*=9.

Diets	**D1**	**D2**	**D3**	**D4**	**D5**
Species			**Villi height (µm)**		
O. mossambicus	201.00±6.92[a]	193.00±4.04[a]	177.33±4.09[a]	182.66±7.51[a]	179.66±2.96[a]
C. gariepinus	138.39±3.48[a]	109.19±4.86[b]	173.06±5.02[c]	140.87±4.39[a]	162.41±3.58[d]
			Villi thickness (µm)		
O. mossambicus	64.66±5.20[a]	61.33±0.88[a]	61.00±1.52[a]	54.33±3.84[a]	62.66±0.88[a]
C. gariepinus	44.57±2.24[a]	33.15±3.20[b]	68.63±6.27[c]	57.89±4.04[d]	64.57±6.57[c]
			Goblet cell (no)		
O. mossambicus	45.00±3.05[a]	50.00±3.00[a]	50.00±4.61[a]	46.00±2.96[a]	48.00±1.76[a]
C. gariepinus	61.00±2.10[a]	63.00±2.00[a]	60.00±3.14[a]	64.00±2.30[a]	66.00±1.20[a]
			Hepatocyte score		
O. mossambicus	1	1	1	1	1
C. gariepinus	1	1	1	1	1

The inclusion of Stinkbug meal in the diets of *O. mossambicus* and *C. gariepinus* did not affect the distal intestinal histology (Figure 3.3 and Figure 3.4). The micrographs showed a normal intestine with distinguishable villi, lamina propria, goblet cells, epithelial lifting, serosa, and submucosa. Furthermore, the micrographs showed normal liver histology at different inclusion levels in both species across all diets (Figure 3.5 and Figure 3.6).

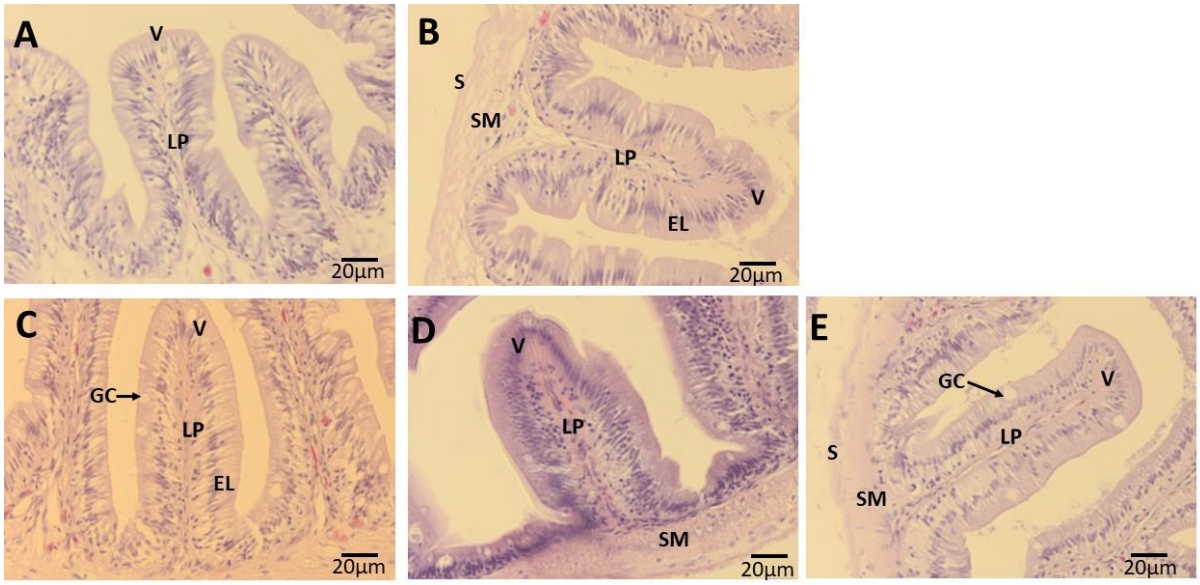

Figure 3.4: Micrographs of *O. mossambicus* fed Stinkbug meal as fishmeal replacement at different inclusion levels stained with H& E. A: Diet D1 (0%), B: Diet D2 (10%), C: Diet D3 (30%), D: Diets D4 (50%), E: Diet D5 (70%). V: Villi, LP: Lamina propria, GC: Goblet cell, EL: Epithelial layer, SM: Submucosa, S: Serosa. Scale bar: 20 µm.

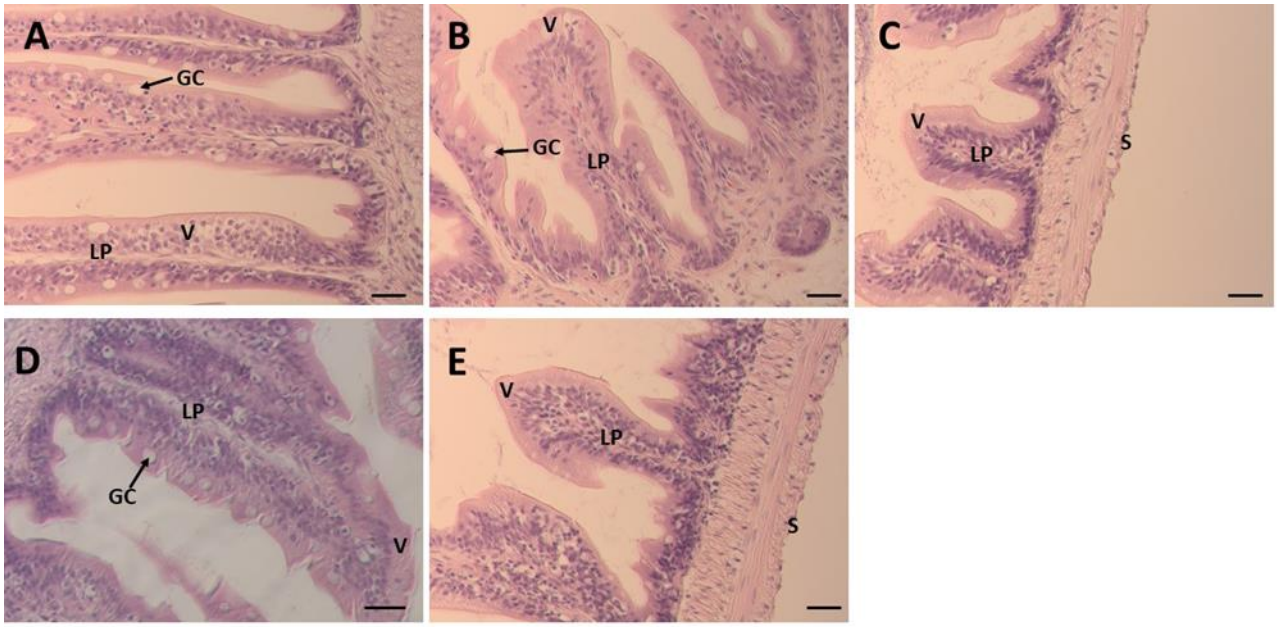

Figure 3.5: The micrographs of *C. gariepinus* distal intestine fed diets with Stinkbug meal as a fishmeal replacement at different inclusion levels. A (Diet 1), B (Diet 2), C (Diet 3), D (Diet 4), and E (Diet 5). GC: Goblet cell; V: Villus; LP: Lamina propria; S: Serosa. Scale bar: 20 µm.

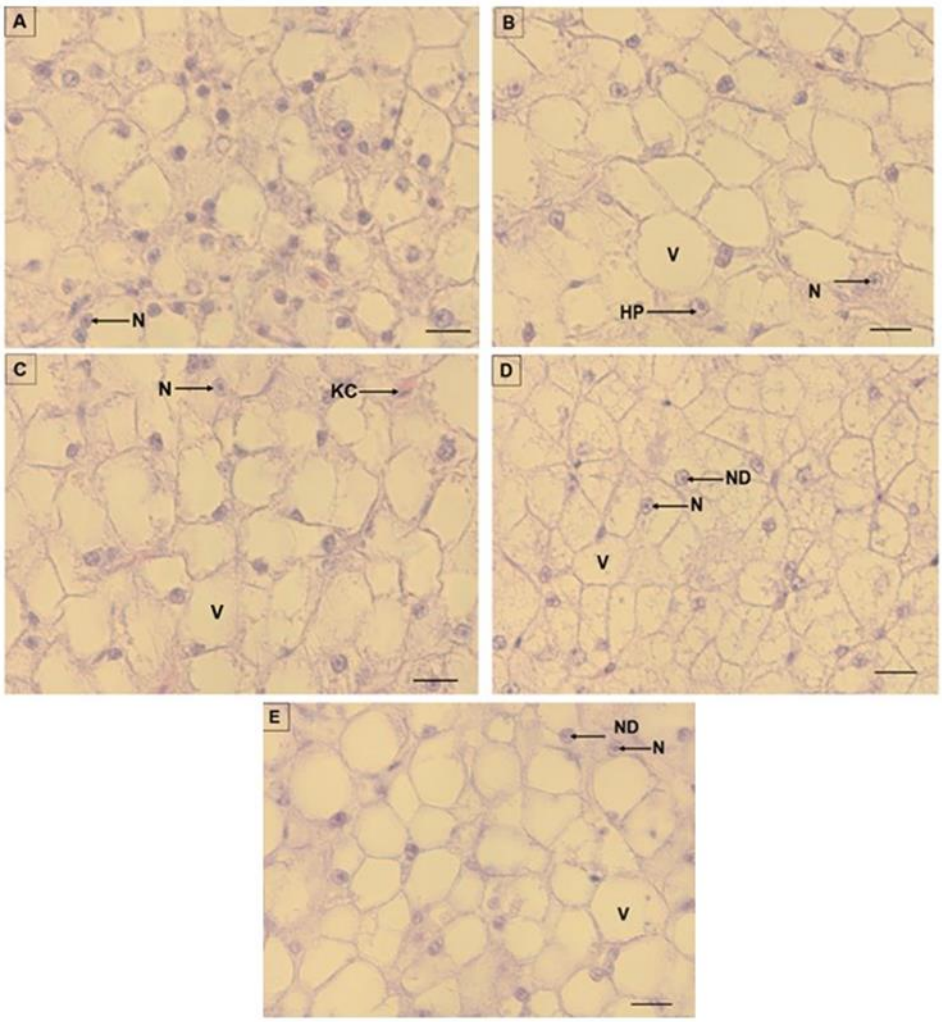

Figure 3.6: Liver micrographs of *O. mossambicus* fed diets with Stinkbug meal as a fishmeal replacement. A (Control: 0%), B (Diet D2; 25%), C (Diet D3: 30%), D (Diet D4: 50%), and E (Diet D5; 75%). V: Vacuolisation, N: Nucleus, ND: Nuclear displacement, HP: Hepatic plate, KC: Kupffer cell. Scale bar: 20 µm

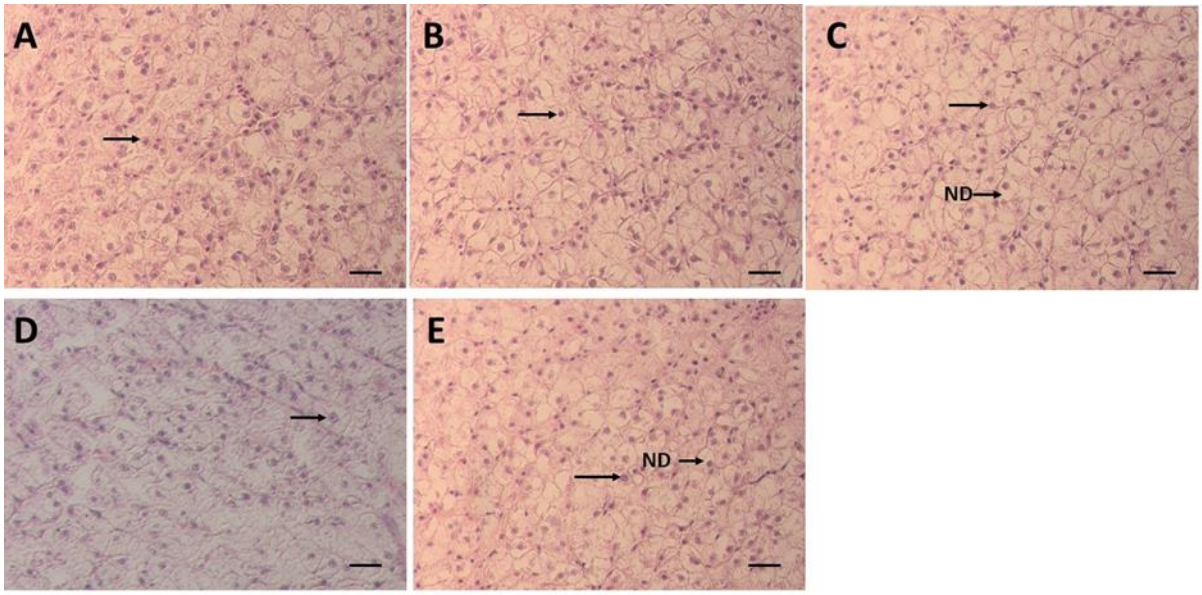

Figure 3.7: The micrographs of *C. gariepinus* liver fed diets with Stinkbug meal as a fishmeal replacement at different inclusion levels. A (Diet 1), B (Diet 2), C (Diet 3), D (Diet 4), and E (Diet 5). Long arrow: Nucleolus; ND: Nuclear displacement. Scale bar: 20 µm.

3.5.6 Gastrointestinal enzyme activity

Amylase enzyme activity in *O. mossambicus* showed no discernible trend (Figure 3.5, A). This was also the case in *C. gariepinus*. However, amylase activity was higher in *O. mossambicus* than in *C. gariepinus* across all diets.

Protease activity in *O. mossambicus* and *C. gariepinus* also showed no pattern across diets (Figure 3.5, B). The protease activity was higher in *C. gariepinus* than in *O. mossambicus*.

Lipase activity in *O. mossambicus* showed no significant difference ($P>0.05$) (Figure 3.5, C). *C. gariepinus* showed no pattern in lipase activity and the levels were higher than in *O. mossambicus*.

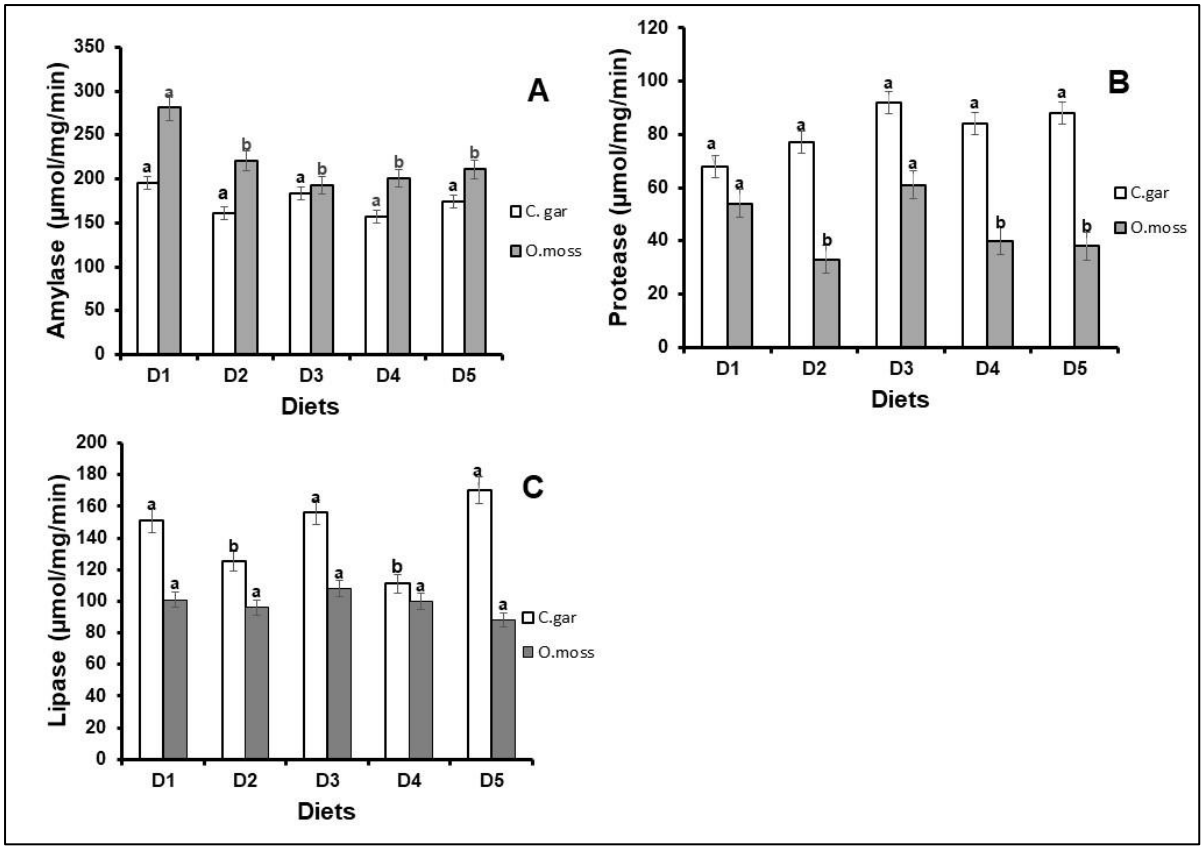

Figure 3.8: Amylase (A), Protease (B), and Lipase (C) enzyme activity in the distal intestine of *O. mossambicus* (O.moss) and *C. gariepinus* (C.gar) fed diets with Stinkbug meal as a fishmeal replacement. Bars represent standard error. Different letters in bars of the same species shows significant difference (P<0.05).

3.5.7 Cost benefit analysis

A decline in incidence cost with an increase in Stinkbug meal inclusion was observed (Table 3.10). On the other hand, the inclusion of Stinkbug meal in the diets of *O. mossambicus* and *C. gariepinus* increased the profit index.

Table 3.10: Cost benefit analysis of replacing fishmeal with Stinkbug in the diets of *O. mossambicus* and *C. gariepinus*.

Cost benefit indices	D1 (0%)	D2 (10%)	D3 (30%)	D4 (50%)	D5 (70%)
Incidence cost	0.16	0.10	0.07	0.06	0.06
Profit index	1.23	1.26	1.31	1.33	1.37

3.6 DISCUSSION

There was a decline in the growth performance of *O. mossambicus* and *C. gariepinus* with increasing Stinkbug meal inclusion. A decline in growth performance was more evident in inclusion levels above 30% in both species. This is consistent with most previous studies where inclusion levels above 25% led to a decline in the growth performance of most fish species when insect meal was used to replace fishmeal (Xiao *et al*., 2018; Gasco *et al*., 2016). The decline in growth performance with higher insect meal inclusion levels has been attributed to different factors such as chitin levels. Chitin interferes with the digestion and absorption of nutrients in fish species, thus compromising fish growth (Gu *et al*., 2022; Tran *et al*., 2022). Stinkbug meal protein level was 29%. This is much lower than in most insect species where the protein levels were mainly above 50% (Ghosh and Mandal, 2019; Elia *et al*., 2018; Anvo *et al*., 2017). Although fish do not have specific requirements for protein levels, higher protein levels are indicative of the nutritional quality of the diet (Henry *et al*., 2015). The linear regression model showed that the decline in growth performance was more evident in *O. mossambicus* than in *C. gariepinus*. This can possibly be explained by the amino acid requirements of different fish species. *O. mossambicus* requires higher levels of methionine and lysine. It is thus plausible that the low methionine and lysine levels in the Stinkbug meal affected the growth performance of *O. mossambicus*. High methionine and lysine levels have been recorded in *Hermetia illucens* meal and growth performance was not compromised (Abdel-Tawwab *et al*., 2020). Moreover, the Stinkbug meal showed low EAAI compared to other terrestrial insects.

One of the unique features of the Stinkbug meal is its high-fat content. In comparison to commonly used insects in aqua feed, Stinkbug meal fat content is much higher (Nogales-Mérida *et al*., 2019). The high-fat level might also have led to a decline in growth performance in both species. Lipids are an important source of energy, and they also assist in the absorption of fat-soluble vitamins (NRC, 1993). However, high-fat levels are known to affect nutrient digestibility. Moreover, the Stinkbug meal showed an imbalance in the fatty acid profile. Although it contains a polyunsaturated omega-6 fatty acid (linoleic acid), this insect lacks omega-3 fatty acids such as α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which are essential fatty acids for growth and health maintenance. A more pronounced

decline in the growth performance of *O. mossambicus* may be due to the digestive enzyme profile. It must be noted that lipase activity was higher in *C. gariepinus* than in *O. mossambicus*. This shows that *C. gariepinus* was probably able to tolerate a high-fat content diet than *O. mossambicus*. Opportunistic predator species such as *C. gariepinus* are known to possess higher lipase activity than herbivorous species (Sankar *et al*., 2014; Tengjaroenkul *et al*., 2000). Similar observations were recorded when *C. gariepinus* was fed an insect-based diet (Rapatsa and Moyo, 2019).

There was a noticeable reduction in feed intake with an increase in Stinkbug meal inclusion in both fish species. A decline in voluntary FI was more evident in *O. mossambicus* than in *C. gariepinus*. The reduced feed intake may be attributed to trans-2-decenal and trans-2-octenal compounds that the Stinkbug releases as a defence mechanism (Sagun *et al*., 2016). These compounds are known to cause an unpleasant odour that repels predators. The pungent smell was also observed in diets with Stinkbug meal. It is speculated that the odour reduced voluntary feed intake. Diet palatability is one major factor that has the potential to affect feed intake in fish species. *C. gariepinus* is known as an opportunistic predator and utilises a variety of feed items in the wild. Thus, it has a greater tolerance to diets that have poor palatability. At a high inclusion level, *O. mossambicus* was occasionally observed grabbing and spitting the diet. This may imply poor palatability of the diet when the Stinkbug meal inclusion level is high. A decline in growth performance due to poor palatability has been observed when insect meal is incorporated into fish diets of different species and feeding habits (Coutinho *et al*., 2021).

The study also observed a decline in nutrient utilisation with an increase in Stinkbug meal inclusion in both *O. mossambicus* and *C. gariepinus*. The PER and APDC were used as protein utilisation measures. The protein utilisation was more efficient in *C. gariepinus* than *O. mossambicus*. The PER in *O. mossambicus* declined significantly from a 30% inclusion level whilst the APDC declined from a 10% inclusion level. On the other hand, *C. gariepinus* showed that the control and diet D3 (30%) APDC were comparable. The higher protein utilisation in *C. gariepinus* may be due to its protease enzyme activity. *C. gariepinus* recorded higher protease activity than *O. mossambicus*. This observation reflects the feeding habit of *C. gariepinus*. Its digestive system is designed to utilise a high-protein diet. Protease activity in fish species is also affected

by feed, fish size and pH (Lazzari *et al*., 2010; Hidalgo *et al*., 1999). Dietary protein plays an important role in determining the rate of fish growth in fish species. For maximum growth, fish species should efficiently synthesise protein. Protein digestibility is highly influenced by the protein quality of the dietary ingredients, and it is usually the leading factor affecting fish performance (Rahman *et al*., 2016).

Although there was a decline in the growth performance, organosomatic indices (CF, HSI, VSI) were not significantly different across all diets in both species. This shows that fish from all experimental diets were in good health condition. Furthermore, utilising diets with Stinkbug meal as a fishmeal replacement did not cause any metabolic or liver deficiencies in *O. mossambicus* and *C. gariepinus*. The VSI confirmed that no excessive hepatic fat was accumulated in fish from all diets. Similar findings were observed in fish that have been fed insect meal as a fishmeal substitute (Tippayadara *et al*., 2021; Xiao *et al*., 2018; Renna *et al*., 2017). This suggests that Stinkbug meal had no negative effect on the health status of the two fish species.

The AST and ALT levels further confirmed that *O. mossambicus* and *C. gariepinus* were in good health. The ALT and AST are liver enzymes that often leak into the bloodstream when there is hepatic damage or increased stress level (Adeoye *et al*., 2019). One of the factors that makes these two species to be commonly cultured is their stress resistance (El-Sayed, 2020). *Oreochromis mossambicus* and *C. gariepinus* feed on insects as part of their diet in the wild (El-Sayed, 2020; Dadebo *et al*., 2014). It is speculated that these species are pre-adapted to utilise an insect-based diet. Belghit *et al*. (2018) indicated that feeding an Atlantic salmon (*Salmo salar*) a diet with *H. illucens* as a fishmeal substitute did not affect the ALT and AST levels. *Salmo salar* is one of the fish species that feed on aquatic insects in nature, and it is reportedly pre-adapted to utilising insect-based diets (Henry *et al*., 2015). However, at a higher inclusion level (70%), these enzymes were elevated. This shows that the inclusion of Stinkbug meal at a 70% inclusion level may pose mild stress to fish species.

Substituting fishmeal with Stinkbug meal did not affect the intestine histology of *O. mossambicus* from all experimental diets. In *C. gariepinus,* the villi height and thickness showed no discernible pattern. The intestine is an organ where feed digestion, nutrient absorption and transportation of nutrients take place (Elia *et al*., 2018). Its change in morphological structure may signal a nutritional deficiency. The

goblet cell prevalence was also not influenced by the Stinkbug meal in all experimental diets in both species. Goblet cells are responsible for secreting mucus as a protective compound (Kim and Ho, 2010). Stinkbug-based diet did not affect the gastrointestinal tract of both species. Furthermore, no sign of inflammation was observed. This was affirmed by the normal histology micrographs which showed distinguishable villi, lamina propria, serosa, submucosa, and epithelial lifting.

The inclusion of the Stinkbug meal had no influence on the liver health of *O. mossambicus* and *C. gariepinus*. The hepatocyte of fish across all diets scored 1. Grade 1 score reflects a normal liver with vacuoles that follow the cell boundary, distinguishable nuclei, and Kupfer cells (McFadzen *et al*., 1997). The liver is an organ involved in nutrient metabolism (Rašković *et al*., 2011). It is also a good bioindicator of the nutritional status of the fish. Changes in its morphological structure are paramount in determining the capacity of a species to digest and effectively utilise a diet. Similar observations were reported when *O. mossambicus* was fed an insect-based diet (Rapatsa and Moyo, 2017). The study recorded normal liver histology in fish across all diets. The lack of liver histological alterations shows that these species can utilise an insect-based diet.

The cost benefit analysis showed that the inclusion of Stinkbug meal in the diets of *O. mossambicus* and *C. gariepinus* may yield higher returns than fishmeal-based diets. Although a decline in growth performance was observed, the study shows that Stinkbug meal inclusion in fish diets is economically viable and superior to fishmeal-based diet. Stinkbug is one of the cheap insects available at the Thohoyandou open market. This insect can also be harvested for free at Thohoyandou, Ga-Modjadji, and Bushbuckridge. Fishmeal is not only expensive but it’s becoming less available and no longer sustainable.

The limitation associated with using Stinkbug meal as a fishmeal substitute is its limited availability and seasonal variation. Preservation of Stinkbugs when they are in season is recommended. This may extend their shelf-life and increase their availability even when they are out of season. Moreover, it is important to search for other insects within our geographical area that are harvested during the rainy season. They will thus alternate with the Stinkbug, granting fish farmers access to insects throughout the year. Another limitation of using Stinkbug meal is that it contains high fat content which

may cause oxidation in the diets. It is thus recommended to defatten the Stinkbug meal prior to use. Defattening insects have been reported to improve their nutritional profile (Li *et al*., 2022; Renna *et al*., 2017). Unlike *H. illucens* and *M. domestica*, the Stinkbug is not easy to culture. However, rearing trials have been attempted in Haenertsburg, Mopane District, Limpopo Province, South Africa (Dzerefos *et al*., 2009). It is thus recommended to improve trials for mass production. During mass production, different substrates may be introduced to improve its nutritional composition. Digestibility of the Stinkbug-based diets may be improved by introducing microbes that have the potential to synthesise chitin.

To the best of the author's knowledge, no study evaluated using Stinkbug meal as a fishmeal replacement in aqua feeds. Thus, there are no comparable results for this study. Inclusion levels above 30% negatively affected the growth performance of both *O. mossambicus* and *C. gariepinus*. The decline in growth performance was more pronounced in *O. mossambicus*. It therefore appears that *C. gariepinus* is physiologically better equipped to handle the Stinkbug meal. The organosomatic indices did not vary between the species. They were also not significantly affected by the high Stinkbug meal inclusion level. The intestine and liver histology did not show any significant alterations with a high Stinkbug meal inclusion level. This shows that the health status of both species was not affected by high Stinkbug meal inclusion levels. However, AST and ALT were significantly higher at inclusion levels of 70%. This suggests that the fish were stressed at high inclusion levels. However, the health status was not affected. Despite the decline in growth performance, cost-benefit analysis shows that Stinkbug meal can sustainably replace fishmeal. Replacing fishmeal with *E. delegorguei* meal had an effect on growth performance, liver and intestine histology, digestive enzyme profile and cost benefit analysis in *O. mossambicus* and *C. gariepinus*. Thus, the null hypothesis is rejected.

CHAPTER 4:

A comparative study on the utilisation of *Macrotermes falciger* (winged termite) diet by a herbivorous fish (*Oreochromis mossambicus*) and an opportunistic predator (*Clarias gariepinus*).

CHAPTER 4: A comparative study on the utilisation of *Macrotermes falciger* (winged termite) diet by a herbivorous fish (*Oreochromis mossambicus*) and an opportunistic predator (*Clarias gariepinus*).

4.1 INTRODUCTION

From the previous chapter, it was clear that growth performance declined at high Stinkbug meal inclusion levels. This was partly attributed to its protein content which is relatively lower than commonly used insect meals. The Stinkbug meal had several limitations which included seasonal variation. It is thus prudent to search for alternative insects that are found locally and have the potential to substitute fishmeal in the diets of commonly cultured freshwater species in southern Africa. In this chapter, winged termites of *M. falciger* species will be evaluated as a potential replacement for fishmeal in the diets of herbivorous (*O. mossambicus*) and opportunistic predator (*C. gariepinus*).

In Africa, over 1000 termite species have been recorded. The family Termitidae is divided into five subfamilies, of which Macrotermitinae is the most popular. The Genus *Macrotermes* is the most preferred and well-known (Egan *et al*., 2021). From the *Macrotermes* genus, *Macrotermes falciger*, *Macrotermes subhyalinus*, *Macrotermes bellicosus*, *Macrotermes natalensis*, *Macrotermes vitrialatus*, and *Macrotermes nigeriensis* have been reported to be widely consumed in Africa (Egan *et al*., 2021). Winged termites are the second most consumed insect group in the world, surpassed by grasshoppers, crickets, and locusts (De Figueirédo *et al*., 2015). The most consumed species in South Africa are *M. falciger*, *M. michaelseni*, and *M. nigeriensis* (Netshifhefhe and Duncan, 2022). Among these three species, *M. falciger* is the most preferred, mainly due to its larger size and nutritional composition (Netshifhefhe and Duncan, 2022).

Several studies on the nutritional composition of Alates termite of the *Macrotermes* species have been carried out in western Africa (Igwe *et al*., 2011; Banjo *et al*., 2006; Mbah and Elekima, 2007). However, in southern Africa, very limited studies have been carried out. There is limited published literature on the nutritional composition of winged *M. falciger* species. One of the few studies conducted in southern Africa showed that *M. falciger* Alates contains 41.8% crude protein content (Phelps *et al*.,

1975), which is relatively higher than the Stinkbug meal (29%). The *M. falciger* is one of the winged termite species that have a higher protein content than most *Macrotermes* species (Fombong and Kinyuru, 2018). This makes it one of the species that has the potential to substitute fishmeal in aqua feed.

One of the unique features of the Stinkbug meal is its high-fat content (48%) and defatting was recommended. However, *M. falciger* Alates contain a lower fat content (44%) than Stinkbug meal. Although it contains a lower fat content than Stinkbug meal, its fat content is still higher than the requirement for *O. mossambicus* (5-6%) and *C. gariepinus* (9-12%) (Hecht *et al*., 1988; Jauncey *et al*., 1983). The *M. falciger* Alates also contains essential and non-essential amino acids and a valuable amount of minerals that are more bioavailable than plant-based minerals (Siulapwa *et al*., 2012). Moreover, the fatty acid profile of *M. falciger* Alates is poorly documented.

The nutritional composition of insects is influenced by their stage of metamorphosis, insect origin, and their diet (Inje *et al*., 2018). Alates termites are the reproductive caste of the colony and have a unique feeding mode. They depend on Workers for nutrients since they cannot feed themselves directly. Termites feed mainly on cellulose, which is obtained from wood, grass, leaves, humus, lichens, and soil rich in organic matter (Siddaiah *et al*., 2023). Digestion of cellulose depends on symbiotic flagellate protozoa, which is found in the termite hindgut and secretes cellulase and cellobiase that breaks down cellulose into glucose and acetic acid. The Workers transfer food to Alates through mouth or anal feeding. Feed transferred by mouth may consist of either paste-like regurgitated chewed wood and saliva or a clear liquid. The liquid food consists of hindgut fluid containing protozoans, products of digestion, and wood fragments (O'Brien and Slaytor, 1982). Thus, the nutritional composition of Alates termites is dependent on the nutritional composition of the Workers.

The lifecycle of termites comprises eggs, larvae, nymphs, workers, and soldiers. The nymph develops into a reproductive caste, which are alates (Figure 4.1). Incubated eggs hatch and develop into larvae which then develop into any member of the castes (soldier, worker, nymph). The caste differentiation is mainly determined during the postembryonic development and is influenced by abiotic and biotic factors (Watanabe *et al*., 2014). The nymphs develop into Alates. The matured Alates swarm during the onset of the rainy season (October and April), and male and the female Alates pair,

shed their wings, and start searching for a place to initiate a new colony. The availability of Alates termites is one factor that has to be considered when they are used in fishmeal replacement studies.

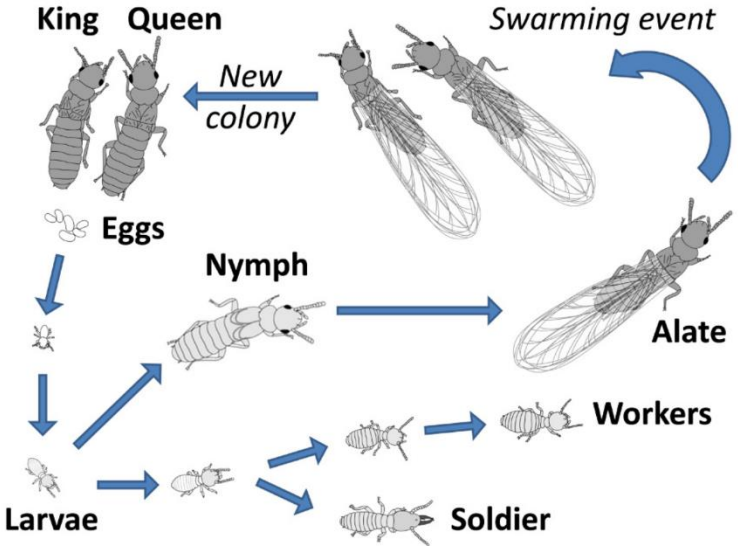

Figure 4.1: The life cycle of *M. falciger*.

The distribution of *M. falciger* is wider than Stinkbug. In South Africa, stinkbugs are mainly harvested at three localities in the Limpopo and Mpumalanga Provinces. On the other hand, termites are widely distributed in tropical and subtropical regions, spreading from humid forests to savannas and even arid areas. The *Macrotermes falciger* species are known to be predominantly found in woodlands and are widely distributed in eastern and southern Africa (Mujinya *et al*., 2014). The wide distribution of *M. falciger* makes it a viable insect to replace fishmeal in aquafeed.

The harvest of *M. falciger* Alates is much more convenient and less laborious than harvesting Stinkbugs. Van Huis (2013) investigated various methods of Alates termite collection around Africa. The most popular and easy method was placing a basin of water under the light source. Alates termites are attracted to a source of illumination at night during their swarming season, making it easier to harvest. In comparison to the Stinkbug, Alates termites can easily be processed. They are collected, fried in a pan with salt, de-winged and sun-dried. On the other hand, Stinkbugs are prepared by shaking vigorously in a bag, rinsing with hot water, and stirring to release the unpleasant odour (Dzerefos *et al*., 2013).

Although *M. falciger* Alates contain a good nutritional composition, their use as feed and as a fishmeal replacement in the animal feed industry is not documented. Thus, Alates of the *M. falciger* may be used as a novel ingredient in the diet of *O. mossambicus* and *C. gariepinus*. These are the two most widely cultured warm freshwater fish species in South Africa.

4.2 OBJECTIVES AND NULL HYPOTHESIS

Objectives

I. To compare growth performance of *O. mossambicus* and *C. gariepinus* fed a diet with an Alates termite meal as a fishmeal substitute.
II. To compare the blood serum chemistry of *O. mossambicus* and *C. gariepinus* fed a diet with Alates termite meal as a fishmeal substitute.
III. To determine the effect of Alates termite meal as a fishmeal replacement on the liver and intestine histology of a herbivorous and an opportunistic predator fish species.
IV. To determine the effect of Alates termite meal in digestive enzyme profile of a herbivorous and an opportunistic predator species
V. To determine the cost benefit analysis of replacing fishmeal with Alates termite meal in *O. mossambicus* and *C. gariepinus* diets.

Null hypothesis

I. There is no difference in growth performance of *O. mossambicus* and *C. gariepinus* fed diet with an Alates termite meal as a fishmeal substitute.
II. The is no difference in blood serum chemistry of *O. mossambicus* and *C. gariepinus* fed a diet with an Alates termite meal as a fishmeal substitute.
III. Replacing fishmeal with Alates termite meal has no effect on the liver and intestine histology of a herbivorous and an opportunistic predator fish species
IV. Replacing fishmeal with Alates termite meal has no effect on the digestive enzyme profile of a herbivorous and an opportunistic predator fish species.
V. There is no effect on the cost benefit analysis of replacing fishmeal with Alates termite meal on the diets of *O. mossambicus* and *C. gariepinus*.

4.3 MATERIALS AND METHODS

4.3.1 Diet formulation

The Alates termites (de-winged) were purchased from the Thohoyandou Open Market in the Vhembe District of Limpopo Province, South Africa. The Alates termites were ground to powder and replaced fishmeal at 0, 10, 30, 50, and 70%, which were designated as D1, D2, D3, D4, and D5, respectively (Table 4.1). The diets were formulated to be isonitrogenous (30% protein), isolipidic (12% fat), and isocaloric (15 MJ/Kg), this was done using Winfeed 3, EFG (Natal) software. The control diet contained 30% fishmeal and no insect meal. The feed ingredients were weighed and mixed using a planetary mixer (Hobart, Troy, OH, USA). During mixing, water (10-20% v/w) was added as required until the desired dough thickness was reached. The mixture was pelleted into a 3 mm diameter size using a meat mincer connected to the planetary mixer. The pellets were labelled and separately sun-dried and stored in polyethylene buckets at 4°C. Yellow maize, wheat bran, sunflower meal, and sunflower oil were purchased from a local market. Fishmeal, Binder, and Vitamin/Mineral premix were purchased from Irvin's Africa (Pty) Ltd, Gauteng Province, South Africa. Lysine and Methionine were purchased from Nutroteq, Gauteng Province, South Africa.

Table 4.1: Feed ingredients (g/kg) mixed to formulate experimental diets where Alates termite meal substituted fishmeal at increasing levels. The proximate composition of the diets is listed.

Inclusion levels	0%	10%	30%	50%	70%
Ingredients	**D1**	**D2**	**D3**	**D4**	**D5**
Fishmeal	300	270	210	150	90
Alates meal	0	30	90	150	210
Yellow Maize	205	205	205	185	155
Wheat bran	242.7	234.0	212.7	202.9	229.4
Sunflower meal	102.0	110.7	131.8	161.3	164.9
Sunflower oil	60.3	60.3	60.5	60.8	60.7
Methionine	20	20	20	20	20
Lysine	20	20	20	20	20
Vitamin/Mineral premix[a]	20	20	20	20	20
Binder	30	30	30	30	30
Total	1000	1000	1000	1000	1000
Proximate composition					
Crude protein (%DM)	30.00	30.00	30.00	30.00	30.00
Fat (%DM)	12.49	12.13	12.68	12.32	12.27
Gross Energy (MJ/Kg)	15.02	15.08	15.00	15.01	15.03
Dry matter (%DM)	92.89	93.01	93.49	93.81	94.05
Ash (%DM)	10.53	10.20	9.62	9.00	8.71

[a]Vitamin/Mineral premix: Vit. A, 12 000 IU; Vit. D3, 1 200 IU; Vit. E, 120 IU; Vit. B4, 10000g; Vit. C, 120g; Vit. B3, 25g; Vit. B5, 15g; Vit. B2, 6g; Vit. B6, 5g; Vit. B1, 4g; Vit. K3, 2g; Vit. B9, 1g; Vit. H, 0.25g; Vit. B12, 0.04g. ZnO, 200g; $FeSO_4$; $CuSO_4$, 7g; MnO, 5g; KI, 2g; Na_2SeO_3, 0.15g; $CoSO_4$, 0.05g.

4.3.2 Proximate composition of the Alates termite meal and experimental diets.

The Alates termite meal and experimental diets were analysed for the proximate composition according to AOAC (2003) and as described in Chapter 3, section 3.3.2.

4.3.3 Mineral content

The mineral content of the Alates termite meal was determined using Inductively coupled plasma-optical emission spectrometry (ICP-OES) as described by Manditsera *et al*., (2019).

4.3.4 Amino acid and fatty acid analysis

Alates termite meal was analysed for amino acids and fatty acids profiles at the Central Analytical Facilities (CAF) of Stellenbosch University, Western Cape Province (South Africa) as described in Chapter 3, section 3.3.4 and 3.3.5.

The Essential amino acid Index (EAAI) of the Alates termite meal was calculated using the Peńaflorida (1989) formula:

$$\mathrm{EAAI} = \sqrt[n]{\frac{\mathrm{aa1}}{\mathrm{AA1}} \times \frac{\mathrm{aa2}}{\mathrm{AA2}} \times \cdots \cdots \frac{\mathrm{aa}n}{\mathrm{AA}n}},$$

Where aa is the amount of the amino acid in the Alates termite, AA is the amino acid requirement of the fish, and *n* is the total number of amino acids used in the calculation.

4.3.5 Fish stocking and experimental design

The experiment was undertaken at the University of Limpopo, Aquaculture Research Unit (ARU), Limpopo Province, South Africa. The Mozambique tilapia (*O. mossambicus*) juveniles were collected from the hatchery at ARU. The African catfish (*C. gariepinus*) sub-adults were also collected from the holding tanks in the ARU hatchery. These species were both bred at the ARU. The study was approved for ethical clearance by the University of Limpopo Animal Research Ethical Committee (AREC/09/2022:PG).

The experiment was conducted in a Recirculating aquaculture system (RAS) using 500 L size fibreglass tanks filled to the 400 L mark. The tanks were connected to a sump and a pump that supplied the tanks with water at 10 L/min. Each tank was heated with a submersible aquarium heater (ViaAqua Glass heater, 200W) and aerated with airstones. The tanks were fitted with the Guelph system with mild modifications for faecal matter collection (Cho *et al*., 1982). Fish species were sedated with 2-phenoxyethanol (1 ml/L) prior to stocking in experimental tanks. A completely randomised design was used to stock the fish in experimental tanks. *Clarias gariepinus* (205 ± 4.3 g/fish) were stocked at 5 fish per tank in triplicates, whilst *O. mossambicus* (7.4 ±3.1 g/fish) were stocked at 10 fish per tank in triplicates. The initial body weight of fish per diet ranged between 61.18 ± 3.19 g and 76.06 ± 4.77 g for *O. mossambicus* and 997.19 ± 6.36 g – 1060.50 ± 3.50 g for *C. gariepinus*.

4.3.6 Feeding procedure

Both fish species were fed a commercial diet (Aqua-plus, Avi Products (pty) ltd for two weeks during the acclimatization period. Fish were then weighed (Initial weight) per tank and re-stocked back to experimental tanks and fed allocated diets to apparent satiation for 8 weeks at 09h:00 and 15h:00. Feed intake was recorded on a daily basis for each tank. Faecal matter samples were collected from the collection tube 1 week after the commencement of the experiment and stored at -20°C until the desired quantity was reached. The faecal matter was used for apparent digestibility coefficient analysis.

4.3.7 Water quality management

Water quality parameters were monitored on a weekly basis using a handheld multiparameter meter (Professional plus YSI 605000). The measured parameters were temperature, dissolved oxygen, ammonia, and pH to meet the optimal requirements for *O. mossambicus* and *C. gariepinus*.

4.3.8 Growth performance parameters

Fish were weighed at the commencement of the experiment, after every two weeks, and at the end of the feeding trial. Fish were starved for 24 h before final growth parameters were taken. The following parameters were measured for both *O.*

mossambicus and *C. gariepinus*: SGR, TGC, FCR, PER, WG, FI, APDC and Fish survival as described in Chapter 3, section 3.3.9.

4.3.9 Organosomatic indices

Three fish from each dietary replicate (9 fish/diet) were used to measure organosomatic indices. The condition factor (CF), Hepato-somatic index (HSI), and Viscero-somatic index (VSI) were calculated as described in Chapter 3, section 3.3.10.

4.3.10 Blood serum chemistry

Three fish samples from each dietary replicate were randomly selected and sedated with 2-phenoxyethanol (1 ml/L). Blood samples were collected and analysed as described in Chapter 3, section 3.3.11. The same procedure was employed for both experiments (*O. mossambicus* and *C. gariepinus*).

4.3.11 Liver and intestinal histological analysis of *O. mossambicus* and *C. gariepinus*

Three fish from each dietary replicate (9 fish/diet) were used for liver and intestinal histology. The liver samples from each fish were separately fixed in a 10% formalin solution using sampling bottles and analysed and quantified as described in Chapter 3, section 3.3.12 and 3.3.13.

4.3.12 Digestive enzyme profile of *O. mossambicus* and *C. gariepinus*

Three fish from each dietary replicate were sedated in a 2-phenoxyethanol (1 ml/l) solution and sacrificed. Fish samples were dissected, and distal intestines were removed and stored in sterile sampling bottles at -80°C until analysis. Samples were analysed for amylase, protease and lipase activity as described in Chapter 3, section 3.3.14.

4.3.13 Cost benefit analysis

The cost benefit analysis was determined according to Bahnasawy *et al*. (2003).

$$\text{Incidence cost} = \frac{\text{Cost of feed}}{\text{Quantity of fish produced (kg)}}$$

$$\text{Profit index} = \frac{\text{Local market value of fish}}{\text{Cost of feed}}$$

The underlying assumption is that all operating costs are constant, and the cost of ingredients was the only variable cost. The prices used were described in chapter 3, section 3.3.14.

4.4 STATISTICAL ANALYSIS

Statistical analysis was carried out as described in Chapter 3, section 3.4.

4.5 RESULTS

4.5.1 Proximate composition

Alates termite meal contained 40.46% protein content, 40.40% fat content, and 6.14% ash content. The NDF and ADF of the Alates termite meal were 56.19% and 22.09%, respectively. The highest concentrations of essential amino acids recorded in the Alates termite meal were lysine (12.99 g/kg), leucine (6.98 g/kg), and histidine (5.15 g/kg), respectively (Table 4.2). The non-essential amino acids were dominated by cystine (22.82 g/kg), glutamic acid (12.48 g/kg), and aspartic acid (8.17). The Alates termite meal recorded an Essential amino acid index (EAAI) of 1.87 (Table 4.2). Sodium constituted the highest value for minerals (41.6 mg/l), followed by potassium (33.8 mg/l), and iron (1.93 mg/l). The fatty acids that dominated the Alates termite meal were palmitic (6.5781 mg/g), oleic (6.5714 mg/g), and stearic (3.1547 mg/g), which are all SFA. This insect also contained linoleic acid and α-linolenic acid, which are PUFA. The fatty acid profile of the experimental diets was dominated by C18:1 cis, C18:2 cis, and C16 (Table 4.3).

Table 4.2: Proximate composition, mineral content, fatty acids profile and amino acid profile of the Alates termite meal.

Proximate composition	**%DM**	**Fatty acids**	**mg/g**	**Essential AA**	**g/kg (DM)**
Dry matter (%)	93.51	C12:0	0.02	Lysine	12.99
Ash (%DM)	6.14	C14:0	0.27	Methionine	3.76
Fat (%DM)	40.40	C16:0	6.57	Phenylalanine	4.79
Protein (%DM)	40.46	C18:0	3.15	Valine	4.69
Carbohydrates (%DM)	6.54	C16:1	0.34	Tryptophan	0.37
Crude fibre (%DM)	8.70	C17:1n-10	0.07	Threonine	4.94
NDF (%DM)	56.19	C18:1n-9(cis)	6.57	Isoleucine	4.25
ADF (%DM)	22.09	C18:2n-6 (cis)	2.73	Histidine	5.15
Energy (KJ/100g)	2301	C18:3n-3	0.32	Leucine	6.98
Minerals	**(mg/l)**	Σ SFA	10.03	**Non-essential AA**	
Iron	1.93	Σ MUFA	6.99	Tyrosine	3.86
Potassium	33.8	Σ PUFA	3.06	Glycine	4.99
Sodium	41.6			Aspartic acid	8.17
				Serine	6.04
				Proline	6.29
				Glutamic acid	12.48
				Alanine	6.09
				Cystine	22.82
				EAAI	1.87

NDF: Neutral detergent fibre; ADF: Acid detergent fibre; SFA: saturated fatty acids, MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. AA: Amino acids; EAAI: Essential amino acid Index.

Table 4.3: Fatty acid profile in Alates termite-based experimental diets (g/kg).

Fatty acid	D1	D2	D3	D4	D5
C12	0.07	0.03	0.04	0.03	0.07
C14	1.21	1.30	1.13	0.85	1.27
C15	0.34	0.18	0.20	0.19	0.36
C16	42.53	17.96	22.74	23.40	44.60
C16:1	2.29	1.29	1.43	1.34	2.10
C17	0.30	0.16	0.18	0.20	0.32
C17:1	0.20	0.08	0.07	0.05	0.02
C18	15.98	6.63	8.39	8.86	16.76
C18:1 cis	85.08	31.65	42.83	46.37	89.22
C18:2 cis	80.35	50.11	54.24	50.12	84.26
C20	0.80	0.44	0.52	0.47	0.84
C20:1 & C18:3n3	3.82	3.34	3.15	2.52	4.00
C21	0.02	0.01	0.01	0.01	0.02
C20:2	0.04	0.05	0.04	0.03	0.05
C22	0.04	0.05	0.04	0.03	0.05
C22:1	0.37	0.84	0.61	0.37	0.39
C20:3n3	0.01	0.04	0.02	0.01	0.01
C20:4n6	0.20	0.17	0.14	0.09	0.21
C23	0.05	0.03	0.03	0.02	0.05
C20:5n3	0.89	2.30	1.65	0.95	0.94
C24	0.26	0.18	0.19	0.17	0.27
C24:1	0.05	0.17	0.10	0.06	0.05
C22:6n3	1.47	3.89	2.89	1.56	1.54

4.5.2 Growth performance indices

The SGR significantly declined when the inclusion level was above 50% ($P<0.05$) in *O. mossambicus* (Table 4.4). The WG, TGC, FI, PER, and APDC followed a similar trend.

The FCR increased at inclusion level higher than 50%. The CF and VSI declined at the inclusion level beyond 50%. However, HSI did not show a discernible pattern.

In *C. gariepinus*, SGR also significantly declined at inclusion level above 50% ($P<0.05$) (Table 4.5). A similar trend was observed in WG, TGC, FI, PER, and APDC. The CF, VSI, and HSI also declined at an inclusion level above 50%.

Table 4.4: Growth performance indices of *O. mossambicus* fed diets with Alates termite meal as a fishmeal replacement. Values are expressed as mean ± standard error. Different superscripts in a row show a significant difference (P<0.05, ANOVA). *n*=9.

Dietary groups	**D1 (0%)**	**D2 (10%)**	**D3 (30%)**	**D4 (50%)**	**D5 (70%)**
IBW (g)	61.18±3.19	73.84±3.82	76.06±4.77	71.43±2.90	74.04±1.25
FBW (g)	137.31±3.28	165.29±4.17	165.49±6.33	174.12±5.39	133.15±4.34
WG (g)	76.13±3.52	91.45±2.59	93.16±2.05	102.69±3.35	59.11±2.38
SGR (%/day)	1.45±0.06^{a}	1.44±0.19^{a}	1.40±0.06^{a}	1.60±0.01^{b}	1.06±0.16^{c}
FCR	1.65±0.82^{a}	1.72±0.32^{a}	1.72±0.37^{a}	1.66±0.45^{a}	1.77±0.12^{a}
TGC	0.73±0.08^{a}	0.79±0.32^{a}	0.75±0.12^{a}	0.85±0.37^{b}	0.53±0.45^{c}
FI (g/fish/day)	0.42±0.00^{a}	0.49±0.01^{b}	0.53±0.04^{b}	0.53±0.00^{b}	0.30±0.01^{c}
PER	2.53±0.11^{a}	3.04±0.61^{a}	2.97±0.12^{a}	3.43±0.87^{a}	1.96±0.37^{a}
APDC (%)	72.11±2.01^{a}	68.05±1.14^{a}	68.01±2.32^{a}	67.35±1.11^{a}	60.56±2.14^{b}
Organosomatic indices					
CF	2.26±0.14^{a}	1.43±0.10^{a}	1.69±0.01^{a}	1.66±0.12^{a}	1.50±0.04^{a}
VSI (%)	11.92±1.10^{a}	9.82±1.91^{a}	9.02±1.13^{a}	10.34±1.69^{a}	9.25±0.04^{a}
HSI (%)	1.28±0.23^{a}	2.53±0.72^{a}	1.96±0.75^{a}	1.60±0.55^{a}	2.43±0.19^{a}
Survival (%)	100	100	100	100	100

Table 4.5: Growth performance indices of *C. gariepinus* fed diets with Alates termite meal as a fishmeal replacement. Values are expressed as means ± standard error. Different superscripts in a row show a significant difference (P<0.05, ANOVA). *n* = 9

Dietary groups	**D1 (0%)**	**D2 (10%)**	**D3 (30%)**	**D4 (50%)**	**D5 (70%)**
IBW (g)	1060.50±3.50	1060.10±4.00	1000.24±4.50	1020.13±3.25	997.19±6.36
FBW (g)	2083.61±3.22	1810.39±2.12	1830.85±5.38	2010.59±4.18	1581.04±6.58
WG (g)	1023.11±3.23	750.29±2.00	830.61±5.63	990.46±7.21	584.85±2.41
SGR (%/day)	$1.20±0.06^{a}$	$1.03±0.10^{b}$	$1.07±0.05^{b}$	$1.22±0.12^{a}$	$0.86±0.23^{c}$
FCR	$1.68±0.01^{a}$	$1.96±0.02^{b}$	$1.85±0.00^{c}$	$1.78±0.02^{c}$	$2.40±0.04^{d}$
TGC	$1.61±0.02^{a}$	$1.24±0.06^{b}$	$1.39±0.04^{c}$	$1.60±0.01^{a}$	$1.03±0.06^{e}$
FI (g/fish/day)	$6.14±0.22^{a}$	$5.18±0.15^{a}$	$5.39±0.12^{a}$	$6.18±0.09^{a}$	$4.9±0.14^{a}$
PER	$3.41±0.04^{a}$	$2.50±0.03^{a}$	$2.76±0.06^{a}$	$3.30±0.03^{a}$	$1.94±0.11^{a}$
APDC (%)	$67.58±2.74^{a}$	$65.41±1.25^{a}$	$64.36±2.05^{a}$	$64.10±1.51^{a}$	$60.88±3.63^{b}$
Organosomatic indices					
CF	$0.67±0.01^{a}$	$0.74±0.05^{a}$	$0.75±0.02^{a}$	$0.77±0.01^{a}$	$0.66±0.02^{a}$
VSI (%)	$7.08±0.44^{a}$	$8.02±1.23^{a}$	$7.37±1.72^{a}$	$7.74±1.03^{a}$	$7.20±0.48^{a}$
HSI (%)	$1.82±0.03^{a}$	$2.14±0.27^{a}$	$2.07±0.39^{a}$	$1.99±0.09^{a}$	$1.75±0.21^{a}$
Survival (%)	100	100	100	100	100

4.5.3 Regression analysis of SGR, FCR and APDC of *O. mossambicus* and *C. gariepinus*.

A negative linear relationship between SGR and inclusion levels of Alates termite meal was observed in both *O. mossambicus* and *C. gariepinus* (Figure 4.2 A & B). *Oreochromis mossambicus* showed higher SGR than *C. gariepinus*. The FCR showed a positive linear relationship with Alates termite meal inclusion levels in both species (Figure 4.2 C & D). The FCR was higher in *C. gariepinus* than *O. mossambicus*. The APDC recorded a negative relationship with inclusion of Alates termite meal and was similar in both species (Figure 4.2 E & F).

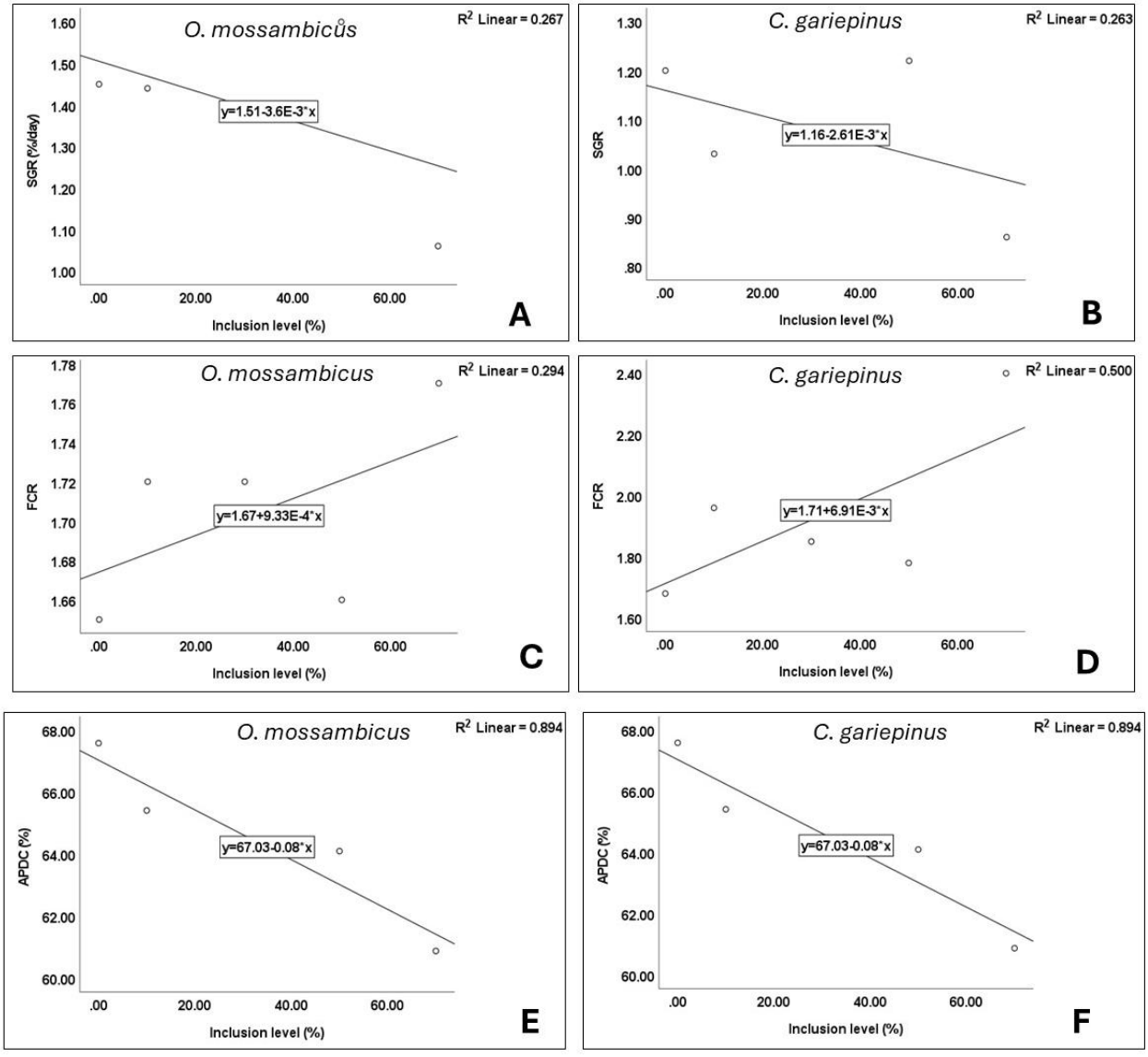

Figure 4.2 : Linear regression analysis of SGR, FCR and APDC of *O. mossambicus* and *C. gariepinus*.

4.5.4 Blood serum chemistry

The AST level in *O. mossambicus* was not influenced by the diets (P>0.05, Figure 4.2A). However, the AST level in *C. gariepinus* increased at an inclusion level higher than 50%.

In *O. mossambicus*, the ALT level did not change with a higher inclusion level (P>0.05, Figure 4.2B). However, the *C. gariepinus* ALT level significantly increased at an inclusion level above 50% (P<0.05).

Triglyceride levels did not change with an increase in inclusion levels in both species (P>0.05, Figure 4.2C). Cholesterol followed a similar trend (Figure 4.2D).

The glucose level in *O. mossambicus* showed no discernible pattern (Figure 4.2E). Similar findings were observed in *C. gariepinus*.

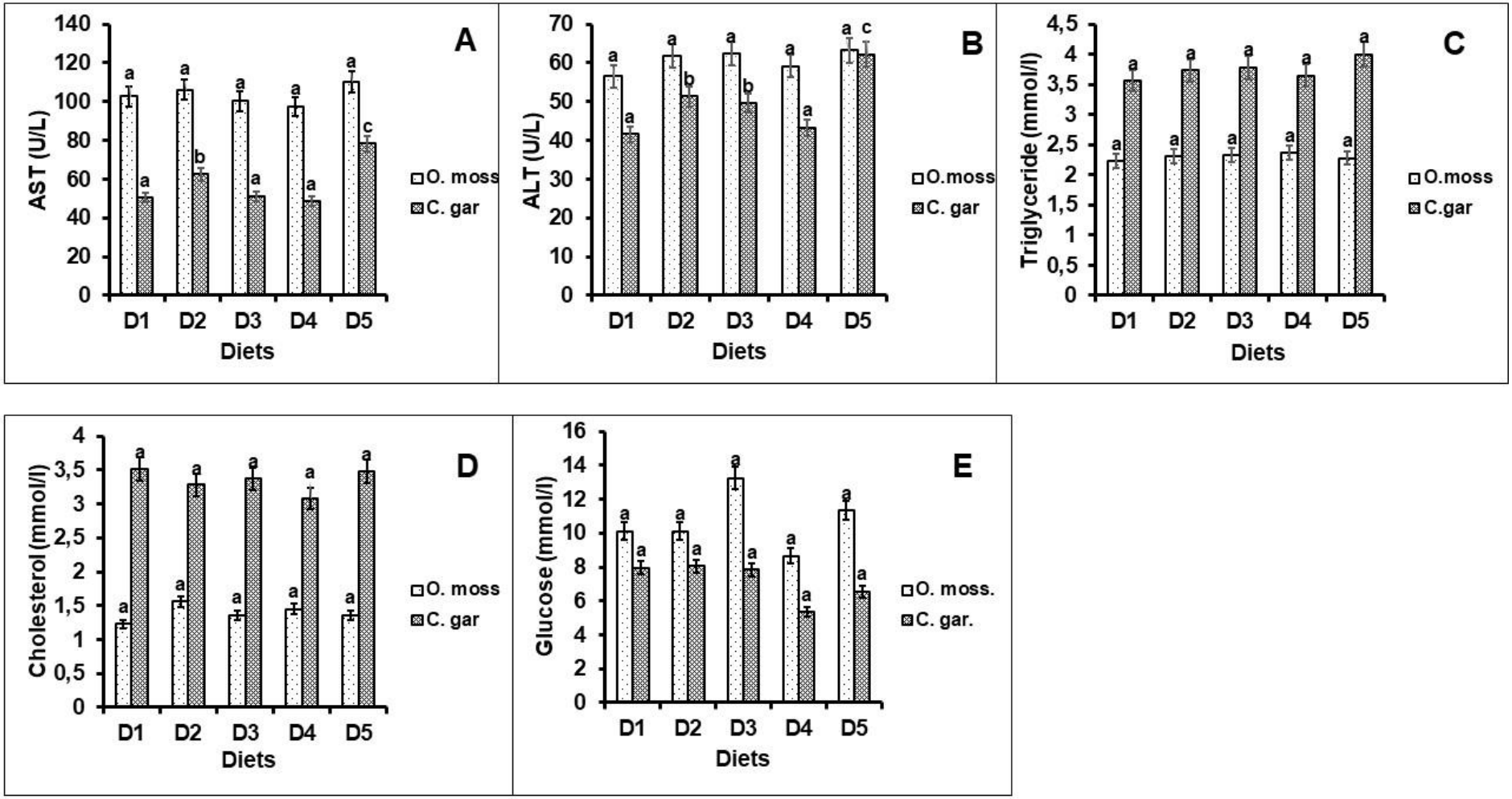

Figure 4.3: Blood serum chemistry of *O. mossambicus* (O. moss) and *C. gariepinus* (C. gar) fed diets with Alates termite meal as a fishmeal replacement at increasing levels. *n* = 9. Different letters on each species bar represent a significant difference ($P<0.05$, ANOVA). AST: alanine aminotransferase; ALT: aspartate aminotransferase. Bars represent standard error.

4.5.5 Liver and intestine histology

In *O. mossambicus*, the villi height and villi thickness significantly decreased above 50% inclusion level (Table 4.6). A similar trend was observed in *C. gariepinus*.
The goblet cell in *O. mossambicus* increased at an inclusion level above 50%. In *C. gariepinus*, goblet cells increased significantly at inclusion level above 50% ($P<0.05$).
The hepatic score in *O. mossambicus* liver was scored 1 across all diets. But above 50% inclusion level, hepatic score in *C. gariepinus* was scored 2.

Table 4.6: Intestine histology and the liver hepatocyte score of *O. mossambicus* and *C. gariepinus* fed diets with Alates termite meal as a fishmeal replacement at increasing inclusion levels. *n* = 9. Values are expressed as mean ± standard error.

Diets	**D1**	**D2**	**D3**	**D4**	**D5**
Species			**Villi height (µm)**		
O. mossambicus	212.25±2.41^{a}	121.60±7.41^{b}	136.99±3.44^{c}	215.80±8.83^{a}	112.95±3.65^{d}
C. gariepinus	205.26±4.28^{a}	158.06±5.00^{b}	193.59±2.34^{c}	230.31±8.30^{d}	103.05±4.05^{e}
			Villi thickness (µm)		
O. mossambicus	126.10±8.56^{a}	98.76±9.18^{b}	117.37±7.79^{c}	141.08±4.56^{d}	93.98±6.53^{b}
C. gariepinus	51.56±2.41^{a}	43.32 ±3.85^{b}	49.36±3.57^{ab}	62.21±2.36^{c}	34.56±1.56^{d}
			Goblet cell (no)		
O. mossambicus	19±1.93^{a}	20±3.81^{a}	18±2.59^{a}	17±2.48^{a}	22±2.59^{a}
C. gariepinus	54±5.05^{a}	63±3.70^{a}	60±5.00^{a}	58±6.68^{a}	74±2.72^{b}
			Hepatocyte score		
O. mossambicus	1	1	1	1	1
C. gariepinus	1	1	1	1	2

Different superscripts in a row are significantly different (P<0.05, ANOVA)

The hepatocyte scores of *O. mossambicus* and *C. gariepinus* were confirmed by the normal liver morphology sections (Figure 4.3 and Figure 4.4). However, *C. gariepinus* fed diet D5 showed nuclear displacement alteration (Figure 4.4E).

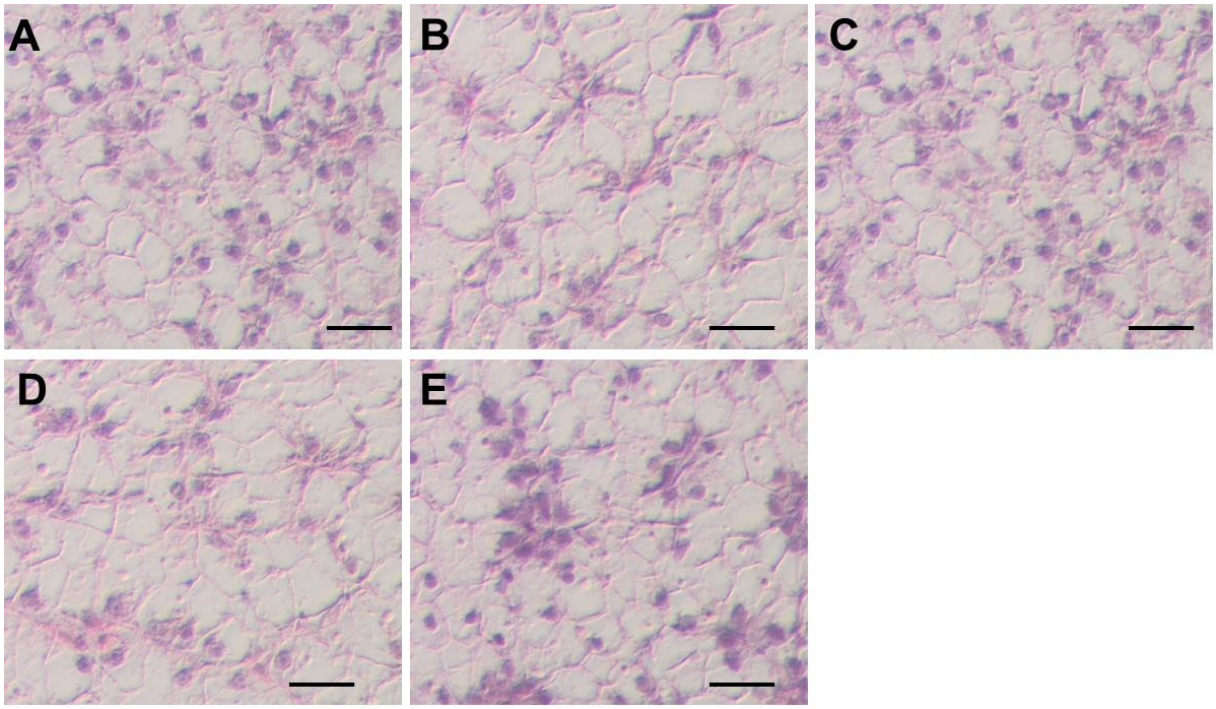

Figure 4.4: Morphological sections of the liver of *O. mossambicus* (stained with H&E) fed diets with Alates termite meal as a fishmeal replacement at increasing inclusion levels. A denotes diet D1, B: diet D2, C: diet D3, D: diets D4, and E: diet D5. Scale bar :20 µm.

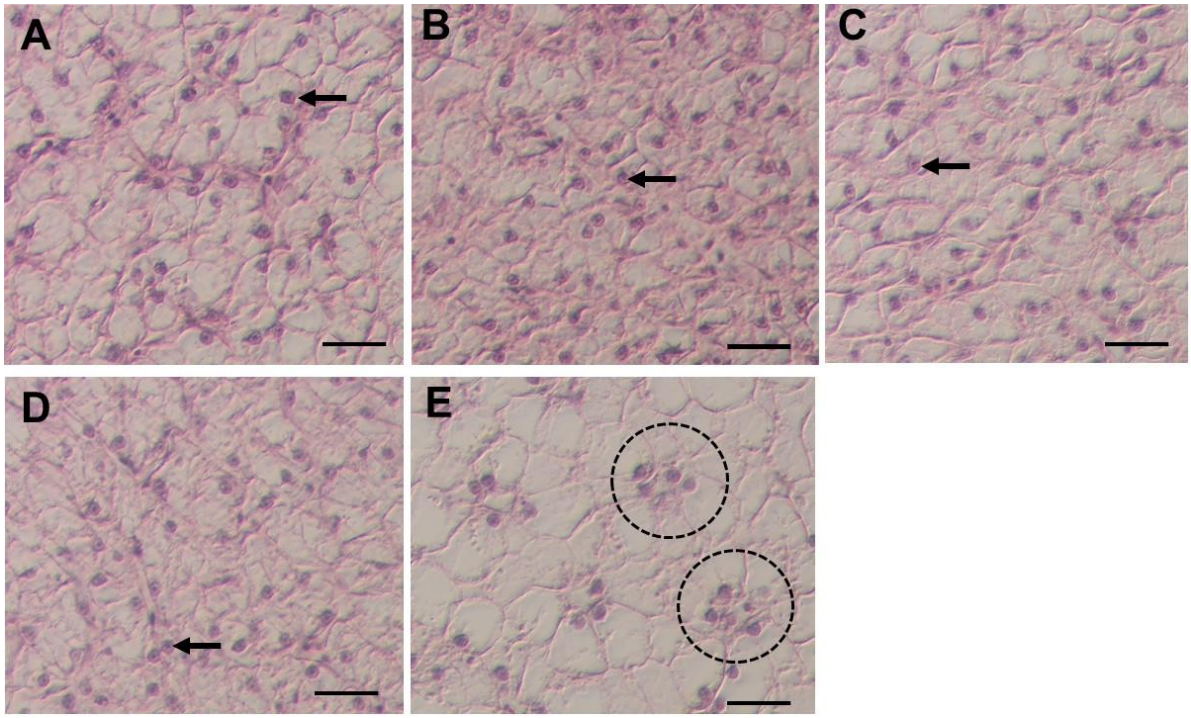

Figure 4.5: Morphological sections of the liver of *C. gariepinus* (stained with H&E) fed diets with Alates termite meal as a fishmeal replacement at increasing inclusion levels. A denotes diet D1, B: diet D2, C: diet D3, D: diet D4, and E: diet D5. Scale bar :20 µm. Arrows: nuclei, dotted circle: nuclear displacement.

The inclusion of the Alates termite meal in the diet of *O. mossambicus* and *C. gariepinus* had no effect on intestinal morphology across all diets (Figure 4.5; Figure 4.6). The intestinal morphology showed normal morphology, which had distinguishable nuclei, lamina propria, epithelia lifting, villus, goblet cell, epithelial cell, internal muscular layer, and external muscular layer in both species (Figure 4.5 and Figure 4.6).

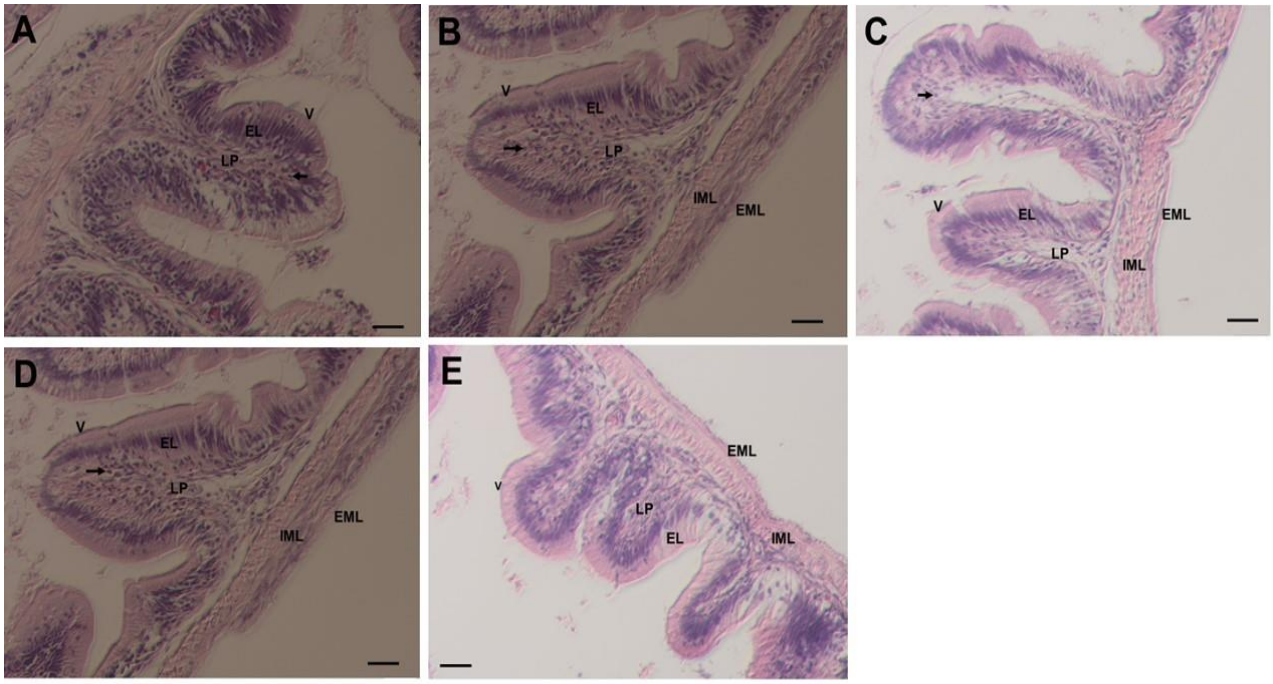

Figure 4.6: Morphological sections of the distal intestine of *O. mossambicus* (Stained with H&E) fed diets with Alates termite meal as a fishmeal replacement at increasing inclusion levels. A denotes diet D1, B: diet D2, C: diet D3, D: diet D4, and E: diet D5. Scale bar :20 µm. V: Villus, EL: Epithelial lifting, LP: Lamina propria, IML: Internal muscular layer, EML: External muscular layer. Arrow: Epithelial cell.

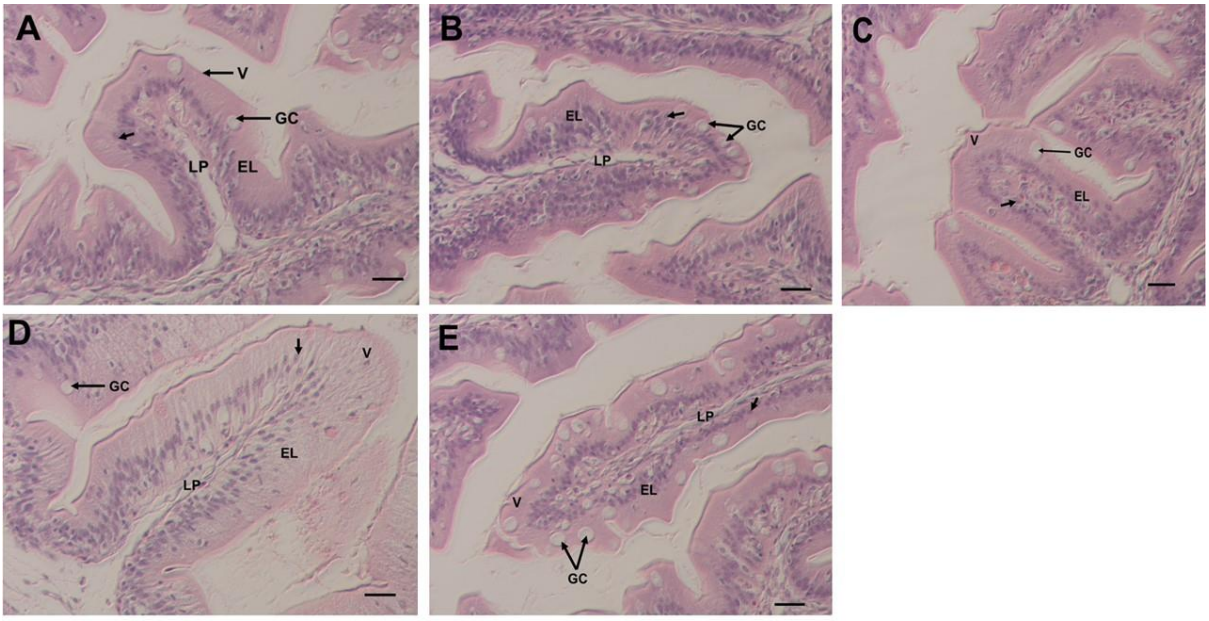

Figure 4.7: Morphological sections of the distal intestine of *C. gariepinus* (Stained with H&E, 200X) fed diets with Alates termite meal as a fishmeal replacement at increasing inclusion levels. A denotes diet D1, B: diet D2, C: diet D3, D: diet D4, and E: diet D5. Scale bar :20 µm. V: Villus, GC: Goblet cell, EL: Epithelial lifting, LP: Lamina propria, Short arrow: Epithelial cell.

4.5.6 Digestive enzymes

Protease activity recorded in *O. mossambicus* did not differ significantly across diets (P>0.05) (Figure 4.7A). *C. gariepinus* also showed no discernible pattern.

Amylase activity increased with Alates termite inclusion level in *O. mossambicus* (Figure 4.7B). In *C. gariepinus*, no pattern was observed.

In *O. mossambicus*, there was no discernible pattern with respect to lipase activity (Figure 4.7C). However, *C. gariepinus* lipase activity significantly declined above 50% inclusion level.

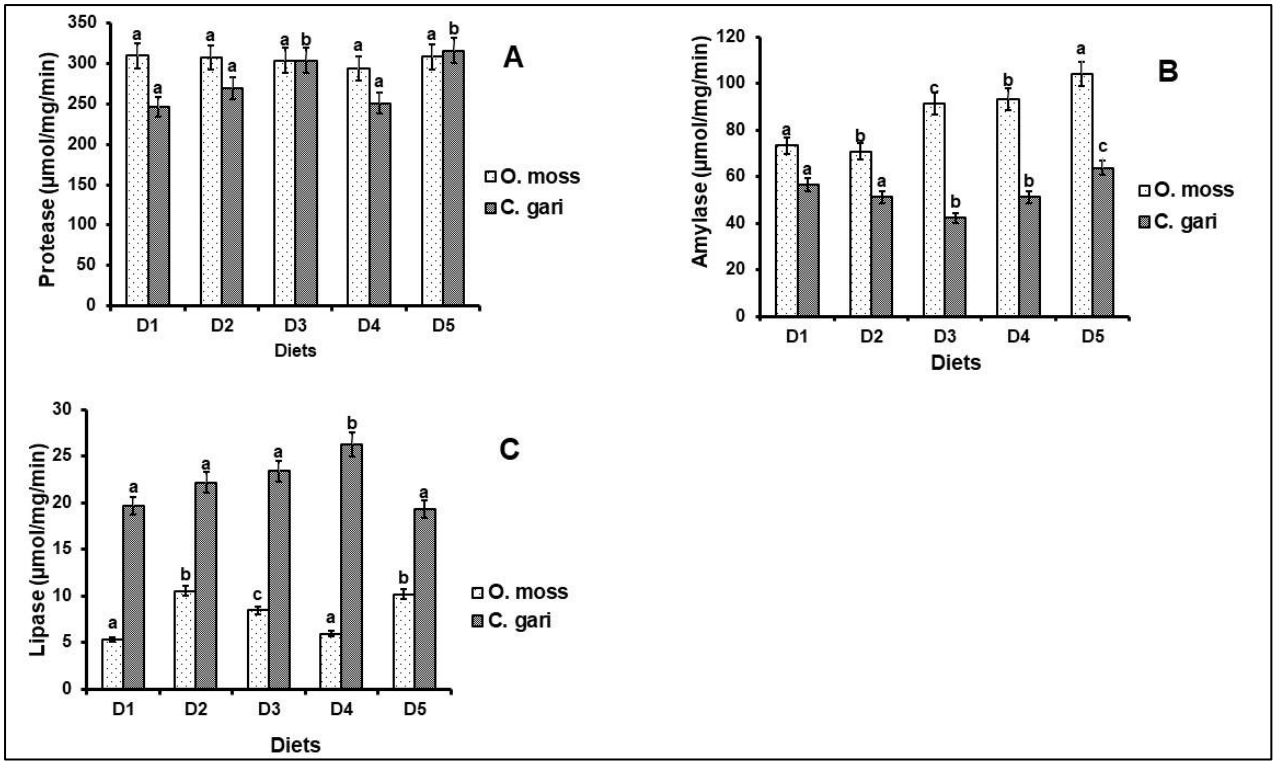

Figure 4.8: Protease (A), amylase (B), and lipase (C) enzyme activity in the distal intestine of *O. mossambicus* (O. moss) and *C. gariepinus* (C. gari) fed diets with Alate termite meal as a fishmeal replacement. Bars represent standard error. Different letters in bars of the same species shows significant difference (P<0.05, ANOVA).

4.5.7 Cost benefit analysis

The incidence cost decreased with an increase in inclusion of Alates termite meal (Table 4.7). On the other hand, profit index increased with increasing inclusion level of Alates termite meal.

Table 4.7: Cost benefit analysis of replacing fishmeal with Alates termite in the diets of *O. mossambicus* and *C. gariepinus*.

Cost benefit indices	D1(0%)	D2 (10%)	D3 (30%)	D4 (50%)	D5 (70%)
Incidence cost	0.16	0.09	0.07	0.05	0.05
Profit index	1.23	1.31	1.36	1.39	1.42

4.6 DISCUSSION

Growth performance and nutrient utilisation declined at an inclusion level above 50% in both *O. mossambicus* and *C. gariepinus*. This inclusion level limit is higher than the Stinkbug meal inclusion level limit (30%). The higher inclusion level in Alates termite meal may be partly attributed to its protein content. The Alates termite meal recorded a protein content of 40.46%, which meets the requirements for *O. mossambicus* (30%) and *C. gariepinus* (40%) (Santiago and Lovell, 1988). Protein requirement in aquafeed is an essential component for the optimal growth of fish species. Moreover, the Alates termite meal recorded an EAAI (1.87) that was higher than the Stinkbug meal (0.11). This shows that the Alates termite meal provided fish species with a better amino acid profile than the Stinkbug meal. The higher inclusion levels in Alates termite meal may also be ascribed to its polyunsaturated fatty acids (PUFA). It contained 3.06 mg/g of the total PUFA, which is above the PUFA recorded in the Stinkbug meal (1.17 mg/g). The PUFA play an important role in fish growth, cellular synthesis, ionic regulation, proper development, and functioning of the neural system (Carr *et al*., 2023).

Inclusion levels of 50% have also been observed in previous studies when *O. mossambicus* and *C. gariepinus* were fed insect-based diets. The Mozambique tilapia has been reported to utilise insect-based diets up to 60% inclusion level without compromising growth performance (Rapatsa and Moyo, 2017). The study used the Mopane worm (*Imbrasia bellina*) as a fishmeal replacement and the maximum inclusion level was 60%. The growth performance of *O. mossambicus* continued to increase even at the highest (60%) inclusion level. However, it is not immediately clear if the inclusion levels above 60% will lead to a decline in growth performance. The linear regression analysis showed that *O. mossambicus* utilised Alates-based diet better than *C. gariepinus*. Fawole *et al*. (2020) recorded a decline in growth performance at an inclusion level above 50% in *C. gariepinus* fed *Hermetia illucens*-based diet. The decline in growth performance at an inclusion level above 50% in *C. gariepinus* was attributed to chitin. However, the study did not measure the chitin content in *H. illucens* and experimental diets.

The organosomatic indices (CF, VSI, and HSI) declined at inclusion level beyond 50% in both species. This shows that Alates termite meal had no negative effect on the health status of *O. mossambicus* and *C. gariepinus* at inclusion level below 50%. The

organosomatic indices have been widely used to evaluate the health status of fish species. The limitation of using organosomatic indices is their lack of standard ranges. Piccolo *et al*. (2017) reported that the normal range for hepatosomatic index is between 1 and 2%. Values higher than these show that the liver health status has been compromised. However, this range is not species-specific. Different fish species respond differently to fish diets. Thus, their range will differ based on fish species. For the condition factor, Bagenal and Tesch (1978) stated that a fish is in good condition when the condition factor is ≥0.5. The condition factor reflects the well-being of fish with the hypothesis that heavier fish of a given length are in better physiological condition than the less robust in relation to their welfare (Begenal and Tesch, 1978). However, this may not be the case with all fish species. Furthermore, the condition factor cannot be compared between different species. The relationship between organosomatic indices and fish health status is not apparent in most studies (Piccolo *et al*., 2017). Thus, organosomatic indices are not a reliable tool to evaluate the health status of fish species. It was therefore prudent to evaluate the fish's health status using blood serum chemistry.

The AST and ALT levels in *O. mossambicus* were not influenced by Alates termite meal inclusion. The AST and ALT enzymes are biomarkers of liver damage in fish species (Belghit *et al*., 2018). This implies that *O. mossambicus* maintained a healthy liver during the feeding trial. *O. mossambicus* is one of the tilapia species that is resistant to stress, and it has been reported to be pre-adapted to utilising insect-based diets (Rapatsa and Moyo, 2022; El-Sayed, 2020). These findings agree with the AST and ALT results observed in the previous chapter. The inclusion of the Stinkbug meal in the diets of *O. mossambicus* did not influence the liver status of the fish. Although these liver enzymes increased at the highest inclusion level, they did not affect the fish's health status. When *H. illucens* replaced fishmeal in the diet of a herbivorous (*O. niloticus*) species, the ALT and AST levels did not differ across diets (Kishawy *et al*., 2022). This shows that the ALT and AST levels may be influenced by the insect used. However, the AST and ALT levels in *C. gariepinus* increased significantly at the inclusion level above 50%. This entails that replacing fishmeal with Alates termite meal above 50% may cause a mild effect on the health status of the liver of *C. gariepinus*. Thus, *O. mossambicus* was more tolerant to Alates termite meal-based diet than *C. gariepinus*. An elevation in AST and ALT at inclusion levels above 50% has also been

recorded when *C. gariepinus* was fed *H. illucens* based diets (Fawole *et al*., 2020). Generally, predatory/ carnivorous species can tolerate lower levels of insect inclusion in their diet than herbivorous species (Mohan *et al*., 2022; Devic *et al*., 2017). In the previous chapter, AST and ALT levels also increased at inclusion levels above 50%.

The histological assessment of the intestine morphology in *O. mossambicus* showed no discernible pattern. A decline in villi height and thickness at inclusion level above 50% was observed in *O. mossambicus*. A short villi height and thickness reduces the surface area for digestion and absorption of nutrients in the intestine, thus compromising growth performance in fish species (Zhang *et al*., 2022). *Clarias gariepinus* also recorded a decline in villi height and thickness at inclusion levels above 50%. Furthermore, substituting fishmeal with Alates termite meal did not have an influence on the number of goblet cells in *O. mossambicus*. This shows that higher inclusion levels of Alates termite meal did not compromise its gastrointestinal tract. On the other hand, *C. gariepinus* recorded a significantly higher number of goblet cells in fish-fed the highest inclusion level (70%). This may suggest that inclusion levels that exceed 50% may induce irritation and inflammation in the gastrointestinal tract of *C. gariepinus*. Goblet cells play a role in maintaining the functional and structural integrity of the intestinal epithelium (Fawole *et al*., 2020). Thus, changes in their density may signal intestinal damage.

The liver hepatocytes of *O. mossambicus* scored 1 across all diets, which implies a healthy liver. The liver hepatocytes were polygonal in shape, with centrally located nuclei and clear cell boundaries in healthy fish. This was affirmed by the normal liver micrographs observed. Thus, substituting fishmeal with Alates termite meal did not have a negative influence on the liver health of *O. mossambicus*. Normal liver micrographs were also observed in *O. mossambicus* fed a Stinkbug meal-based diet. In *C. gariepinus*, diets D1 to D4 scored 1 which implies a normal liver micrograph. However, *C. gariepinus* fed diet D5 scored 2, indicating an intermediate liver. This was further affirmed by the slight alteration observed in the micrographs of *C. gariepinus* liver in D5. Substituting fishmeal with Alates termite meal at inclusion levels exceeding 50% may have a slight effect on the liver tissue of *C. gariepinus*. Contrary observations were recorded in *C. gariepinus* fed Stinkbug meal-based diets. The liver histology analysis is consistent with the results of the blood serum chemistry. Although *O.*

mossambicus and *C. gariepinus* showed similar growth performance and nutrient utilisation, their response in liver histology marginally differed.

Digestive enzymes explain the capacity of the fish to utilise feed (Li *et al*., 2019). They are also essential in optimising diet formulation. Protease, amylase, and lipase enzymes play a pivotal role in the digestive process of nutrients in fish species. Enzyme activity differs in different fish species. The protease activity in both species showed no discernible pattern across diets. This is consistent with the protease activity observed in the previous chapter, where there was no discernible pattern across diets. Opportunistic predator like *C. gariepinus* exhibit higher proteolytic enzyme activity in their gastrointestinal tract (Adewumi *et al*., 2019). This is mainly due to their high protein requirement in their diet. The protease activity in *O. mossambicus* was marginally higher than in *C. gariepinus* in several diets. Although *O. mossambicus* is an herbivore, it has been reported that this species can adjust its diet to accommodate the prevailing food availability. Doupé *et al*. (2009) reported piscivory behaviour in *O. mossambicus*. This then explains its high protease enzyme activity. The amylase activity was higher in *O. mossambicus* than in *C. gariepinus*. The amylase activity was also higher in *O. mossambicus* than in *C. gariepinus* when fed a Stinkbug meal-based diet. Herbivorous fish species have been reported to produce higher amylase activities in their gut than omnivorous (El-Sayed. 2020; Moore *et al*., 1986). This is mainly due to their diet that fundamentally comprises carbohydrates. Thus, *O. mossambicus* may be physiologically adapted to utilise high carbohydrate-based diets. The lower amylase activity recorded in *C. gariepinus* reflects its opportunistic predator feeding habit. It is principally dependent on animal-based diets and not equipped to produce amylase enzymes. Although *O. mossambicus* showed no discernible pattern in lipase activity, the values were lower than those recorded in *C. gariepinus*. Similar observations were also reported in the previous chapter. *C. gariepinus* is an opportunistic feeder that is known to feed on a variety of items (Adewumi *et al*., 2014). Its high lipase activity may be attributed to its high consumption of fat-rich food (Moor *et al*., 1986). Moreover, herbivorous species such as *O. mossambicus* are known to excrete lower lipase activity than carnivorous species. Thus, higher lipase activities are expected in *C. gariepinus* than in *O. mossambicus*. Digestive enzymes have a positive influence on feed utilisation in fish species and their activities differ from species to species. These two species have the pre-requisite enzymes to digest an

insect-based diet. The cost-benefit analysis showed that replacing fishmeal with Alates termite meal in the diets of *O. mossambicus* and *C. gariepinus* is economically viable. It is noted that the price of Alates termite is lower than the Stinkbug. The wide distribution, easy harvesting and processing method makes Alates termites cheaper than Stinkbug in the market. Thus, their profit index is higher than Stinkbug meal inclusion.

The study showed that Alates termite meal can replace fishmeal up to 50% inclusion level without significantly affecting the growth performance and feed utilisation of *O. mossambicus* and *C. gariepinus*. The decline in growth performance was not distinguishable between fish species. The organosomatic indices declined at inclusion levels above 50% in both species. Thus, at inclusion levels above 50%, the health status of *O. mossambicus* and *C. gariepinus* may be compromised. In *O. mossambicus*, the blood serum chemistry was not affected by the inclusion of Alates termite meal. On the other hand, AST and ALT significantly declined at inclusion levels above 50%. Thus, *O. mossambicus* was more tolerant to Alates termite meal-based diets than *C. gariepinus*. This was more evident on the liver histology of *C. gariepinus* which recorded mild alterations. The cost-benefit analysis showed that the inclusion of Alates termite meal in the diets of these two species has economic benefits. Replacing fishmeal with Alates termite meal had an effect on growth performance, liver and intestine histology, digestive enzyme profile and cost benefit analysis in *O. mossambicus* and *C. gariepinus*. Thus, the null hypothesis is rejected.

CHAPTER 5:

Partial replacement of fishmeal with Soldier termite (*Macrotermes falciger*) meal in *Oreochromis mossambicus* and *Clarias gariepinus*: A comparative study

CHAPTER 5: Partial replacement of fishmeal with Soldier termite (*Macrotermes falciger*) meal in *Oreochromis mossambicus* and *Clarias gariepinus*: A comparative study.

5.1 INTRODUCTION

The previous chapter showed that Alates termite meal has the potential to replace fishmeal up to 50% inclusion level in the diets of *O. mossambicus* and *C. gariepinus* without affecting growth performance and health status. Although Alates termites had a good nutritional profile, their main limitation is seasonal variability. Alates termites are the reproductive cast of the colony. It is thus interesting to evaluate other castes in the colony as potential fishmeal substitutes in the diets of commonly cultured warm freshwater species. This chapter will thus evaluate Soldier termites as a potential fishmeal substitute in the diets of the Mozambique tilapia (*O. mossambicus*) and the African catfish (*C. gariepinus*). Soldier and Alates termites (*M. falciger*) were formerly under the Isoptera order but have now been reclassified into the Blattodea order (Evangelista *et al*., 2019).

Soldier termites have the same feeding mode as Alates termites. Their nutritional profile is dependent on what they receive from the workers' caste. Proximate and nutritional composition data are scarce on Soldier termites (*M. falciger*) in the published literature in contrast with the Alates termites. The Soldier termites are an ideal insect to use in aqua feed due to their high protein content, vitamins, minerals, and good amino acid profile (Hlongwane *et al*., 2022; Netshifhefhe and Duncan, 2022).

A high (69.75%) protein content in Soldier termites of *Macrotermes* species has been reported (Hlongwane *et al*., 2022), which is comparable to fishmeal. The Soldier termites have a higher protein content than Alates termites (40.46%). These castes (Alates and Soldier termites) depend on workers for feeding and they receive the same food. However, the Soldier termites have a higher protein content. This may be due to their different digestive system. The Soldier termites have a higher nutrient utilisation efficiency, particularly with regard to protein. Protein is one of the most expensive dietary sources in aquafeed. It represents over 50% of the total feed costs (El-Sayed, 2020). Therefore, it is essential to evaluate its potential as a fishmeal substitute. The amino acid profile of the Soldier termites of *Macrotermes* species is not well

documented. The few studies that evaluated their nutritional composition showed that they contain a good amino acid profile, minerals, and vitamins (Hlongwane *et al*., 2022; Ntukuyoh *et al*., 2012).

The fat content of Soldier termites ranges between 2.03% and 7.97% (Hlongwane *et al*., 2021; Ntukuyoh *et al*., 2012). The fat content range of Soldier termites is relatively lower than Alates termites (40.40%) and Stinkbugs (48.59%). It will thus not be necessary to defatten the Soldier termites. This factor makes it an ideal insect to replace fishmeal in aqua feed. The fatty acid profile of Soldier termites is also not well documented. However, this insect contains high levels of linoleic acid, which is one of the essential fatty acids in fish growth. Soldier termites are also one of the insects that are easy to prepare. They are prepared by blanching in boiling water and then drying in the sun (Kinyuru *et al*., 2013).

One major advantage of Soldier termites is that they are harvested throughout the year, in contrast to the Alates termites which are harvested at the onset of the rainy season during their swarming phase (Netshifhefhe and Duncan, 2022). Soldier termites are harvested by inserting grass into the openings or shafts of a termite mound. The Soldier termites attack the intruding grass with their mandibles and get entrapped on the grass (Figure 5.1).

Figure 5.1: Soldier termites entrapped on the succulent grass (Musundire *et al*., 2021)

Different countries employ different insect harvesting techniques (Dao *et al*., 2022). Soldier termites may also be harvested by removing part of the termitarium (Boafo *et al*., 2019). The method of trapping Soldier termites by using containers such as terra cotta pots, calabashes or baskets and filling them with plant material such as crop residues has been reported in Burkina Faso (Dao *et al*., 2020).

The distribution of the *M. falciger* Soldier termites is the same as that of Alates termites discussed in the previous chapter. Thus, they are more widely distributed than the Stinkbugs. Most terrestrial insects such as Mopane worm (*Imbrasia belina*) and Stinkbug (*E. delegorguei*) are confined to specific regions. However, Soldier termites are widely distributed across Africa. This makes them easily accessible to farmers both in urban and rural communities.

Amongst the local terrestrial insects that are found at the market, Soldier termites are one of the cheap and affordable insects. The use of Soldier termites as feed in poultry has been reported in Burkina Faso and Ghana (Boafo *et al*., 2019; Sankara *et al*., 2018). This shows that Soldier termites have the potential to replace fishmeal in the animal feed industry. However, their potential in aqua feed has not been explored. Thus, the current chapter will evaluate the growth performance, nutrient utilisation, blood serum chemistry, liver and intestinal histology, and digestive enzyme profile of *O. mossambicus* and *C. gariepinus* diets.

5.2 OBJECTIVES AND HYPOTHESES

The objectives of this chapter were:

I. To determine the effect of substituting fishmeal with Soldier termite meal on the growth performance of *O. mossambicus* and *C. gariepinus*.
II. To determine blood serum chemistry of *O. mossambicus* and *C. gariepinus* fed diets with Soldier termite meal as fishmeal replacement.
III. To determine the effect of Soldier termite meal as a fishmeal replacement in the liver and intestine histology of *O. mossambicus* and *C. gariepinus*
IV. To determine the digestive enzyme profile in *O. mossambicus* and *C. gariepinus* fed diets with Soldier termite meal as fishmeal replacement.
V. To determine the cost-benefit analysis of replacing fishmeal with Soldier termite in *O. mossambicus* and *C. gariepinus*.

Hypotheses:

I. Substituting fishmeal with Soldier termite meal has no effect on the growth performance of *O. mossambicus* and *C. gariepinus*.
II. There is no effect in blood serum chemistry of *O. mossambicus* and *C. gariepinus* fed diets with Soldier termite meal as a fishmeal replacement.
III. Substituting fishmeal with Soldier termite meal has no effect on the liver and intestine histology of *O. mossambicus* and *C. gariepinus.*
IV. There is no effect in the digestive enzyme profile of *O. mossambicus* and C. gariepinus fed diets with Soldier termite meal as a fishmeal replacement.
V. There is no effect on the cost benefit analysis of replacing fishmeal with Soldier termite meal in the diets of *O. mossambicus* and *C. gariepinus.*

5.3 MATERIAL AND METHODS

5.3.1 Diet preparation

The Soldier termites (sun-dried) were purchased from the Thohoyandou Open Market in the Vhembe District of Limpopo Province, South Africa. Soldier termites were ground to powder and replaced fishmeal at 0, 10, 30, 50, and 70%, which were labelled as D1, D2, D3, D4, and D5, respectively (Table 5.1). The diets were formulated to be isonitrogenous (30% protein), isolipidic (12% fat), and isocaloric (15 MJ/Kg) using Winfeed 3, EFG (Natal) software. The control diet contained 30% fishmeal and no insect meal. The feed ingredients were weighed and mixed using a planetary mixer (Hobart, Troy, OH, USA). During mixing, water (10-20% v/w) was added as required until the desired dough thickness was reached. The mixture was pelleted into a 3 mm diameter size using a meat mincer connected to the planetary mixer. The pellets were separately collected in trays labelled with the respective diets and sun-dried. After drying, the dried pellets were stored in polyethylene buckets which were also marked with the corresponding diet.

Table 5.1: Ingredients (g/kg) and proximate composition of experimental diets replacing fishmeal with Soldier termite meal at different inclusion levels.

Fishmeal replacement	0% (Control)	10%	30%	50%	70%
Diets	D1	D2	D3	D4	D5
Fishmeal[a]	300	270	210	150	90
Soldier termites	0	30	90	150	210
Maize	211.8	204.8	219	224.8	200
Wheat bran	196	185.9	166.1	180.1	160.1
Sunflower meal	78.5	96	102.3	82.8	126
Soybean meal	50	50	50	50	50
Sunflower oil	63.7	63.3	62.6	62.3	63.9
Methionine[b]	20	20	20	20	20
Lysine[b]	20	20	20	20	20
Vit/Min premix[c]	20	20	20	20	20
Binder[d]	30	30	30	30	30
Chromic oxide[e]	10	10	10	10	10
Total	1000	1000	1000	1000	1000
Proximate composition					
Crude protein	30.56	30.87	30.08	30.19	30.39
Fat	12.32	12.07	12.02	12.03	12.39
Gross Energy	15.12	15.08	15.41	15.03	15.33
Dry matter	91.60	91.86	92.33	92.56	92.80

[a]Fishmeal :65.5%; 12% fat; 18% ash (Irvine's Africa pty ltd; South Africa)

[b]Methionine and lysine (Nutroteq: South Africa)

[c]Vitamin/Mineral premix: Vit. A, 12 000MIJ; Vit. D3, 1 200 MIJ; Vit. E, 120 MIJ; Vit. B4, 10000g; Vit. C, 120g; Vit. B3, 25g; Vit. B5, 15g; Vit. B2, 6g; Vit. B6, 5g; Vit. B1, 4g; Vit. K3, 2g; Vit. B9, 1g; Vit. H, 0.25g; Vit. B12, 0.04g. ZnO, 200g; FeSO4; CuSO4, 7g; MnO, 5g; KI, 2g; Na2SeO3, 0.15g; CoSO4, 0.05g (Irvine's Africa pty ltd: South Africa)

[d]Binder (Irvine's Africa pty ltd: South Africa)

[e]Chromium (III) oxide (Sigma-Aldrich)

5.3.2 Fish stocking

The experiment was undertaken at the Aquaculture Research Unit (ARU), University of Limpopo, Limpopo Province, South Africa. The Mozambique tilapia (*O. mossambicus*) juveniles and the African catfish (*C. gariepinus*) fingerlings were obtained from the ARU hatchery.

Both groups of fish were fed a commercial diet (Aqua-plus, Avi Products (pty) ltd) for two weeks during the acclimatisation period. During stocking, fish species were sedated in a 2-phenoxyethanol (1ml/5L) solution prior to stocking in experimental tanks. Fish were weighed prior commencement of the experimental feeding trials.

A total of 150 juvenile *O. mossambicus* with uniform size (average individual weight 9.70 ± 1.2 g/fish) were randomly assigned to 5 diets (D1, D2, D3, D4, and D5) in triplicates at a stocking density of 10 fish per tank. The initial body weight of 10 fish in each tank was recorded in bulk. The average initial body weight of *O. mossambicus* in the diets ranged between 97.5±3.58 g and 97.83±2.23 g.

On the other hand, a total of 75 *C. gariepinus* fingerlings (average individual weight 5.0± 0.8 g) were also randomly assigned to 5 diets in triplicates at a stocking density of 5 fish per tank. The average initial body weight of *C. gariepinus* in the diets ranged between 23.85±0.57 g and 25.61±2.49 g.

5.3.3 Experimental design

The experiment was conducted in an indoor Recirculating Aquaculture System (RAS) using 500L size fibreglass tanks filled to the 400L mark. The tanks were connected to a sump and a pump that supplied the tanks with water at 10 L/min. Each tank was heated with a submersible aquarium heater (ViaAqua Glass heater, 200W) and aerated with air stones. Fish were fed allocated diets to apparent satiation for 8 weeks at 09h:00 and 15h:00. Fish were regarded as satiated when 2 sinking pellets were left uneaten for 5 minutes. The experiment was conducted under natural photoperiod. Feed intake was recorded on daily basis for each tank. The study was approved for ethical clearance by the University of Limpopo Animal Research Ethical Committee (AREC/09/2022:PG).

5.3.4 Water quality management

Water quality parameters were monitored on a weekly basis using a handheld multiparameter meter (Professional plus YSI 605000). The water temperature was kept between 27 and 29°C, dissolved oxygen (6.5-8.0 mg/l), ammonia (<1 mg/l), and pH (6.8 - 8.0).

5.3.5 Proximate composition

The dry matter, moisture, ash content, fat content, crude protein and gross energy were analysed according to AOAC (2012). Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) were determined by boiling the sample with a neutral detergent solution and acid detergent solution, respectively (van Soest *et al*.,1991)

5.3.6 Mineral composition

The mineral content was determined using Inductively coupled plasma-optical emission spectrometry (ICP-OES) as described by Manditsera *et al*., (2019). Samples were hydrolyzed with 65% concentrated nitric acid and 37% hydrochloric acid. Hydrogen peroxide was used to remove nitrous vapours. The analysis was performed using standard solutions of known concentrations. All chemicals used were analytical reagent grade.

5.3.7 Amino acids and fatty acids analyses

Soldier termite meal was analysed for amino acids and fatty acids profile at the Central Analytical Facilities (CAF) of Stellenbosch University (South Africa). The amino acid profile and fatty acid profile were analysed as described in Chapter 3, sections 3.3.4 and 3.3.5.

The Essential amino acid Index (EAAI) of the Soldier termite was calculated using the Peńaflorida (1989) formula:

$$\mathrm{EAAI} = \sqrt[n]{\frac{\mathrm{aa1}}{\mathrm{AA1}} \times \frac{\mathrm{aa2}}{\mathrm{AA2}} \times \cdots\cdots \frac{\mathrm{aa}n}{\mathrm{AA}n}},$$

Where aa is the amount of the amino acid in the Soldier termite, AA is the amino acid requirement of the fish, and *n* is the total number of amino acids used in the calculation.

5.3.8 Growth performance indices

Growth parameters such as specific growth rate (SGR), thermal-unit growth coefficient (TGC), feed conversion ratio (FCR), protein efficiency ratio (PER), and apparent protein digestibility coefficient (APDC) were calculated using formulas listed in Chapter 3, section 3.3.9.

5.3.9 Organosomatic indices

Condition factor (CF), hepatosomatic index (HSI) and viscerosomatic index (VSI) were also calculated using formulas listed in Chapter 3, section 3.3.10.

5.3.10 Blood serum chemistry

The blood serum chemistry was assessed as described in Chapter 3, section 3.3.11.

5.3.11 Liver and intestinal histomorphology analysis

Three fish from each dietary replicate (9 fish/diet) were used for liver and intestinal histomorphology. The liver samples from each fish were separately fixed in a 10% buffered formalin solution in sampling bottles. The distal intestine of each fish was also separately preserved in 10% formalin solution in sampling bottles. Analysis was performed as described in Chapter 3, section 3.3.12.

5.3.12 Liver and intestinal histomorphology assessment

Liver and intestine slides were assessed using a light microscope (Leica Microsystems model DM750, Leica, Bannockburn, IL, USA) which was equipped with a digital camera (Digital Video Camera Company, Austin, TX, USA). This was done as described in Chapter 3, section 3.3.13.

5.3.13 Gastrointestinal enzyme profile

Three fish from each dietary replicate were sedated in a 2-phenoxyethanol solution and sacrificed. Fish samples were dissected, distal intestines were removed and stored in sampling bottles at -80°C until analysis. The analysis of amylase, lipase, and protease was done as described in Chapter 3, Section 3.3.14.

5.3.14 Cost benefit analysis

The cost benefit analysis was determined according to Bahnasawy *et al.* (2003).

$$\text{Incidence cost} = \frac{\text{Cost of feed}}{\text{Quantity of fish produced (kg)}}$$

$$\text{Profit index} = \frac{\text{Local market value of fish}}{\text{Cost of feed}}$$

The underlying assumption is that all operating costs are constant, and the cost of ingredients was the only variable cost. The prices used were listed in chapter 3, section 3.3.15.

5.4 STATISTICAL ANALYSIS

The data was statistically analysed as described in Chapter 3, Section 3.4.

5.5 RESULTS

5.5.1 Proximate composition

The Soldier termite contained 57.58% protein content, 7.29% fat, and 5.05% ash content (Table 5.2). The NDF and ADF were 61.99% and 20.27%, respectively. The mineral content that dominated the Soldier termite meal were potassium and sodium, respectively.

The fatty acid profile showed that saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids dominated the Soldier termite meal (Table 5.2). Oleic acid (0.47 g/kg), stearic acid (0.28 g/kg), and linoleic acid (0.22g/kg) dominated the Soldier termite meal fatty acid profile (Table 5.2). Although in small quantity, the Soldier termite meal recorded α-linolenic acid (0.02 g/kg), which is one of the limiting fatty acids in fish diets.

Table 5.2: Proximate composition, mineral content, and the fatty acid profile of Soldier termite meal.

Proximate composition	**%DM**	**Fatty acids**	**g/kg**
Dry matter (%)	85.47	C14:0 Myristic acid	0.01
Ash (%DM)	5.05	C16:0 Palmitic acid	0.17
Fat (%DM)	7.29	C17:0 Margaric acid	0.04
Protein (%DM)	57.58	C18:0 Stearic acid	0.28
Carbohydrates (%DM)	16.25	C20:0 Arachidic acid	0.02
NDF (%DM)	61.99	C17:1n-10 Heptadecenoic acid	0.02
ADF (%DM)	20.27	C18:1n-9(cis) Oleic acid	0.47
Energy (KJ/100g)	1 581	C18:2n-6 (cis) Linoleic acid	0.22
Minerals	**(mg/l)**	C18:3n-3 α-linolenic acid	0.02
Iron	3.4	Σ Saturated fatty acid	0.54
Potassium	46.3	Σ Monounsaturated fatty acid	0.49
Sodium	23.3	Σ Polyunsaturated fatty acid	0.24

Soldier termites meal contained both essential and non-essential amino acids (Table 5.3). Lysine (14.06 g/kg), histidine (9.57 g/kg) and leucine (8.11 g/kg) dominated the essential amino acid profile, respectively. The Soldier termite also contained 5.50 g/kg methionine (Table 5.3). Soldier termite amino acid profile was relatively lower than in fishmeal. Its profile did not meet the requirement for *O. mossambicus*. However, amino acids such as histidine, tryptophan, and methionine were comparable to fishmeal. On the other hand, lysine, isoleucine, histidine, and leucine amino acids in Soldier termite meal met the requirements for *C. gariepinus* (Table 5.3). The EAAI recorded in the Soldier termite meal was 6.78 (Table 5.3).

Table 5.3: Amino acid profile of the Soldier termite meal, fishmeal, requirements for *O. mossambicus*, and requirements for *C. gariepinus* (g/kg).

Essential AA	g/kg (DM)	Fishmeal*	Requirements for *O. mossambicus***	Requirements for *C. gariepinus****
Lysine	14.06	42.2	37.8	12-14.3
Methionine	5.50	19.4	09.9	6-6.4
Phenylalanine	7.39	37.4	25.0	12-14
Valine	6.43	27.7	22.0	7.1-8.4
Tryptophan	0.31	5.7	0.43	1.2-14
Threonine	7.00	23.1	29.3	5-5.6
Isoleucine	6.45	24.5	20.1	6-7.3
Histidine	9.57	17.5	10.5	4-4.2
Leucine	8.11	37.9	34.0	8-9.8
EAAI	6.78			
Non-essential AA				
Tyrosine	5.89			
Glycine	6.79			
Aspartic acid	8.98			
Serine	9.24			
Proline	6.24			
Glutamic acid	24.88			
Alanine	7.68			
Cystine	17.77			

*Djssou et al., (2016); **Santiago and Lovell (1988); ***NRC (1993)

Table 5.4:Fatty acid profile of experimental diets (g/kg).

Fatty acid (g/kg)	D1	D2	D3	D4	D5
C14 Myristic acid	1.25	1.23	0.91	0.53	0.54
C15 Pentadecanoic acid	0.14	0.14	0.13	0.09	0.07
C16 Palmitic acid	10.33	11.43	11.73	8.75	14.36
C16:1 Palmitoleic acid	1.37	1.45	0.94	0.50	0.62
C17 Margaric acid	0.15	0.15	0.10	0.07	0.10
C17:1 Heptadecenoic acid	0.16	0.12	0.07	0.04	0.05
C18 Stearic acid	2.43	3.32	5.47	4.71	8.00
C18:1 (t) Elaidic acid	0.05	0.01	0.00	0.00	0.01
C18:1 (c) Oleic acid	9.13	12.51	20.15	16.06	30.06
C18:2 (t) Linoleic acid	0.00	0.02	0.07	0.11	0.12
C18:2 (c) Linoleic acid	16.11	24.01	47.36	39.28	71.34
C20 Arachidic acid	0.15	0.26	0.44	0.33	0.55
C18:3n6 y-Linolenic acid	0.01	0.31	0.96	1.17	2.14
C20:1 & C18:3n3	2.30	2.43	2.06	1.41	2.48
C20:2 Decosadienoic acid	0.05	0.05	0.04	0.02	0.05
C22 Decosanoic acid	0.21	0.31	0.65	0.52	0.98
C22:1 Erucic acid	0.79	0.83	0.58	0.29	0.34
C20:3n3 Eicosatrienoic acid	0.04	0.07	0.04	0.03	0.03
C20:4n6 Arachidonic acid	0.18	0.22	0.13	0.05	0.06
C23 Tricosanoic acid	0.01	0.02	0.04	0.03	0.07
C20:5n3 Eicosapentaenoic acid	3.07	3.08	2.10	1.02	1.12
C24 Lignoceric acid	0.09	0.09	0.18	0.11	0.24
C24:1 Nervonic acid	0.21	0.18	0.12	0.05	0.07
C22:6n3 Docosahexaenoic acid	4.97	4.88	3.26	1.60	1.56

5.5.2 Growth performance

In *O. mossambicus*, fish fed the control diet recorded the best growth performance (SGR, TGC, FI, PER, and APDC) (Table 5.5). Amongst the diets with Soldier termite meal, diet D4 showed the best growth performance. However, growth performance

significantly declined at inclusion levels above 50% ($P<0.05$). Feed intake did not differ significantly across all diets ($P>0.05$) in *O. mossambicus*.

In *C. gariepinus*, an increase in Soldier termite meal resulted in a decrease in growth performance parameters (WG, SGR, TGC, FI, PER, and APDC) (Table 5.6). The diet with the highest inclusion level (D5) recorded the lowest growth performance parameters. In *C. gariepinus* feed intake declined significantly at inclusion level above 30%.

Somatic indices showed no discernible pattern across diets (Table 5.5). Moreover, the CF, VSI, and HSI were not influenced by Soldier termite meal inclusion ($P>0.05$). Somatic indices in *C. gariepinus* followed a similar trend (Table 5.6)

There was no mortality recorded across all diets in the *O. mossambicus* and *C. gariepinus* experimental trials.

Table 5.5:Growth performance and somatic indices (Mean ± SE) of *O. mossambicus* fed Soldier termite meal as fishmeal replacement at different inclusion levels for 8 weeks. *n* = 9.

Diets	**D1 (0%)**	**D2 (10%)**	**D3 (30%)**	**D4 (50%)**	**D5 (70%)**
IBW (g)	97.83±2.23	98.00±3.80	98.73±2.32	97.5±3.58	98.4±1.91
FBW (g)	310.50±3.99	207.91±4.57	239.08±2.88	268.17±2.66	173.00±3.76
WG (g)	212.67±2.24^{a}	109.92±2.52^{d}	140.27±2.44^{c}	171.24±3.05^{b}	74.68±1.53^{e}
SGR (%/day)	2.02±0.09^{a}	1.31±0.05^{d}	1.55±0.11^{c}	1.77±0.07^{b}	0.98±0.03^{e}
FCR	1.58±0.09^{e}	2.93±0.05^{b}	2.22±0.11^{c}	1.96±0.07^{d}	3.84±0.03^{a}
TGC	1.35±0.10^{a}	0.82±0.04^{d}	0.99±0.05^{c}	1.15±0.11^{b}	0.59±0.04^{e}
FI (g/fish/day)	3.16±0.03^{a}	2.02±0.06^{a}	2.19±0.02^{a}	2.89±0.06^{a}	1.62±0.01^{a}
PER	1.53±0.11^{a}	1.31±0.05^{a}	1.34±0.27^{a}	1.43±0.21^{a}	1.24±0.03^{a}
APDC (%)	80.20±1.96^{a}	78.57±3.25^{a}	77.36±2.21^{a}	75.33±3.81^{a}	75.16±1.47^{a}
		Somatic Indices			
CF	2.31±0.96^{a}	1.48±0.02^{a}	1.32±0.35^{a}	1.63±0.04^{a}	1.37±0.11^{a}
VSI (%)	11.69±1.43^{a}	12.26±1.78^{a}	10.51±1.70^{a}	12.30±2.35^{a}	7.66±1.62^{a}
HSI (%)	1.79±0.24^{a}	1.80±0.19^{a}	3.00±0.81^{a}	2.61±0.26^{a}	2.47±1.04^{a}
Survival (%)	100	100	100	100	100

IBW: Initial body weight, FBW: Final body weight, WG: Weight gain, SGR: Specific growth rate, FCR: Food conversion ratio, TGC: Thermal-growth coefficient, FI: Feed intake, PER: Protein efficiency ratio: CF: Condition factor, VSI: Viscerosomatic Index, HSI: Hepatosomatic Index. D1: Diet 1 (0%), D2: Diet 2 (10%), D3: Diet 3 (30%), D4: Diet 4 (50%), D5: Diet 5 (70%).

Table 5.6:Growth parameters and somatic indices (Mean ± SE) of *C. gariepinus* fed diets with Soldier termite meal as a fishmeal replacement at different inclusion levels for 8 weeks. *n* = 9.

Dietary groups	D1 (0%)	D2 (10%)	D3 (30%)	D4 (50%)	D5 (70%)
IBW (g)	24.86±2.02	24.44±1.04	25.04±1.54	23.85±0.57	25.61±2.49
FBW (g)	46.80±1.16	44.41±2.91	41.97±0.45	37.28±1.62	34.99±3.61
WG (g)	21.94±2.10^{a}	19.97±2.49^{a}	16.94±1.31^{ab}	13.43±0.26^{ab}	10.38±1.31^{b}
SGR (%/day)	1.26±0.17^{a}	1.19±0.23^{a}	1.03±0.10^{b}	0.89±0.01^{c}	0.71±0.18^{d}
FCR	1.44±0.02^{a}	1.44±0.04^{a}	1.29±0.01^{b}	1.50±0.00^{ac}	1.56±0.08^{ac}
TGC	0.50±0.01^{a}	0.47±0.01^{a}	0.40±0.02^{b}	0.34±0.01^{c}	0.26±0.0^{d}
FI (g/fish/day)	0.79±0.01^{a}	0.75±0.08^{a}	0.73±0.05^{a}	0.68±0.04^{b}	0.59±0.07^{c}
PER	1.27±0.14^{a}	0.92±0.17^{b}	0.84±0.08^{b}	0.83±0.01^{b}	0.36±0.08^{c}
APDC (%)	78.41±1.36^{a}	61.10±2.14^{b}	57.95±1.25b	56.47±2.44^{b}	51.02±1.68^{b}
			Somatic Indices		
CF	0.55±0.01^{a}	0.57±0.01^{a}	0.56±0.09^{a}	0.52±0.01^{a}	0.53±0.01^{a}
VSI (%)	8.18±0.18^{a}	7.83±1.33^{a}	6.54±0.69^{a}	7.37±2.07^{a}	6.99±1.42^{a}
HSI (%)	1.22±0.16^{a}	1.47±0.22^{a}	1.63±0.17^{a}	1.82±0.65^{a}	1.12±0.11^{a}
Survival (%)	100	100	100	100	100

IBW: Initial body weight, FBW: Final body weight, WG: Weight gain, SGR: Specific growth rate, FCR: Food conversion ratio, TGC: Thermal-growth coefficient, FI: Feed intake, ADC: Apparent digestibility coefficient, CF: Condition factor, VSI: Viscero-somatic Index, HSI: Hepato-somatic Index. D1: Diets 1 (0%), D2: Diet 2 (10%), D3: Diet 3 (30%), D4: Diet 4 (50%), D5: Diet 5 (70%).

5.5.3 Regression analysis of SGR, FCR and APDC of *O. mossambicus* and *C. gariepinus*.

The SGR in *O. mossambicus* was higher than in *C. gariepinus* (Figure 5.2 A & B). Furthermore, the SGR showed a negative linear relationship with inclusion of Soldier termite meal in both species. On the other hand, a positive relationship between FCR and inclusion of Soldier termite meal was recorded in both species (Figure 5.2 C & D). Regression analysis showed that *O. mossambicus* digested Soldier termite-based diets better than C*. gariepinus* (Figure 5.2 E & F).

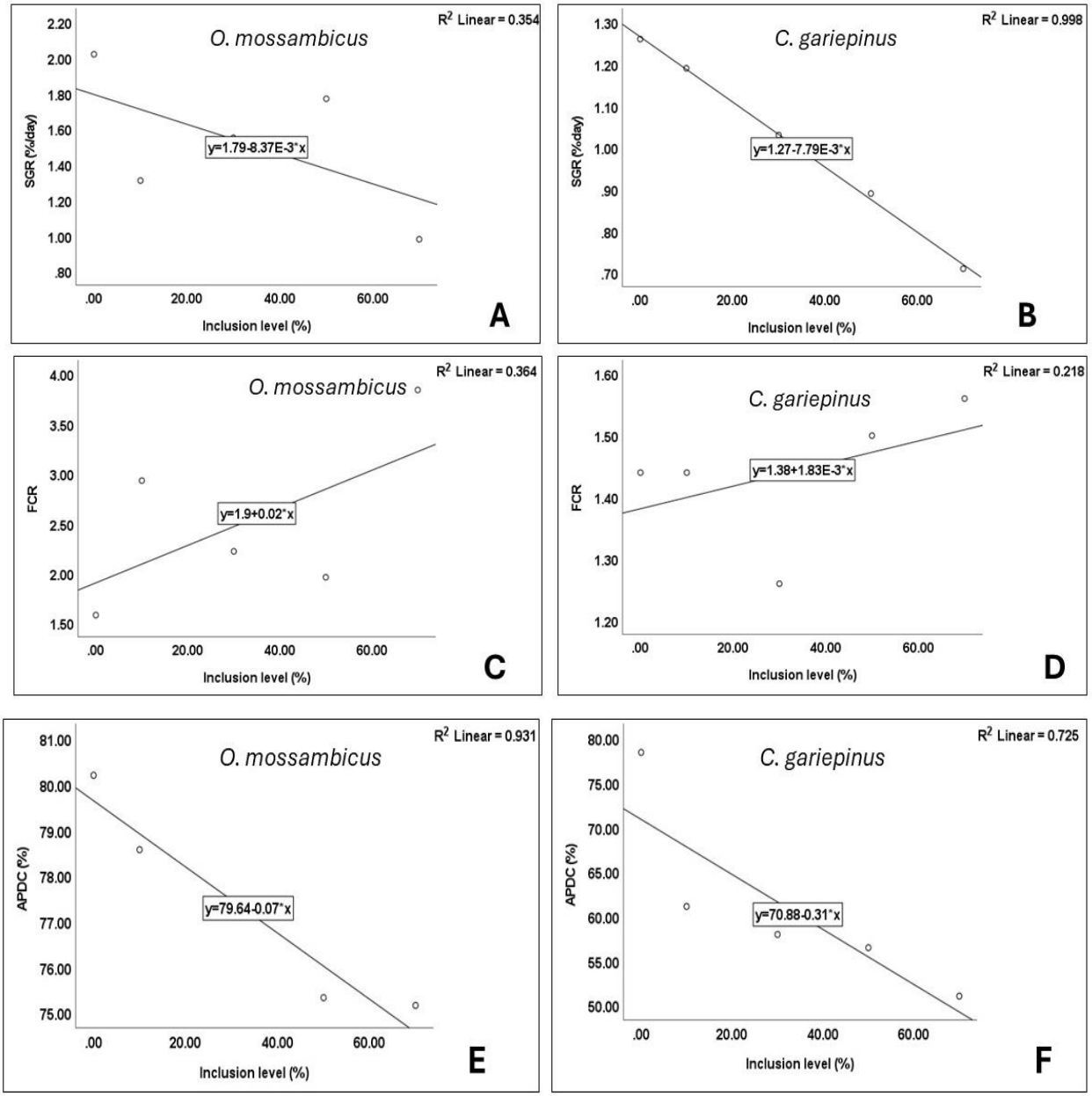

Figure 5.2: Linear regression analysis of SGR, FCR and APDC of *O. mossambicus* and *C. gariepinus*.

5.5.4 Blood serum chemistry

The AST and ALT levels in *O. mossambicus* increased significantly (P<0.05) at inclusion levels above 50% (Table 5.7).

In *C. gariepinus*, increasing Soldier termite meal resulted in an increase in ALT and AST values (Table 5.7). At the highest inclusion level (70%), the ALT and AST levels significantly increased (P<0.05).

The cholesterol, triglyceride, and glucose levels had no significant difference (P>0.05) across diets in both *O. mossambicus* and *C. gariepinus* (Table 5.7).

Table 5.7:Blood serum chemistry (Mean ± SE) of *O. mossambicus* and *C. gariepinus* fed diets with Soldier termite meal as fishmeal replacement at different inclusion levels. *n* = 9.

Diets	ALT (U/L)	AST (U/L)	Chol (mmol/l)	Trig (mmol/l)	Glu (mmol/l)
O. mossambicus					
D1	30.00±2.33^{a}	101.14±3.24^{a}	1.32±0.02^{a}	2.05±0.01^{a}	4.37±0.14^{a}
D2	81.30±3.17^{b}	131.11±4.65^{b}	1.29±0.02^{a}	2.04±0.02^{a}	5.35±0.12^{a}
D3	54.10±2.40^{c}	122.64±1.76^{c}	1.40±0.01^{a}	2.10±0.02^{a}	3.32±0.11^{a}
D4	35.21±5.56^{a}	107.51±3.28^{a}	1.41±0.02^{a}	2.04±0.01^{a}	4.39±0.12^{a}
D5	100.41±3.47^{d}	141.5±2.33^{d}	1.29±0.09^{a}	2.26±0.01^{a}	4.74±0.12^{a}
C. gariepinus					
D1	77.36±3.11^{a}	105.26±6.20^{a}	1.73±0.01^{a}	1.34±0.02^{a}	5.05±0.10^{a}
D2	125.33±5.68^{b}	120.14±4.00^{b}	2.08±0.03^{a}	1.67±0.04^{a}	5.95±0.13^{a}
D3	131.45±4.57^{c}	149.54±3.41^{c}	3.25±0.05^{a}	1.11±0.01^{a}	4.05±0.14^{a}
D4	139.92±4.36^{d}	232.36±4.65^{d}	3.34±0.02^{a}	1.13±0.01^{a}	5.55±0.11^{a}
D5	160.16±3.88^{e}	303.54±6.85^{e}	2.87±0.01^{a}	2.14±0.02^{a}	6.15±0.12^{a}

ALT: alanine aminotransferase, AST: aspartate aminotransferase, Chol.: cholesterol, Trig.: triglyceride, Glu.: glucose. Different superscripts in a column are significantly different (P<0.05).

5.5.5 Liver and intestinal histomorphology

In *O. mossambicus*, the villi showed no discernible pattern, whilst the villi thickness was not influenced by the incorporation of Soldier termite meal across all diets (P>0.05). In *C. gariepinus*, both the villi height and villi thickness showed no discernible pattern (Table 5.8).

The goblet cell number in *O. mossambicus* was not influenced by Solider termite meal inclusion (P>0.05) (Table 5.8). In *C. gariepinus*, the goblet cell number increased with an increase in inclusion levels (Table 5.8).

The liver hepatocyte of fish across all diets scored 1 in both *O. mossambicus* and *C. gariepinus* (Table 5.8).

The inclusion of Soldier termite meal in the diets of *O. mossambicus* and *C. gariepinus* had no marked effects on the intestine histomorphology (Figure 5.2; Figure 5.3). Fish across all diets showed normal intestinal histomorphology with distinguishable villus, lamina propria, submucosa, and epithelial cells.

The liver histomorphology was also normal in both *O. mossambicus* and *C. gariepinus* across all diets (Figure 5.4; Figure 5.5). The liver showed well-defined nucleus, Kupfer cells and vacuolation in D5 (Figure 5.4E).

Table 5.8:Intestine histology and the liver hepatocyte score of *O. mossambicus* and *C. gariepinus* fed diets with Soldier termite meal as a fishmeal replacement at increasing inclusion levels. *n*=9. Values are expressed as mean±SE.

Diets	**D1**	**D2**	**D3**	**D4**	**D5**
Species			**Villi height (µm)**		
O. mossambicus	98.12±1.62^{a}	100.25±3.11^{a}	104.63±2.69^{a}	144.85±1.25^{b}	116.25±1.30^{c}
C. gariepinus	236.32±3.25^{a}	189.35±2.58^{b}	180.38±2.00^{c}	217.75±2.78^{d}	230.80±2.44^{a}
			Villi thickness (µm)		
O. mossambicus	56.36±1.20^{a}	52.66±1.36^{a}	50.10±1.55^{a}	52.41±1.62^{a}	55.15±1.45^{a}
C. gariepinus	65.30±2.00^{a}	78.03±2.71^{b}	86.97±2.14^{c}	67.70±2.69^{a}	98.57±3.27^{d}
			Goblet cell (no)		
O. mossambicus	12±2.36^{a}	14±1.20^{a}	16±3.41^{a}	15±2.00^{a}	18±1.17^{a}
C. gariepinus	41±3.57^{a}	46±3.69^{ab}	48±2.48^{b}	48±4.21^{b}	58±3.60^{c}
			Hepatocyte score		
O. mossambicus	1	1	1	1	1
C. gariepinus	1	1	1	1	1

Different superscripts in a row are significantly different (P<0.05).

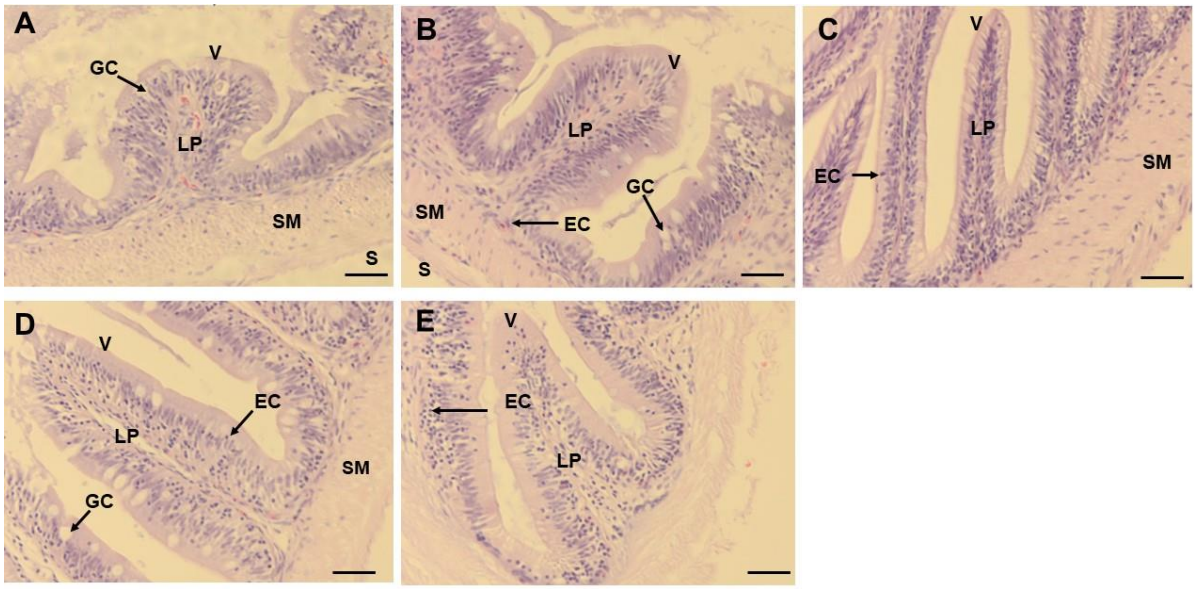

Figure 5.3: Micrographs of *O. mossambicus* fed diets with Soldier termite meal as a fishmeal replacement. A (diet D1), B (Diet D2), C (Diet D3), D (Diet D4) and E (Diet 5). V: Villi, LP: Lamina propria, GC: Goblet cell, SM: Submucosa, S: Serosa, EC: Epithelial cells, Scale bar: 20 µm.

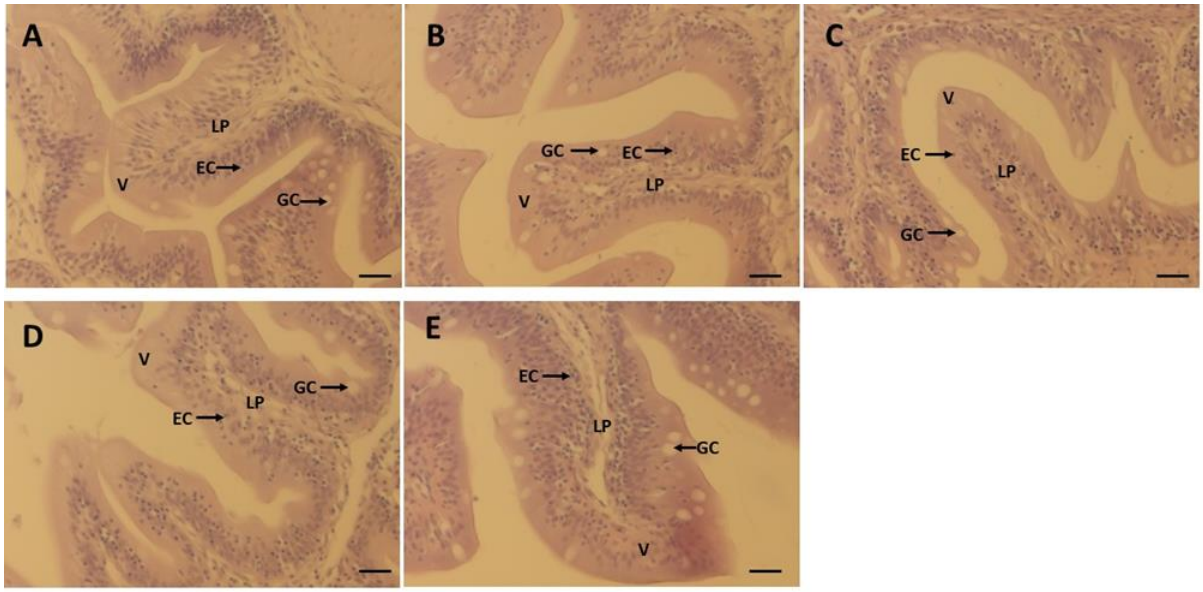

Figure 5.4: Micrographs of *C. gariepinus* fed diets with Soldier termite meal as a fishmeal replacement. A (diet D1), B (Diet D2), C (Diet D3), D (Diet D4) and E (Diet 5). V: Villi, LP: Lamina propria, GC: Goblet cell, EC: Epithelial cells, Scale bar: 20 µm.

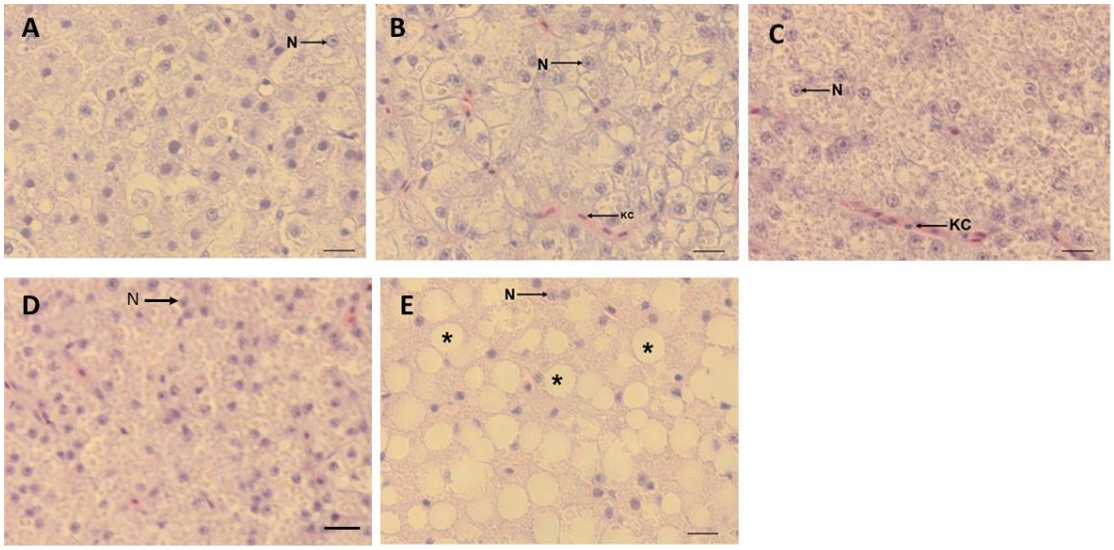

Figure 5.5: Liver micrographs of *O. mossambicus* fed diets with Soldier termite meal as a substitute for fishmeal. A (Diet 1, control,), B (Diet 2), C (Diet 3), D (Diet 4), E (Diet 5). N: Nucleous, KC: Kupfer cell, *: Vacuolation. Scale bar: 20 µm.

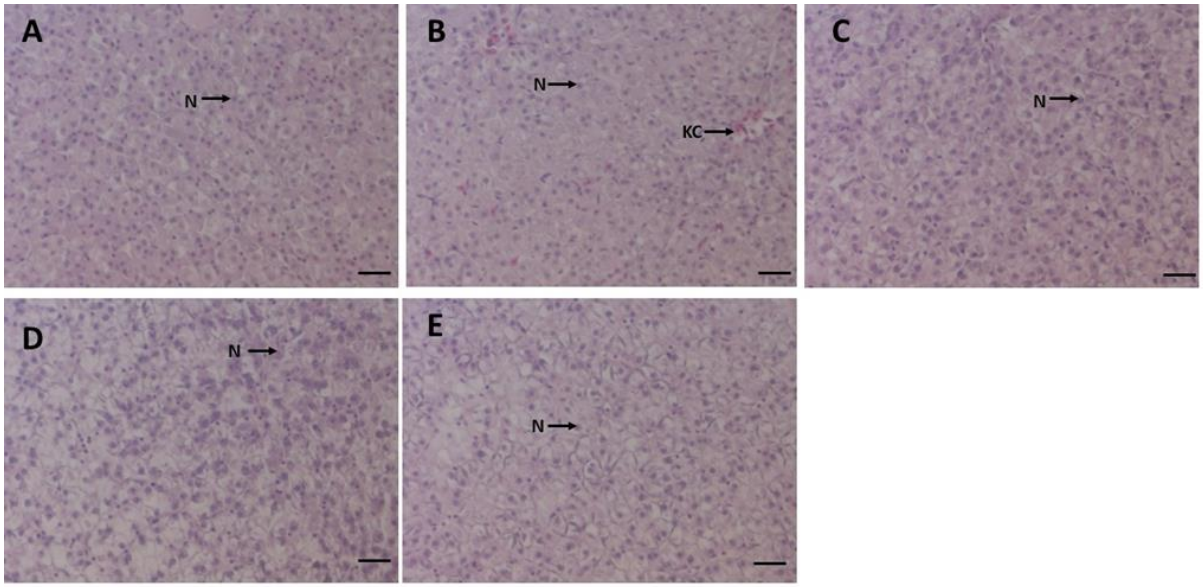

Figure 5.6: Liver micrographs of *C. gariepinus* fed diets with Soldier termite meal as a substitute for fishmeal. A (Diet 1, control,), B (Diet 2), C (Diet 3), D (Diet 4), E (Diet 5). N: Nucleous, KC: Kupfer cell. Scale bar: 20 µm.

5.5.6 Gastrointestinal enzyme profile

Oreochromis mossambicus showed increasing amylase activity with an increase in Soldier termite meal inclusion (Figure 5.6A), whilst *C. gariepinus* showed no discernible pattern. However, amylase activity was higher in *O. mossambicus* than in *C. gariepinus*.

Protease activity was the lowest in the control diet in *O. mossambicus* (Figure 5.6B). Diets with Soldier termite meal (D2, D3, D4 and D5) showed protease activity that did not differ significantly ($P>0.05$).

Clarias gariepinus recorded an increase in protease activity with an increase in Soldier termite meal inclusion (Figure 5.6B). The protease activity was higher in *O. mossambicus* than in *C. gariepinus*.

Both *O. mossambicus* and *C. gariepinus* showed lipase activity increasing with an increase in Soldier termite meal inclusion (Figure 5.6C). However, lipase activity was higher in *C. gariepinus* than *O. mossambicus*.

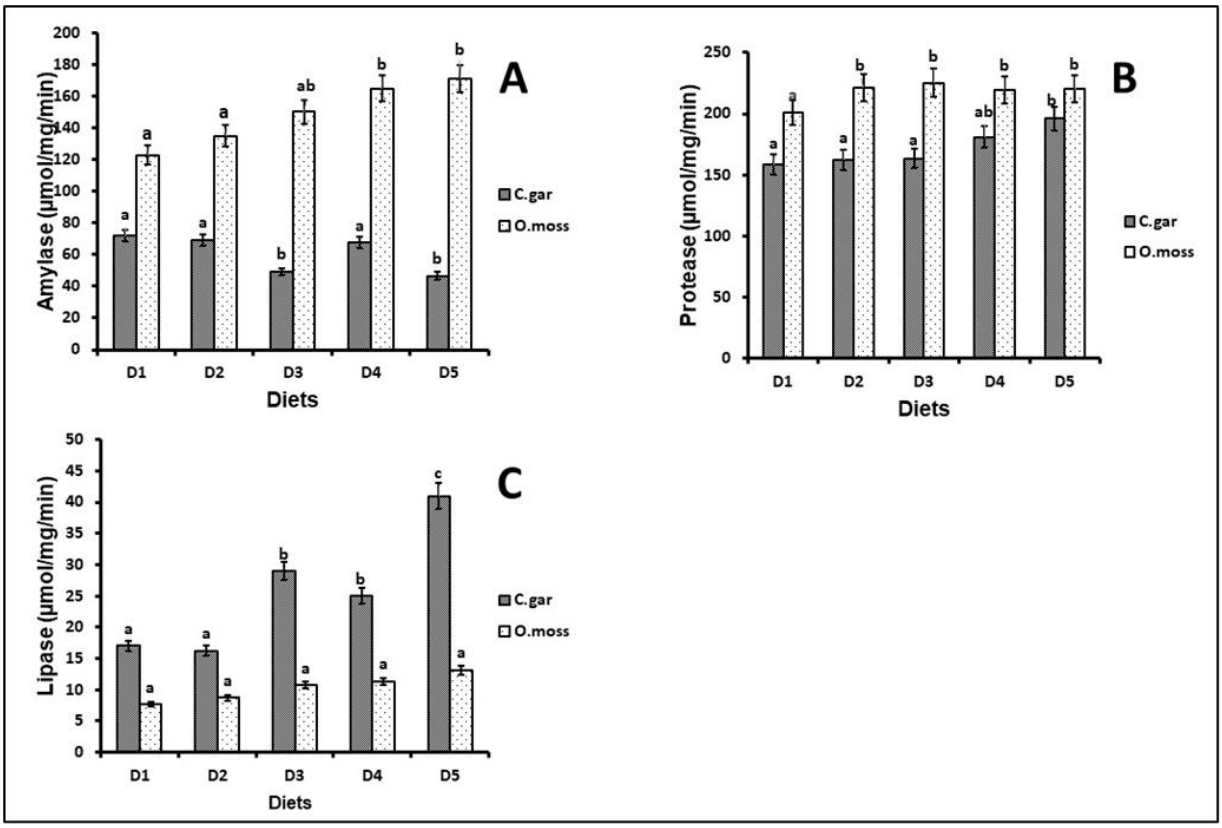

Figure 5.7: Amylase, protease and lipase activity of *O. mossambicus* and *C. gariepinus* fed diets with Soldier termite meal at increasing inclusion levels. C. gar: *C. gariepinus*; O. moss: *O. mossambicus*. D1 (0%), D2 (10%), D3 (25%), D4 (50%), D5 (70%).

5.5.7 Cost benefit analysis

The incidence cost decreased with an increase in the inclusion of Soldier termite meal (Table 5.9). On the other hand, the profit index increased with increasing inclusion level of Soldier termite meal.

Table 5.9:Cost benefit analysis of replacing fishmeal with Soldier termite in the diets of *O. mossambicus* and *C. gariepinus*.

Cost benefit indices	D1(0%)	D2 (10%)	D3 (30%)	D4 (50%)	D5 (70%)
Incidence cost	0.15	0.11	0.09	0.07	0.06
Profit index	1.18	1.21	1.26	1.32	1.38

5.6 DISCUSSION

The study showed that the best inclusion level of Soldier termite meal in the diets of *O. mossambicus* is 50%. The growth performance of fish fed 50% inclusion level was not significantly different from the control. This implies that the Soldier termite meal may be a potentially viable alternative in the replacement of fishmeal. However, it must be noted that growth performance declined at the highest inclusion level. Linear regression analysis showed that the decline in growth performance was more evident in *C. gariepinus*. However, in *O. mossambicus* the decline only became evident at 70% inclusion level. The 50% inclusion level has also been observed in the previous chapter, where Alates termite replaced fishmeal in the diets of *O. mossambicus*.

The difference in growth performance of these two species may be ascribed to their feeding habit. Herbivorous fish species have shown the potential to utilise diets with higher insect meal inclusion levels. A complete replacement of fishmeal with insect meal without adverse effects on growth performance and nutrient utilisation has been reported in *O. niloticus* (Tippayadara *et al*., 2021). A decline in growth performance with an increase in insect meal in *C. gariepinus* has also been previously reported (Rapatsa and Moyo, 2019). The study partly attributed the decline in growth performance to the presence of anti-nutritional factors in the Mopane worm. To the best of the author's knowledge, there is no study that recorded a complete replacement of insect meal without compromising the growth performance and nutrient utilisation in the diet of an opportunistic predator.

A decline in growth performance in *C. gariepinus* may also be partly attributed to a lack of taurine in Soldier termite meal. Soldier termites belong to the order Isoptera/Blattodea. Insects from this order have not been explored as potential fishmeal substitutes in aqua feed. However, in *O. mossambicus* its enzyme profile played a significant role in overcoming the lack of taurine. Taurine (2-aminoethane sulfonic acid) is a non-proteinaceous beta-amino acid that is essential for growth in fish species (Hameed *et al*., 2022). Moreover, it has been shown that *O. mossambicus* exhibits chitinase enzyme that breakdown chitin. It is known that age and or stage of development significantly influences the digestive enzyme activity in different fish species.

The blood serum chemistry showed that the incorporation of Soldier termite meal as a fishmeal replacement has no influence on the cholesterol, triglyceride, and glucose levels in *O. mossambicus* and *C. gariepinus*. This shows that fish fed diets with Soldier termite meal were not exposed to stress. Glucose levels are known to increase when organisms are under stressful conditions, probably due to catecholamine action on stored glycogen in fish tissues. One of the good traits of *O. mossambicus* is its ability to tolerate stressful conditions (El-Sayed, 2022). Similar observations were recorded when *O. niloticus* was fed an insect-based diet (Ogunji *et al*., 2008). Triglyceride and cholesterol were also not influenced by Soldier termite inclusion in both species. The serum triglyceride and total cholesterol are predominant free lipids that are distributed in the fish, and they serve as indicators of fat metabolism (Hu *et al*., 2020). This shows that the incorporation of Soldier termite meal did not affect fat metabolism. Moreover, Soldier termites contain low (7.29%) fat content, which is lower than Alates termite meal and the Stinkbug. The Solder termite meal influenced the AST and ALT levels in *O. mossambicus* and *C. gariepinus*. At higher inclusion levels (70%), AST and ALT levels were significantly elevated. The AST and ALT are the main indices used to evaluate liver injury. When the liver is damaged, it increases the cell membrane permeability, and these two enzymes are secreted from cells into the bloodstream. Thus, the elevation of these two enzymes signals liver damage (Belghit *et al*., 2018). A decline in feed intake was also observed in *C. gariepinus* and this suggests that Soldier termite meal might have anti-nutritional factors that might have caused an elevation in AST and ALT. Ntukuyoh *et al*. (2012) reported anti-nutritional factors (hydrocyanic acid, oxalate, phytic acid) in *Macrotermes bellicosus*. The presence of anti-nutritional factors in *Macrotermes* species may be linked to the undigested cellulose in the intestine of Soldier termites. Soldier termites receive unprocessed food from the workers, it is possible that the food contains anti-nutritional factors. Although anti-nutritional factors were not determined in this study, it is important for anti-nutritional factors to be determined in the insect. Histomorphology showed that fish across all diets in both species scored 1, which represents a healthy liver. The micrographs also showed no marked alterations.

The intestine histomorphology was not influenced by the incorporation of Solider termite meal in both species across all diets. This indicates that the gastrointestinal tract of both species was not affected even at the highest inclusion level. This is consistent with most studies where insect meal replaced fishmeal (Bosi *et al*., 2021; Zarantoniello *et al*., 2021). The intestine is a region where nutrient digestion and assimilation take place. The intestine also reflects the nutritional status of the fish when a novel ingredient is introduced into fish diets. Moreover, *O. mossambicus* possesses pre-requisite enzymes to digest an insect-based diet. Although *C. gariepinus* growth performance declined with an increase in Soldier termite meal, the intestine histomorphology supports that its health status was not compromised. The goblet cell number in *O. mossambicus* was not affected by the inclusion of Soldier termite meal. On the other hand, goblet cell number increased with an increase in Solder termite inclusion level. This is consistent with the decline in growth performance observed in *C. gariepinus*. It further implies that *C. gariepinus* may be prone to inflammation in the gastrointestinal tract with higher Soldier termite inclusion level.

Higher amylase activity was observed in *O. mossambicus* than *C. gariepinus*. An increase in amylase activity in the gut of *O. mossambicus* is a common phenomenon (Hlophe *et al*., 2014). Tilapias are herbivorous and are well equipped to utilise dietary carbohydrates more efficiently than carnivorous species (El-sayed, 2020). Tilapias are also known to utilise digestible carbohydrate of between 35% and 40% (El-Sayed and Garling, 1988). The diet fed these species comprised mainly of ingredients with high carbohydrate levels (maize, wheat bran). De Silva and Anderson (1995) also reported that amylase activity levels increased in *O. mossambicus* fed a starch rich diet. A higher amylase activity in *O. mossambicus* than in *C. gariepinus* was also reported in the previous chapter.

Clarias gariepinus showed amylase activity with no discernible pattern. *Clarias gariepinus* is an opportunistic predator and it has a limited capacity to digest a high carbohydrate-based diet. On the other hand, its protease activity showed an increasing trend. The anatomy and physiology of *C. gariepinus* reflect its dependency on animal-based diets for growth. Opportunistic predators are well-equipped to digest a rich-protein diet. Moreover, *C. gariepinus* feeds on rich-protein items in nature. Its protein requirement (40%) is also higher than *O. mossambicus* (30%) (Koch *et al*., 2017). Both species showed that fat content in the diets did not significantly affect its

metabolism. Thus, these fish species are capable of utilising lipids in their diets. However, *O. mossambicus* recorded a lower level of lipase activity than *C. gariepinus*. Opuszynski and Shireman (1995) also reported a lower lipase activity in herbivorous fish species than carnivorous ones.

The cost benefit analysis showed that the incorporation of Soldier termite meal in the diets of *O. mossambicus* and *C. gariepinus* may yield higher profit margins than fishmeal-based diets. This suggests that Soldier termites may be a viable substitute for fishmeal in these two fish species.

The study showed that Soldier termite meal may substitute fishmeal up to 50% inclusion level without adverse effects on growth performance and nutrient utilisation of *O. mossambicus*. On the contrary, the inclusion of Soldier termite in the diets of *C. gariepinus* resulted in a decline in growth performance. This was attributed to the feeding habits and digestive enzyme profiles of these species. Although a decline in growth performance was observed in *C. gariepinus*, its health status was not affected. The AST and ALT levels significantly increased at the highest inclusion level in both species. Reflecting that higher inclusion levels may expose the fish to stress. The liver and intestinal histomorphology confirmed that both fish species were in good health. Replacing fishmeal with Soldier termite meal has higher profit potential than fishmeal-based diets. Replacing fishmeal with Soldier termite meal had an effect on growth performance, liver and intestine histology, digestive enzyme profile and cost benefit analysis in *O. mossambicus* and *C. gariepinus*. Thus, the null hypothesis is rejected.

General discussion, recommendations, and conclusion

CHAPTER 6: General discussion, recommendations, and conclusion.

In recent years, insect meals have received growing attention as a sustainable ingredient for aquafeed production. The use of insect meals in the animal feed industry was approved by the European Commission (Regulation 2017/893/EC, 2017) in 2017. Several studies have used insects as fishmeal substitutes in different fish species. This thesis looked at the potential of using locally available insects (*Encosternum delegorguei*, *Macrotermes falciger*-Alates, *Macrotermes falciger*-Soldier) within the context of southern Africa. The sub-tropical temperatures in southern Africa are ideal for the proliferation of different insect species including the ones used in this study. Previous studies mainly focused on insects from the orders Diptera, Orthoptera, Lepidoptera, and Coleoptera. Insects from these orders have been widely evaluated as potential fishmeal substitutes and their nutritional composition is well known. This is the first study that focused on the order Isoptera/Blattodea and Hemiptera. The insects used in this study are commonly consumed by people. However, their nutritional composition is poorly documented.

In southern Africa, one of the terrestrial and locally available insects that have been widely evaluated is the Mopane worm. This insect has a high nutritional index that is comparable to fishmeal (Rapatsa and Moyo, 2022). The main limitation of the Mopane worm is that it is prohibitively expensive, and its current price is higher than fishmeal. From an economic perspective, the use of Mopane worms in aqua feed is unsustainable. Furthermore, in the last few years, poor Mopane worm harvest have been recorded particularly in the Limpopo Basin. This has been attributed to climate change. The insects that have been investigated in this study have been consistently available at the local insect market in southern Africa.

The insects evaluated in this study have different benefits and limitations (Table 6.1). These factors make it easy to identify the suitable insect to use at different seasons.

Table 6.1: Benefits, limitations, and possible solutions of the Stinkbug, Alates termites, and Soldier termites.

Insect	Benefits	Limitations	Possible solutions
Stinkbug	Locally available Protein content meets the requirements for Tilapia and catfish	High-fat content Laborious processing method Unpleasant odour High price at the market Seasonal variability Limited distribution Poor amino acid profile	Defatting
Alates termites	High protein content Simple processing method Simple harvesting method Good amino acid and fatty acid profiles	High-fat content Seasonal variability	Defatting
Soldier termite	High protein content Good amino acid and fatty acid profiles Availability and wide distribution Simple harvesting process Simple processing method	Harvesting method not suitable for large quantities Abundance differs with season	Develop new harvesting methods Preservation during season of abundance

The study showed that Stinkbug meal can replace fishmeal up to 30% inclusion level in the diets of *O. mossambicus* and *C. gariepinus*. The Alates termite meal can replace up to 50% inclusion level in both species and Soldier termite can replace 50% in *O. mossambicus*. The Stinkbug and Alates termites are seasonal insects. On the other hand, Soldier termites are available throughout the year. This gives farmers an opportunity to have access to insects throughout the year. Among the evaluated insects, the Soldier termite showed higher protein content, lower fat content, and higher EAAI (Table 6.2). It is one of the insects that have few limitations. It is thus the best-performing insect in this study. It is recommended to incorporate this insect into the diet of *O. mossambicus*. Significantly, this study showed that different fish species respond differently to insect-based diets. Although the Soldier termite replaced fishmeal up to 50% in *O. mossambicus*, it could only replace up to 10% in *C. gariepinus*. It is thus recommended that Alates termite be used to replace fishmeal in *C. gariepinus*.

Table 6.2: The nutritional profile summary of the Stinkbug meal, Alates termite meal, and Soldier termite meal.

Nutritional profile	Stinkbug	Alates termite	Soldier termite
Crude protein(%DM)	29.53	40.46	57.58
Fat (%DM)	48.59	40.40	7.29
Ash (%DM)	3.14	6.14	5.05
EAAI	0.11	1.87	6.78
Energy (Kj/100g)	2 514	2 301	1 581
Availability	Dry season	Wet season	Dry and wet season

The amino acid profile is more important in fishmeal replacement studies than the protein content. Methionine, lysine, and cysteine are normally the main limiting amino acids in fish diets. It is noted in this study that in all the insects evaluated, methionine levels were below the requirement of both *O. mossambicus* and *C. gariepinus*. In this study, methionine and lysine were added to the formulated diets. It is thus recommended that crystalline methionine and lysine amino acids be added to diets when any of the evaluated insects are used to substitute fishmeal.

In most studies that used insect meals, chitin has been cited as a major limitation that reduces the digestion of insect meals. One of the limitations of this study was that chitin content was not determined. However, surrogate measures of chitin (ADF and NDF) were determined. The NDF was highest in the Soldier termite meal and the ADF was highest in the Stinkbug meal. The digestibility of both the Soldier termite meal and Stinkbug meal can be enhanced if methods of reducing chitin in the insect meal can be implemented. The methods include introducing chitinase in the formulated diets and introducing microbes that have the potential to break down chitin. Hlophe-Ginindza *et al*. (2015) showed that supplementation of kikuyu-based diets with Natuzyme50® improved the digestibility of the kikuyu diets in *O. mossambicus*. Natuzyme50® is a highly effective multi-enzyme feed additive that contains enzymes such as cellulase, xylanase, and phytase that are not naturally produced by fish. The presence of these enzymes releases bound nutrients that would have not been available to the fish. It is thus recommended that Natuzyme50® be added to diets formulated with the evaluated insects.

One of the main limitations of terrestrial insects is their lack of essential fatty acids such as EPA and DHA. The insect that recorded the highest PUFA value is the Alates termite (3.06 mg/g), Stinkbug meal (1.17 mg/g), and Soldier termite (0.24 mg/g). The fatty acid profile of insects may be improved by manipulating the insect diet through introducing high fatty acid feed. The insect that recorded the highest fat content was the Stinkbug (48.59%) and Alates termite (40.40%), respectively. The Solder termite recorded the lowest (7.29%) fat content. The crude fat requirements of *O. mossambicus* and *C. gariepinus* are estimated to be between 5-6% and 9-12%, respectively (Hecht *et al*.,1988; Jauncey *et al*., 1983). Lipid content higher than 20% has been reported to impair fish immunity (Henry and Fountoulaki, 2014). Thus, there is a need to defatten the Stinkbug meal and Alates termites meal prior to incorporating them into fish diets. Moreover, defatting insect meals has been reported to improve the nutritional composition,

Most studies that evaluated insect meals as fishmeal substitutes focused mainly on the nutritional profile of the insect. The insect availability and pricing have been largely ignored. The Stinkbugs and the Alates termites are harvested seasonally. On the other hand, Soldier termites are harvested throughout the year. These insects are also

available at the insect market at an affordable price. Some of the insect markets that have been operational for over a decade are in Thohoyandou and Giyani Towns. These markets have the potential to meet the demand for different insects during both the rainy season and dry season. Moreover, the insect quality in these markets is of good quality.

The use of insect meals is one of the viable solutions to reduce aquaculture production costs. The current study showed that the inclusion of insect meals in the diets of *O. mossambicus* and *C. gariepinus* may reduce incidence costs while increasing profit margins. This will increase aquaculture production, improve the profit margins and transform the livelihood of fish farmers. Moreover, it is recommended that rural fish farmers work hand in hand with extension officers and aquaculture researchers to formulate their own feed at the farm using locally available ingredients. This can be done using the least-cost feed formulation function (The SOLVER) in Excel. This approach requires a basic knowledge of Excel. The government is also encouraged to support farmers with computers and training through extension officers. This approach may reduce production costs, reduce transportation costs, and save time, thus increasing their aquaculture profitability.

The Mozambique tilapia and the African catfish have different nutritional requirements. It is thus advisable to formulate separate diets for each species to meet their different nutritional need. Moreover, protein and amino acid requirements differ with age and fish size in each species. The larval, juvenile, and adult fish species have different protein and amino acid requirements. It is thus important to keep in mind the size, age, and stage of fish before formulating diets for optimal growth. This study showed that *O. mossambicus* and *C. gariepinus* responded differently to an insect-based diet. This is mainly due to their feeding habits. Generally, herbivorous fish species have shown the efficiency to utilise insect-based diets better than opportunistic predators.

When considering feed formulation, the processing of insect meal, particularly defatting is an additional cost. It is important to incorporate an insect that requires a simple processing method for cost-efficiency. Insects with high fat content require defatting before incorporating into fish diets. The use of full-fat insect meals can be a challenge since high lipid content can interfere with the extrusion process

(Weththasinghe *et al*., 2021), hence reducing the pellet quality. Moreover, High-temperature processing can reduce the nutritional quality of protein feed resources. The Stinkbug and Alates termites recorded higher fat content than Soldier termites. The use of Stinkbug meal and Alates termites in aqua feed may require defatting. It is thus recommended to use Soldier termites, which recorded only 7% fat content and require no defatting.

Each of the evaluated insects has its own limitations. All these insects showed no effect on the health status of *O. mossambicus* and *C. gariepinus*. It is recommended to combine two or more insects in fish diets to improve the nutritional composition and compensate for the missing essential components such as amino acids and fatty acids. Combining dietary proteins in fish diets may help prevent deficiencies caused by using a single protein source (Henry *et al*., 2015). Insects such as *Macrotermes bellicosus* are deficient in amino acids such as methionine, lysine and threonine. This insect may be mixed with an insect rich in these lacking amino acids. Thus, equilibrating the amino acid profile of the diets. Insect meals may also be combined with other dietary protein sources that are available within the geographical area of the farmer.

The European Commission only approved the use of insects in the animal feed industry in 2017. One of the factors that discouraged the use of insect meals is the bioaccumulation of metals, especially in terrestrial insects. Greenfield *et al*., (2014) reported the bioaccumulation of metals in Mopane worms harvested from Mopane trees located in an area contaminated with heavy metals. There is no study that assessed the bioaccumulation of heavy metals in Stinkbugs. The Stinkbug mainly feeds on *Dodonaea viscosa*, *Combretum imberbe*, *Combretum molle*, and *Peltophorum africanum* (Dzerefos *et al*., 2009). It is thus recommended to evaluate heavy metal concentrations in insects prior to incorporating them in aqua feed. Another factor that delayed the approval of insect meals in animal feed is the outbreak of Bovine Spongiform Encephalopathy (BSE), which is also known as “mad cow disease” (Renna *et al*., 2022). It is thus advisable to assess the bacterial and viral load in insects prior to use in aqua feed. Insects evaluated in this study, none of them are vectors of any diseases. Insects such as *Musca domestica* are mechanical vectors of

transmission of pathogens including parasites, bacteria, fungi, and viruses (Issa, 2019).

Consumer acceptability is one of the factors to consider when incorporating insects in aqua feed. Fish-fed diets incorporated with *Musca domestica* may raise concerns regarding consumer acceptability. However, insects evaluated in this study are known for human consumption. It is speculated that fish-fed diets incorporated with these insects will be well accepted by consumers. Thus, there is a need to carry out a consumer survey on insect acceptability before incorporating into aqua feed.

CONCLUSION

This study showed the potential for locally available insect meals to substitute fishmeal in the diets of two commonly cultured warm freshwater species. The Stinkbug meal replaced 30% fishmeal in both *O. mossambicus* and *C. gariepinus*. The Alates termite meal could replace 50% of fishmeal in both *O. mossambicus* and *C. gariepinus*. The Soldier termite meal replaced 50% of fishmeal in *O. mossambicus*. However, it could only replace up to 10% in *C. gariepinus*. The Alates termite meal is recommended for inclusion in fish diets at the farm level. The liver and intestinal histology of fish species were not influenced by the inclusion of insect meals. It was noted that *O. mossambicus* and *C. gariepinus* responded differently to an insect-based diet. Herbivorous *O. mossambicus* showed a greater potential to efficiently utilise insect-based diets than an opportunistic predator (*C. gariepinus*). The cost-benefit analysis showed that the incorporation of these insects in aqua feed is economically viable.

RECOMMENDATIONS

It is recommended that Stinkbug meal be defatten prior use in aqua feed. The Natuzyme50® is recommended to be used to enhance digestibility in Alates termite meal and Soldier termite meal. It also recommended that future studies be undertaken to assess the chitin level of the Alates termite meal, Soldier termite meal, and the Stinkbug meal. A more comprehensive study should be undertaken to accurately access the availability and cost of the Stinkbug, Alates termite, and Soldier termite insects. It is also recommended to combine different insect meals to enhance the growth of the fish species.

REFERENCES

CHAPTER 7: REFERENCES

Abdel-Tawwab, M., Khalil, R.H., Metwally, A.A., Shakweer, M.S., Khallaf, M.A. and Abdel-Latif, H.M.R. 2020. Effects of black soldier fly (*Hermetia illucens* L.) larvae meal on growth performance, organs-somatic indices, body composition, and hematobiochemical variables of European sea bass, *Dicentrarchus labrax*. *Aquaculture*. 522, Article ID: 735136.

Abdel-Warith, A.A., Younis, E.M., Al-Asgah, N.A. and Mahboob, S. 2020. Effect of replacing fish meal by full fat soybean meal on growth performance, feed utilization and gastrointestinal enzymes in diets for African catfish *C. gariepinus*. *Brazilian Journal of Biology*. 80(**3**): 535-543.

Achionye-Nzeh, C.G. and Ngwudo, O.S. 2003. Growth response of *C. anguillaris* fingerlings fed larvae of *Musca domestica* and soyabean diet in the laboratory. *Bioscience Biotechnology Research Communications*. 15:221-223

Adeleke, B., Robertson-Andersson, D., Moodley, G. and Taylor, S. 2021. Aquaculture in Africa: A comparative review of Egypt, Nigeria, and Uganda Vis-Á-Vis South Africa. *Reviews in Fisheries Sciences and Aquaculture*. 29(**2**):167-197.

Adeoye, A.A., Akegbejo-Samsons, Y., Fawole, F.J. and Davies, S.J. 2019. Preliminary assessment of black soldier fly (*Hermetia illucens*) larval meal in the diet of African catfish (*C. gariepinus*): Impact on growth, body index, and hematological parameters. *Journal of World Aquaculture Society*.1-10.

Adepoju, O.T. and Omotayo, O.A. 2014. Nutrient Composition and Potential Contribution of Winged Termites (*Marcrotermes bellicosus* Smeathman) to Micronutrient Intake of Consumers in Nigeria. *British Journal of Applied Science & Technology*. 4(**7**): 1149-1158.

Adewolu, M.A., Ikenweiwe, N.B. and Mulero, S.M. 2010. Evaluation of an Animal Protein Mixture as a Replacement for Fishmeal in Practical Diets for Fingerlings of *C. gariepinus* (Burchell, 1822). *The Israeli Journal of Aquaculture- Bamidgeh*. 62(**4**): 237-244.

Adewumi, A.A., Idowu, E.O., Edward, J.B., Iwalaye, O.A., Fadiya, O.O. and Obafemi, B.D. 2019. Some physiological responses of *C. gariepinus* fed graded levels of *Cirina forda* larvae based diets. Food Science and Nutrition Studies. 3(**4**).

Adewumi, A.A., Idowu, O.E. and Bamisile, S.T. (2014). Food and feeding habits of *C. gariepinus* (BURCHELL 1822) in Egbe Reservoir, Ekiti State, Nigeria. Animal Research International. 11(**3**):2041-2047.

Adeyemi, S.O. 2009. Food and feeding habits of some commercially important fish species in Gbedikere Lake, Bassa, Kogi State, Nigeria. *International Journal of Lake & River*. 2:31-36.

Admassu, D., Abera, L. and Tadesse, Z. 2015. The food and feeding habits of the African catfish, *C. gariepinus* (BURCHELL 1822), in Lake Babogaya, Ethiopia, *Global Journal of Fisheries and Agriculture*. 3(**4**):211-220.

Agbo, N.W., Madalla, N. and Jauncey, K. 2011. Effects of dietary cottonseed meal protein levels on growth and feed utilization of Nile tilapia, *O. niloticus* L. *Journal of Applied Sciences and Environmental Management*. 15(**2**): 235-239.

Akinwole, A.O., Dauda, A.B. and Ogunkunle, V.O. 2020. Growth performance, nutrient utilization, survival and body indices of African catfish (*C. gariepinus*, Burchell 1822) reared on maggot meal based diet. *FUDMA Journal of Sciences*. 4 (**2**), 53-59.

Akullo, J., Agea, J.G., Obaa, B.B., Okwee-Acai, J. and Nakimbugwe, D. 2018. Nutrient composition of commonly consumed edible insects in the Lango sub-region of northern Uganda. *International Food Research Journal*. 25(**1**): 159 -165.

Aldrich, J.R. 1988. Chemical ecology of the Heteroptera. *Annual Review of Entomology*. 33:211-238.

Alegbeleye, W.O., Obasa, S.O., Olude, O.O., Otubu, K. and Jimoh, W. 2012. Preliminary evaluation of the nutritive value of the variegated grasshopper (*Zonocerus variegatus* L.) for African catfish *C. gariepinus* (Burchell, 1822) fingerlings. *Aquaculture Research*. 43:412-420.

Alfiko, Y., Xie, D., Astuti, R.T., Wong, J. and Wang, L. 2022. Insects as a feed ingredient for fish culture: Status and trends. *Aquaculture and Fisheries*. 7 (**2**):166-178.

Aniebo, A.O., Erondu, E.S. and Owen, O.J. 2009. Replacement of fish meal with maggot meal in African catfish (*C. gariepinus*) diets. *Revista Científica UDO Agrícola*. 9(**3**): 666-671.

Anvo, M.P.M., Aboua, B.R.D., Compaoré, I., Siaaao, R., Zoungrana-Kaboré, C.Y., Kouamelan, E.P. and Toguyéni, A. 2017. Fish meal replacement by *Cirina butyrospermi* caterpillar's meal in practical diets for *C. gariepinus* fingerlings. *Aquaculture Research*. 48 (**10**): 5243-5250.

Arru, B., Furesi, R., Gasco, L., Madau, F.A. and Pulina, P. 2019. The Introduction of Insect Meal into Fish Diet: The First Economic Analysis on European Sea Bass Farming. *Sustainability*.11, 1697.

Askarian, F., Zhou, Z., Olsen, R.E., Sperstad, S. and Ringø, E. 2012. Culturable autochthonous gut bacteria in Atlantic salmon (*Salmo salar* L.) fed diets with or without chitin. Characterization by 16S rRNA gene sequencing, ability to produce enzymes and in vitro growth inhibition of four fish pathogens. *Aquaculture*. 1(**8**):326-329.

Association of Official Analytical Chemists (AOAC). 2003. Official Methods of Analysis (17th ed). Gaithersburg.

Athey, K.J., Ruberson, J.R., Olson, D.M. and Harwood, J.D. 2019. Predation on stink bugs (Hemiptera: Pentatomidae) in cotton and soybean agroecosystems. *PLoS ONE*. 14(**3**): 02114325.

Azri, N.A.M., Chun, L.K., Hasan, H.A., Jaya-Ram, A., Kari, Z.A. and Hamid, N.K.A. 2022. The effects of partial replacement of fishmeal with hermetia meal on the growth and fatty acid profile of African catfish fry. *Agriculture Reports*. 1(**1**):17-27.

Baeza-Ariño, R., Martĩnez-Llorens, S., Nogales-Merida, S., Jover-Cerda, M. and Tomas-Vidal A., 2014. Study of liver and gut alterations in sea bream, *Sparus aurata* L., fed a mixture of vegetable protein concentrate. *Aquaculture Research*. 47, 460-471.

Bagenal T.B. and Tesch F.W. (1978) 3rd ed. Oxford Blackwell Scientific publications; London: 1978. Methods of Assessment of Fish Production in Fresh Waters. IBP Handbook No. 3; pp. 101-136.

Bahnasawy, M.H., Abdel-Baky, T.E. and Abd-Allah, G.A. 2003. Growth performance of Nile tilapia (*O. niloticus*) fingerlings raised in an earthen pond. *Archives of Polish Fisheries*. 11: 277-285.

Balarin, J.D. and Hatton, J.P. 1979. Tilapia: A guide to their Biology and Culture in Africa. University of Stirling. pp174.

Banjo, A.D., Lawal, O.A. and Songonuga, E.A. 2006. The nutritional value of fourteen species of edible insects in southwestern Nigeria. *African Journal of Biotechnology*. 5 :298-301.

Barlow, S.M. 2003. FISHMEAL. In Encyclopedia of Food Sciences and Nutrition (Second Edition), International Association of Fishmeal and Oil Manufacturers, St Albans, Hertfordshire, UK.

Barragan-Fonseca, K.B., Dicke, M. and van Loon, J.J.A. 2017. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed - a review. *Journal of Insects as Food and Feed*. 3(**2**): 105-120.

Barroso, F.G., de Haro, C., Sánchez-Muros, M.-J., Venegas, E., Martìnez-Sanchez, A. and Pérez-Bañon, C. 2014. The potential of various insect species for use as food for fish. *Aquaculture*. 422:193-201.

Barroso, F.G., Sánchez-Muros, M.J., Rincón, M.A., Rodriguez-Rodriguez, M., Fabrikov, D., Morote, E. and Guil-Guerrero, J.L. 2019. Production of n-3-rich insects by bioaccumulation of fishery waste. *Journal of Food Composition and Analysis*. 82:103237.

Basto, A., Matos, E.L. and Valente, M.P. 2020. Nutritional value of different insect larvae meals as protein sources for European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture*. 521.

Belghit, I., Liland, N.S., Gjesdal, P., Biancarosa, I., Menchetti, E., Li, Y., Waagobo, R. and Krogdahl, E.L. 2018. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). *Aquaculture*. 503:609-619.

Bernfeld, P. 2006. Enzymes of starch degradation and synthesis. In: Nord, F.F. (Ed.), Advances in Enzymology and Related Areas of Molecular Biology, Vol. 12. John Wiley & Sons, Inc, Hoboken, pp379-424.

Bezerra, R.S., Lins, E.J.F., Alencar, R.B., Paiva, P.M.G., Chaves, M.E.C., Luana, C.B.B. and Carvalho Jr., L.B. 2005. Alkaline proteinase from intestines of Nile tilapia (*O. niloticus*). *Process Biochemistry*. 40: 1829-1834.

Biasato, I., Chemello, G., Oddon, S.B., Ferrocino, I., Corvaglia, M.R., Caimi, C., Resconi, A., Paul, A., van Spankeren, M., Capucchio, M.T., Colombino, E., Cocolin, L., Gai, F., Schiavone, A. and Gasco, L. 2022. *Hermetia illucens* meal inclusion in low-fishmeal diets for rainbow trout (*Oncorhynchus mykiss*): Effects on the growth performance, nutrient digestibility coefficients, selected gut health traits, and health status indices. *Animal Feed Science and Technology*. 290: 115341.

Boafo, H.A., Affedzie-Obresi, S., Gbemavo, D.S.J.C., Clottey, V.A., Nkegbe, E., Adu-Aboagye, G. and Kenis, M. 2019. Use of termites by farmers as poultry feed in Ghana. *Insects*. 10:69.

Bosi, A., Banfi, D., Moroni, F., Ceccotti, C., Giron, M.C., Antonini, M., Giaroni, C. and Terova, G. 2021. Effect of partial substitution of fishmeal with insect meal (*Hermetia illucens*) on gut neuromuscular function in Gilthead sea bream (*Sparus aurata*). *Scientific Reports*. 11, Article ID: 21788.

Britz, P.J. and Venter, S. 2016. Aquaculture review: South Africa. *World Aquaculture*.19-27.

Bruton, M.N. 1979. The food and feeding behaviour of *C. gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with emphasis on its role as a predator of cichlids. *The Transactions of the Zoological Society of London*. 35 (**1**): 47-114.

Caimi, C., Gasco, L., Biasato, I., Malfatto, V., Varello, K., Prearo, M., Pastorino, P., Bona, M.C., Francese, D.R., Schiavone, A., Elia, A.C., Dőrr, A.J.M. and Gai, F. 2020. Could Dietary Black Soldier Fly Meal Inclusion Affect the Liver and Intestinal Histological Traits and the oxidative stress biomarkers of Siberian Sturgeon (*Acipenser baerii*) juveniles? *Animals*.10 (**1**):155.

Carr, I., Glencross, B. and Santigosa, E. 2023. The importance of essential fatty acids and their ratios in aquafeeds to enhance salmonid production, welfare, and human health. *Frontiers in Animal Science*. 4, Article ID:1147081.

Chemello, G., Renna, M., Caimi, C., Guerreiro, I., Oliva-Teles, A., Enes, P., Biasato, I., Schiavone, A., Gai, F. and Gasco, L. 2020. Partially Defatted Tenebrio molitor Larva Meal in Diets for Grow-Out Rainbow Trout, *Oncorhynchus mykiss* (Walbaum): Effects on Growth Performance, Diet Digestibility and Metabolic Responses. *Animals*. 10, 299.

Cho, C.Y., Slinger, S.J. and Bayley, H.S. 1982. Bioenergetics of salmonid fishes Energy intake, expenditure, and productivity. *Comparative Biochemistry and Physiology*. 73 (**1**): 25-41.

Chulu, M.C. 2015. Nutrient composition of the termite *Macrotermes Falciger*, collected from Lusaka district, a potential agent against malnutrition. Msc. Dissertation. The University of Zambia. Lusaka, Zambia. 25pp.

Coutinho, F., Castro, C., Guerreiri, I., Rangel, F., Couto, A., Serra, C.R., Peres, H., Pousáo-Ferreira, Rawski, Oliva-Teles, A. and Enes, P. 2021. Mealworm larvae meal in diets for meagre juveniles: Growth, nutrient digestibility and digestive enzymes activity. *Aquaculture*. 535, Article ID: 736362.

Cullen, D.A., Cease, A.J., Latchininsky, A.V., Ayali, A., Berry, K., Buhl, J., De Keyser, R, Foquet, B., Hadrich, J.C., Matheson, T., Ott, S.R, M.A. and Poot-Pech, B.E.

et al. 2017. In: From Molecules to Management: Mechanisms and Consequences of Locust Phase Polyphenism. *Advances in Insect Physiology*. 53:167-285.

Dadebo, E., Aemro, D. and Tekle-Giorgis, Y. 2014. Food and feeding habits of the African catfish *C. gariepinus* (Burchell, 1822) (Pisces: Clariidae) in Lake Koka, Ethiopia. *African Journal of Ecology*. 52:471-478.

DAFF (Department of Agriculture, Forestry and Fisheries). 2010. National Aquaculture Sector Overview. South Africa. National Aquaculture Sector Overview Fact Sheets. Text by Halley K, Semoli B. In: FAO Fisheries and Aquaculture Department, Rome: FAO.

DAFF (Department of Agriculture, Forestry and Fisheries). 2015. Aquaculture Yearbook 2014, South Africa.

DAFF (Department of Agriculture, Forestry and Fisheries). 2016. Aquaculture yearbook 2016, South Africa.

DAFF (Department of Agriculture, Forestry and Fisheries). 2017. Aquaculture Yearbook 2016 South Africa. Department of Agriculture, Forestry and Fisheries.

DAFF (Department of Agriculture, Forestry and Fisheries). 2019. Aquaculture yearbook. Department of Agriculture, Forestry and Fisheries, South Africa, pp 214.

DAFF (Department of Agriculture, Forestry and Fisheries). 2021. National Freshwater (inland) wild capture Fisheries policy for South Africa. Government Gazette. No. 45954 (No. 1790)

Dandadzi, M., Musundire, R., Muriithu, A. and Ngadze, R.T. 2023. Effects of drying on the nutritional, sensory and microbiological quality of edible stinkbug (*Encosternum delegorguei*). *Heliyon*. 9(**8**), Article ID: e18642.

Daniel, N. 2018. A review on replacing fish meal in aqua feeds using plant protein sources. *International Journal of Fisheries and Aquatic Studies*. 6(**2**): 164-179.

Dao, A. N.C., Sankara, F., Pousga, S., Coulibaly, K., Nacoulma, J.P., Somda, I. and Kenis, M. 2022. Sustainable Use of Macrotermes spp. to Improve Traditional Poultry Farming through an Efficient Trapping System in Burkina Faso. *Insects*. 13(**1**):62.

Dao, A.N.C., Sankara, F., Pousga, S., Coulibaly, K., Nacoulma, J.P., Ouedraogo, S., Kenis, M. and Somda, I. 2020. Traditional methods of harvesting termites used as poultry feed in Burkina Faso. *International Journal of Tropical Insect Science*. 40:109-118.

Das, M. and Mandal, S.K. 2014. *Oxya hyla hyla* (Orthoptera: Acrididae) as an Alternative Protein Source for Japanese Quail. *International Scholarly Research Notices*. Article ID 269810.

De Figueirédo, R.E.C.R., Vasconcellos, A., Policarpo, I.S. and Alves, R.R.N. 2015. Edible and medicinal termites: A global overview. *Journal of Ethnobiology and Ethnomedicine*. 11, 29.

de Silva, S.S. and Anderson, T.A. 1995. Fish Nutrition in Aquaculture. Chapter and Hall, London. Spinger Science & Business Media. pp320.

DeFoliart, G.R. 1999. Insects as food: why western attitude is important. Annual Review of Entomology. 44,21-50.

Devic, E., Leschen, W., Murray, F. and Little, D.C. 2017. Growth performance, feed utilization and body composition of advanced nursing Nile tilapia (*O. niloticus*) fed diets containing black soldier fly (*Hermetia illucens*) larvae meal. *Aquaculture Nutrition*. 24(**1**):416-423.

Diener, S., Zurbrugg, C. and Tockner, K. 2009. Conversion of organic material by black soldier fly larvae: Establishing optimal feeding rates. *Waste management Research*. 27: 603-610.

DiGiacomo, K. and Leury, B.J. 2019. Review: Insect meal: A future source of protein feed for pigs? *Animal*.13(**12**): 3022-3030.

Djissou, A.S.M., Adjahouinou, D.C., Koshio, S. and Fiogbe, E.D. 2016. Complete replacement of fish meal by other animal protein sources on growth performance of *C. gariepinus* fingerlings. *International Aquatic research.* 8:333-341.

Doupé, R.G., Knott, M.J., Schaffer, J. and Burrows, D.W. 2009. Investigational piscivory of some juvenile Australian freshwater fishes by the introduced Mozambique tilapia *O. mossambicus*. *Journal of fish biology*. 74(**10**):2386-2400.

Dube, S. and Dube, C. 2010. Towards improved utilisation of macimbi *Imbrasia belina* Linnaeus, 1758 as food and financial resource for people in the Gwanda district of Zimbabwe. *Journal of Science and Technology*. 5: 28-36.

Dzerefos, C.M, Witkowski, E.T.F. and Toms, R. 2009. Life-history traits of the edible stinkbug, *Encosternum delegorguei* (Hem., Tessaratomidae), a traditional food in southern Africa. *Journal of Applied Entomology*. 133:749-759.

Dzerefos, C.M., Witkowski, E.T.F. and Toms, R. 2013. Comparative ethnoentomology of edible stinkbugs in southern Africa and sustainable management considerations. *Journal of ethnobiology and ethnomedicine*. 9:20.

Ebenso, I.E. and Udo, M.T. 2003. Effect of live maggot on growth of the nile perch, *O. niloticus* (Cichlidae) in South Eastern Nigeria. *Global Journal of Agricultural Science*. 2(**2**):72-73.

Egan, B., Nethavhani, Z. and van Asch, B. 2021. Overview of the Genetic Diversity of African *Macrotermes* (Termitidae: Macrotermitinae) and Implications for Taxonomy, Ecology and Food Science. *Insects*. 12(**6**):518.

Egonyu, J.P., Subramanian, S., Tanga, C.M., Dubois, T., Ekesi, S. and Kelemu S. 2021. Global overview of locusts as food, feed and other uses. *Global Food Security*. 31, Article ID:100574.

Ekpo, K.E. and Onigbinde, A.O. 2007. Characterization of Lipids in winged reproductive of the termite Macrotermes bellicosus. *Pakistan Journal of Nutrition*.6(**3**):247-251.

Elia, A.C., Capucchio, M.T., Caldaroni, B., Magara, G., Dörr, A.J.M., Biasato, I., Biasibetti, E., Righetti, M., Pastorino, P., Prearo, M., Gai, F., Schiavone, A. and Gasco, L. 2018. Influence of *Hermetia illucens* meal dietary inclusion on the histological traits, gut mucin composition and the oxidative stress biomarkers in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 496:50-57.

El-Sayed, A.F.M. 2020. Nutrition and feeding. In: Tilapia culture. Academic Press, (2nd ed.).pp135-163.

El-Sayed, A.F.M. and Garling Jr., D.L.1988. Carbohydrate-to-lipid ratio in diets for *Tilapia zillii* fingerlings. *Aquaculture*. 73: 157-163.

English, G., Wanger, G. and Colombo, S.M. 2021. A review of advancements in black soldier fly (*Hermetia illucens*) production for dietary inclusion in salmonid feeds. *Journal of Agriculture and Food Research*. 5, Article ID:100164. Commission Regulation

European Union (EU) .2017. European Commission Regulation 2017/893 of 24 May 2017 Amending Annexes I and IV to Regulation (EC) No. 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No. 142/2011 as Regards the Provisions on Processed Animal Protein; European Union: Luxemburg.

European Union (EU) .2021. European Commission Regulation 2021/1372 of 17 August 2021 Amending Annex IV to Regulation (EC) No. 999/2001 of the European Parliament and of the Council as Regards the Prohibition to Feed Non-Ruminant Farmed Animals, Other Than Fur Animals, with Protein Derived from Animals; European Union: Luxemburg.

Evangelista, D.A., Wipfler, B., Béthoux, O., Donath, A., Fujita, M., Kohli, M.K., Legendre, F., Liu, S., Machida, R., et al., 2019. An integrative phylogenomic approach illuminates the evolutionary history of cockroaches and termites

(Blattodea). Proceedings of the Royal Society B: *Biological Sciences*. 286(1895), Article ID:20182076.

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Samples, S. and Lalander, C. 2020. Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) - Possibilities and limitations for modification through diet. *Waste management*. 102: 40-47.

Ezewudo, B.I., Monebi, C.O. and Ugwumba, A.A.A. 2015. Production and utilization of *Musca domestica* maggots in the diet of *O. niloticus* (Linnaeus, 1758) fingerlings. *African Journal of Agricultural Research*, 10(**23**): 2363-2371.

FAO (Food and Agriculture Organization) .1996. Artificial Reproduction and Pond Rearing of the African Catfish *C. gariepinus* in Sub-Saharan Africa - A Handbook. Rome, Italy. 362, pp142.

FAO (Food and Agriculture Organization). 2010. National Aquaculture Sector Overview. South Africa. National Aquaculture Sector Overview Fact Sheets. Text by Halley K, Semoli B. In: FAO Fisheries and Aquaculture Department. Rome: Italy.

FAO (Food and Agriculture Organization). 2013. Food and agriculture organization of the united nations, Edible insects, Future prospects for food and feed security. ISSN 0258-6150. Rome. Italy.

FAO (Food and Agriculture Organization). 2016. Aquaculture Big Numbers. Fisheries and Aquaculture Technical Paper No. 601. Rome, Italy.

Fasakin, E.A., Balogun, A.M. and Ajayi, O.O. 2003. Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish *C. gariepinus* fingerlings. *Aquaculture Research*. 34:733-738.

Fawole, F.J., Adeoye, A.A., Tiamiyu, L.O., Ajala, K.I., Obadara, S.O. and Ganiyu, I.O. 2020. Substituting fishmeal with *Hermetia illucens* in the diets of African catfish (*C. gariepinus*): Effects on growth, nutrient utilization, haematophysiological response, and oxidative stress biomarker. *Aquaculture.* 518, Article ID: 734849.

Feng, Y., Chen, X.M., Zhao, M., He, Z., Sun, L., Wang, C.Y. and Ding, W.F. 2018. Edible insects in China: Utilization and prospects. *Insect Science*. 25: 184-198.

Finke, M.D. 2007. Estimate of chitin in raw whole insects. *Zoo Biology*. 26: 105-115.

Finke, M.D. and Oonincx, D.G.A.B. 2014. Insects as food for insectivores. In: Mass Production of Beneficial Organisms, pp583-616.

Fischer, H., Romano, N., Renukdas, N., Kumar,V and Sinha, A.K. 2022. Comparing black soldier fly (*Hermetia illucens*) larvae versus prepupae in the diets of largemouth bass, *Micropterus salmoides*: Effects on their growth, biochemical composition, histopathology, and gene expression. *Aquaculture*. 546, Article ID: 737323.

Fisher, H.J., Collins, S.A., Hanson, C., Colombo, S.M. and Anderson, D.M. 2020. Black soldier fly larvae meal as a protein source in low fish meal diets for Atlantic salmon (*Salmo salar*). *Aquaculture*. 521, Article ID:734978.

Folch, J., Lees, M. and Stanley, G.H.S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry.* 226 (**1**): 497-509.

Fombong, F.T. and Kinyuru, J.N. 2018. Termites as food in Africa. In termites and sustainable management, sustainability in plant and crop protection. Springer International Publishing. 1: pp217-240.

Gao, Y., Xu, J., Xu, M., Wang, Z., Yang, J. and Shi, S. 2022. Evaluation of soybean stinkbug (*Riptortus pedestris*) powder, as a food ingredient and its nutritional composition. *Czech Journal of Food Sciences*. 40(1): 51-60.

Gasco, L., Henry, M., Piccolo, G., Marono,S., Gai, F., Renna, M., Lussiana, C., Antonopoulou, E., Mola, P. and Chatzifotis. 2016. *Tenebrio molitor meal* in diets for European sea bass (*Dicentrarchus labrax* L.) juveniles: Growth performance, whole body composition and in vivo apparent digestibility. *Animal Feed Science and Technology*. 220:34-45.

Gasco., L., Józefiak, A. and Henry, M. 2021. Beyond the protein concept: Health aspects of using edible insects on animals. *Journal of Insects as food and feed*. 7: 715-741.

Gbai, M., Ouattara, N., Bamba, Y., Ouattara, M., Ouattara, A. and Yao, K. 2019. Substitution of the fishmeal by maggot meal in the feed of Nile Tilapia *O. niloticus* at different stages of growth. *International Journal of Fisheries and Aquaculture Research*. 5 (**4**): 25-39.

Ghosh, S. and Mandal, D.K. 2019. Nutritional evaluation of a short-horned grasshopper, *Oxya hyla hyla* (Serville) meal as a substitute of fishmeal in the compound diets of rohu, *Labeo rohita* (Hamilton). *The Journal of Basic and Applied Zoology*. 80(**28**):1-8.

Glew, R.H., Jackson, D., Sena, L., VanderJagt, D.J., Pastuszyn, A. and Millson, M. 1999. *Gonimbrasia belina* (Lepidoptera: Saturniidae): a nutritional food source rich in protein, fatty acids and minerals. *American Entomologist*. 45: 250-253.

Greenfield, R., Akala, N. and van der Bank, F.H. 2014. Heavy metal concentrations in two populations of Mopane worms (*Imbrasia belina*) in the Kruger National Park pose a potential human health risk. *Bulletin of environmental contamination and toxicology*. 93(**3**): 316-321.

Gu, J., Liang, H., Ge, X., Xia, D., Pan, L., Mi, H. and Ren, M. 2022. A study of the potential effect of yellow mealworm (*Tenebrio molitor*) substitution for fish meal on growth, immune and antioxidant capacity in juvenile largemouth bass (*Micropterus salmoides*). *Fish and Shellfish Immunology*. *120*: 214-221.

Guerreiro, I., Serra, C.R., Coutinho, F., Couto, A., Castro, C., Rangel, F., Pres, H., Pousão-Ferreira, P., Matos, E., Gasco, L., Gai, F., Olivia-Teles, A. and Enes, P. 2020. Digestive enzyme activity and nutrient digestibility in meagre (*Argyrosomus regius*) fed increasing levels of black soldier fly meal (Hermetia illucens). *Aquaculture Nutrition*. 27(**1**):142-152.

Hameed, A., Majeed, W., Naveed, M., Ramzan, U., Bordiga, M., Hameed, M., Rehman, S.U. and Rana, N. 2022. Success of Aquaculture Industry with New

Insights of Using Insects as Feed: A Review. *Fish Nutrition and Feed Technology*. 7(**6**), 395.

Handley, M.A., Hall, C., Sanford, E., Diaz, E., Gonzalez-Mendez, E., Drace, K., Wilson, R., Villalobos, M. and Croughan, M. 2007. Globalization, binational communities, and imported food risks: Results of an outbreak investigation of lead poisoning in Monterey County, California. *American Journal of Public Health*. 97: 900-906

Hecht T., W. Uys, and P. J. Britz. 1988. The culture of sharptooth catfish in southern Africa. South African National Scientific Programmes Report No.153.

Hecht, T. and Britz, P.J. 1990. Effects of salinity on growth and survival of African sharptooth catfish (*C. gariepinus*) larvae. *Journal of Applied Ichthyology*. 5: 194-202.

Henry, M. and Fountoulaki. M. 2014.Optimal dietary protein/ lipid ratio for improved immune status of a newly cultivated Mediterranean fish species, the shi drum *Umbrina cirrosa*, L. *Fish and Shellfish Immunology*. 37(**2**) 215-219.

Henry, M., Gasco, L., Piccolo, G. and Fountoulaki, E. 2015. Review on the use of insects in the diet of farmed fish: past and future. *Animal Feed Science and Technology*. 203:1-22.

Henry, M.A., Golomazou, E., Asimaki, A., Psofakis, P., Fountoulaki, E., Mente, E., Rumbos, C.I., Athanassiou, C.G. and Karapanagiotidis, I.T. 2022. Partial dietary fishmeal replacement with full-fat or defatted superworm (*Zophobas morio*) larvae meals modulates the innate immune system of gilthead seabream, *Sparus aurata*. *Aquaculture Reports*. 27, Article ID: 101347.

Hidalgo, M.C., Urea, E. and Sanz, A. 1999. Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture*. 170(**3-4**): 267-283.

Hlongwane, Z.T., Siwela, M., Slotow, R. and Munyai, T.C. 2022. Effect of geographical location, insect type and cooking method on the nutritional composition of

insects consumed in South Africa. *Journal of Insects as Food and Feed*. 8(**5**): 537-556.

Hlophe, S.N. and Moyo, N.A.G. 2014. A comparative study on the use of *Pennisetum clandestinum* and *Moringa oleifera* as protein sources in the diet of the herbivorous Tilapia rendalli. *Aquaculture international*. 22:1245-1262.

Hlophe, S.N., Moyo, N.A.G. and Ncube, I. 2014. Postprandial changes in pH and enzyme activity from the stomach and intestines of *Tilapia rendalli* (Boulenger, 1897), *O. mossambicus* (Peters, 1852) and *C. gariepinus* (Burchell, 1822). *Journal of Applied Ichthyology*. 30:35-41.

Hlophe, S.N., Moyo, N.A.G. and Sara, J.R. 2011. Use of Kikuyu grass as a fishmeal substitute in practical diets for *Tilapia rendalli*. *Asian Journal of Animal and Veterinary Advances*. 6(**11**): 1079-1083.

Hlophe-Ginindza, S.N., Moyo, N.A.G. and Ngambi, J.W. 2015. The effect of exogenous enzyme supplementation on growth and digestive enzyme activities in *O. mossambicus* fed kikuyu-based diets. *Aquaculture Research.* 47; 3777-3787.

Hong, J. and Kim, YY. 2022. Insect as feed ingredients for pigs. *Animal Bioscience.* 35(**2**): 347-355.

Houlihan, D., Bouiard, T. and Jobling, M. 2001. Food Intake in Fish. USA: Iowa State University Press. Blackwell Science Ltd. pp418.

Hu, Y., Huang, Y., Tang, T., Zhong, L., Chu, W., Dai, Z., Chen, K., Hu, Y., 2020. Effect of partial black soldier fly (*Hermetia illucens* L.) larvae meal replacement of fish meal in practical diets on the growth, digestive enzyme and related gene expression for rice field eel (*Monopterus albus*). *Aquaculture Reports*. 17, Article ID: 100345.

Hua., K. 2021. A meta-analysis of the effects of replacing fish meals with insect meals on growth performance of fish. *Aquaculture*. 530, Article ID: 735732.

Idowu, A.B., Amusan, A.A.S. and Oyediran, A.G. 2003. The response of *C. gariepinus* fingerlings (Burchell 1822) to the diet containing housefly maggot (*Musca domestica*). *Nigerian Journal of Animal Production*, 30(1),

Idowu, E., Adewumi, A., Ose, J., Edward, J. and Obaronbi, G. 2017. Effects of Varying Levels of *Moringa oleifera* on Growth Performance and Nutrient Utilization of *C. gariepinus* Post-Fingerlings. *American Scientific Research Journal for Engineering, Technology, and Sciences*. 32 (**1**): 79-95.

Idowu, E.O. and Afolayan, E.B. 2013. The Effects of Supplementing of Fish Meal With Maggots At Varying Levels in the Diet Of *C. gariepinus. International Archive of Applied Science and Technology*. 4(**4**):41-47.

Igwe, C.U., Ujowundu, C.O., Nwaogu, L.A. and Okwu, G.N. 2011. Chemical Analysis of an Edible African Termite, *Macrotermes nigeriensis*; a Potential Antidote to Food Security Problem. *Biochemistry and Analytical biochemistry.*1 (1), Article ID:1000105.

Inje, O.F., Olufunmilayo, A.H., Audu, J.A., Ndaman, S.A. and Chidi, E.E. 2018. Protein quality of four indigenous edible insect species in Nigeria. *Food Science and Human Wellness*. *7*(**2**): 175-183.

Ishida, Y., Fujita, T. and Asai, K., 1981. New detection and separation method for amino acids by high-performance liquid chromatography. *Journal of Chromatography*. 204:143-148.

Issa, R. 2019. *Musca domestica* acts as transport vector hosts. *Bulletin of the National Research Centre*. 43 (**73**).

Jabir, A.R., Razak, S.A. and Vikineswary, S. 2012. Nutritive potential and utilization of super worm (*Zophobas morio*) meal in the diet of Nile tilapia (*O. niloticus*) juvenile. *African Journal of Biotechnology*. 11 (**24**): 6592-6598.

Jauncey, K. Tacon, A. G. J. and Jackson, J. 1983. The quantitative essential amino acids of *O.* (Saretherodon) *mossambicus*. In: International Sysposium on

Tilapia in Aquaculture, L. Fishelson and Z. Yaron, Eds., Tel Aviv University, Israel. pp328-337.

Jeong, S., Khosravi, S., Mauliasari, I.R. and Lee, S. 2020. Dietary inclusion of mealworm (*Tenebrio molitor*) meal as an alternative protein source in practical diets for rainbow trout (*Oncorhynchus mykiss*) fry. *Fisheries and Aquatic Sciences*. 23:12.

Kadye, W.T. and Booth, A. 2012. Detecting impacts of invasive non-native sharptooth catfish, *C. gariepinus*, within invaded and non-invaded rivers. *Biodiversity Conservation*. 21:1997-2015.

Kari, Z.A., Kabir, M.A., Mat, K., Rusli, N.D., Razab, M.K.A.A., Ariff, N.S.N.A., Edinur, H.A., Rahim, M.Z.A., Pati, S., Dawood, M.A.O. and Wei, L.S. 2021. The possibility of replacing fish meal with fermented soy pulp on the growth performance, blood biochemistry, liver, and intestinal morphology of African catfish (*C. gariepinus*). *Aquaculture Reports*. 21, Article ID:100815.

Kenis, M., Koné, N., Chrysostome, C.A.A.M., Devic, E., Koko, G.K.D., Clottey, V.A., Nacambo, S. and Mensah, G.A. 2014. Insects used for animal feed in West Africa. *Entomologia*. 2:218.

Khieokhajonkhet, A., Uanlam, P., Ruttarattanamongkol, K., Aeksiri,N., Tatsapong, P. and Kaneko, G. 2022. Replacement of fish meal by black soldier fly larvae meal in diet for goldfish *Carassius auratus*: Growth performance, hematology, histology, total carotenoids, and coloration. *Aquaculture*. 561, Article ID:738618.

Kim, Y.S. and Ho, S.B. 2010. Intestinal goblet cells and mucins in heath and disease: Recent insights and progress. *Current Gastroenterology Reports*. 12: 319-330.

Kinyuru, J.N., Konyole, S.O., Roos, N., Onyango, C.A., Owino, V.O., Owuor, B.O., Estambale, B.B., Friis, H., Aagaard-Hansen, J. and Kenji, G.M. 2013. Nutrient composition of four species of winged termites consumed in western Kenya. *Journal of Food Composition and Analysis*. 30:120-124.

Kishawy, A.T.Y., Mohammed, H.A., Zaglool, A.W., Attia, M.S., Hassan, F.A.M., Roushdy, E.M., Ismail, T.A. and Ibrahim, D. 2022. Partial defatted black solider larvae meal as a promising strategy to replace fish meal protein in diet for Nile tilapia (*O. niloticus*): Performance, expression of protein and fat transporters, and cytokines related genes and economic efficiency. *Aquaculture*, 555, Article ID: 738195.

Kobayashi, T. 1967. The developmental stages of some species of the Japanese Pentatomoidea (Hemiptera) XVI. Homalogonia and an allied genus of Japan (Pentatomidae). *Applied Entomology and Zoology*. 2:1-8.

Koch, R. L., Pezzini, D. T., Michel, A. P. and Hunt, T. E. 2017. Identification, biology, impacts, and management of stink bugs (Hemiptera: Heteroptera: Pentatomidae) of soybean and corn in the Midwestern United States. *Journal of Integrated Pest Management*. 8(**1**).

Kroeckel, S., Harjes, A.G.E., Roth, I., Katz, H., Wuertz, S., Susenbeth, A. and Schulz, C. 2012. When a turbot catches a fly: Evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute-Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). *Aquaculture*. 364-365: 345-352.

Kwiri, R., Winini, C., Muredzi, P., Tongonya, J., Gwala, W., Mujuru, F. and Gwala, S.T., 2014. Mopane worm (*Gonimbrasia belina*) utilisation, a potential source of protein in fortified blended foods in Zimbabwe. A review. *Global Journal of Science Frontier Research*.14:55-67.

Lazzari, R., Neto, J.R., Pedron, F., Loro, V.L., Pretto, A. and Gioda, C.R. 2010. Protein sources and digestive enzyme activities in jundiá (Rhamdia quelen). *Animal Science and Pastures*. 67(**3**):259-266.

Li, X., Qin, C., Fang, Z., Sun, X., Shi, H., Wang, Q. and Zhao, H. 2022. Replacing Dietary Fish Meal with Defatted Black Soldier Fly (*Hermetia illucens*) Larvae Meal Affected Growth, Digestive Physiology and Muscle Quality of Tongue Sole (*Cynoglossus semilaevis*). *Frontiers in Physiology*. 13, Article ID: 855957.

Li, Z., Zhang, X., Aweya, J. J., Wang, S., Hu, Z., Li, S. and Wen, X. 2019. Formulated diet alters gut microbiota compositions in marine fish *Nibea coibor* and *Nibea diacanthus*. *Aquaculture Research*. 50 (**1**):126-138.

Liao, H., Liu, P., Deng, Y., Zhang, W., Pan, C., Jia, Y., Long, F. and Tang, H. 2022. Feeding effects of low-level fish meal replacement by algal meals of *Schizochytrium limacinum* and *Nannochloropsis salina* on largemouth bass (*Micropterus salmoides*). *Aquaculture*. 557, Article ID: 738311.

Lindsay, G.J., Walton, M., Adron, J., Fletcher, T.C., Cho, C. and Cowey, C. 1984. The growth of rainbow trout (*Salmo gairdneri*) given diets containing chitin and its relationship to chitinolytic enzymes and chitin digestibility. *Aquaculture*. 37: 315-334.

Liu, T., Han, T., Wang, J., Liu, T., Bian, P., Wang, Y. and Cai, X. 2020. Effects of replacing fish meal with soybean meal on growth performance, feed utilization and physiological status of juvenile redlip mullet *Liza haematocheila*. *Aquaculture Reports*. 20, 100756.

Llagostera, P.F., Kallas, Z., Reig, L. and de Gea, D.A. 2019. The use of insect meal as a sustainable feeding alternative in aquaculture: Current situation, Spanish consumers' perceptions and willingness to pay. *Journal of Cleaner Production*. 229:10-12.

Madibela, O.R., Mokwena, K.K., Nsoso, S.J. and Thema, T.F. 2009. Chemical composition of Mopane worm sampled at three sites in Botswana and subjected to different processing. *Tropical Animal Health and Production*. 41: 935-942.

Makhado R.A., Potgieter M.J., Wessels D.C.J., Saidi A.T. and Masehela K.K. 2012. Use of mopane woodland resources and associated woodland management challenges in rural areas of South Africa. *Ethnobotany Research Applications*. 10: 369-379.

Makkar, H.P.S., Tran, G., Heuze, V. and Ankers, P. 2014. State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*. 197:1-33.

Makore, T. A., Garamumhango, P., Chirikure, T. and Chikambi, S.D. 2015. Determination of nutritional composition of *Encosternum delegorguei* caught in Nerumedzo community of Bikita, Zimbabwe. *International Journal of Biology*. 7(**4**).

Makuku, S.J. 1993. Community approaches in managing common property forest resources: The case of Norumedzo community in Bikita. In: Pierce G.D. & Gumbo D.J. (eds) The Ecology and Management of Indigenous Forests in Southern Africa. Proceedings of an international symposium, Victoria Falls Zimbabwe, 27-29 July 1992. Forest commission and SAREC, Harare.

Manditsera, F.A., Luning, P.A., Fogliano, V. and Lakemond, C.M.M. 2019. The contribution of wild harvested edible insects (*Eulepida mashona* and *Henicus whellani*) to nutrition security in Zimbabwe. *Journal of Food Composition and Analysis*. 75:17-25.

Manyeula, F., Tsopito C., Kamau J., Mogotsi K.K., Nsoso S.J. and Moreki J.C. 2013. Effect of *Imbrasia belina* (westwood), *Tylosema esculentum* (Burchell) Schreiber and *Vigna subterranean* (L) Verde as protein sources of growth and laying performance of Tswana hens raised under intensive production system. *Scientific Journal of Animal Science*. 2(**1**):1-8.

Mapendembe, A. and Mujere, N. 2014. Management of Edible Stinkbugs (*Encosternum delegorguei*) in Bikita District, Zimbabwe. *African Journal of Governance and Development*. 3(**1**).

Marareni, M. and Mnisi, C.M. 2020. Growth performance, serum biochemistry and meat quality traits of Jumbo quails fed with mopane worm (*Imbrasia belina*) meal-containing diets. *Veterinary and Animal Science*.10, Article ID:100141

Mareko, M.H.D., Nsoso, S.J., Mosweu, K., Mokate, K.K. and Madibela, O.R. 2010. Chemical composition and sensory evaluation of broilers supplemented on two levels of Phane worm (*Imbrasia belina*) meal Botswana. *Journal of Agriculture and Applied Sciences*. 6:223-233.

Mariod, A.A. 2020. African edible insects as alternative source of food, oil, protein and bioactive components. Springer. pp314.

Markweg, H., Lang, M.S. and Wagner, F. 1995. Dodecanoic acid inhibition of lipase from *Acinetobacter* sp. OPA 55. *Enzyme and Microbial Technology*. 17, 512-516.

Mastoraki, M., Katsika, L., Enes, P., Guerreiro, I., Kotzamanis, Y.P., Gasco, L., Chatzifotis, S. and Antonopoulou, E. 2022. Insect meals in feeds for juvenile gilthead seabream (*Sparus aurata*): Effects on growth, blood chemistry, hepatic metabolic enzymes, body composition and nutrient utilization. *Aquaculture*. 561, Article ID:738674.

Mawanza, M. 1999. Studies on the biology, ecology and distribution of *Encosternum* (Haplosterna) *delegorguei* Spin (Hemiptera: Tessaratomidae) in Zimbabwe. Abstract. Paper presented at the ZSSA Symposium. July. University of the North, South Africa.

Mbah, C.E. and Elekima, G.O.V. 2007. Nutrient composition of some terrestrial insects in Ahmadu Bello University, Samaru Zaria Nigeria. *Science world Journal*. (2)2.

McFadzen, I.R.B., Coombs, S.H. and Halliday, N.C. 1997. Histological indices of the nutritional condition of sardine, *Sardina pilchardus* (Walbaum) larvae of the north coast of Spain. *Journal of Experimental Marine Biology and Ecology*. *212* (**2**): 239-258.

Mohammed, F.A., Suliman, H.M.A., Yousif, R.A., Mohamed, A.A., Alhafez, A.M. and Rahma, M.E. 2020. Effect of Different Levels of Locust Meal on Growth, Feed Conversion and Carcass Composition for Nile Tilapia Fry (O. Niloticus). *International Journal of Oceanography and Aquaculture*. 4(**2**), Article ID:000188.

Mohan, K., Rajan, D.K., Muralisankar, T., Ganesan, A.R., Sathishkumar, P. and Revathi, N. 2022. Use of black soldier fly (*Hermetia illucens* L.) larvae meal in aquafeeds for a sustainable aquaculture industry: A review of past and future needs. *Aquaculture*. 553, Article ID: 738095.

Mojeremane, W. and Lumbile, A.U. 2005. The characteristics and economic value of Colophospermum mopane (Kirk ex Benth.) J. Léonard in Botswana. *Pakistan Journal of Biological Sciences*. 8:781-784.

Moore, J.A., Swingle, R.S. and Hale, W.H. 1986. Effects of whole cottonseed, cottonseed oil or animal fat on digestibility of wheat straw diets by steers. *Journal of Animal Science*. 63:1267-1273.

Moreki, J.C., Tiroesele, B. and Chiripasi, S.C. 2012. Prospects of utilizing insects as alternative sources of protein in poultry diets in Botswana: A review. *Journal of Animal Science Advances*. 2: 649-658.

Mousavi, S., Zahedinezhad, S. and Loh, J. 2020. A review on insect meals in aquaculture: the immunomodulatory and physiological effects. *International Aquaculture Research*. 12:100-115.

Moyo N.A.G. and Rapatsa, M.M. 2021. A review of the factors affecting tilapia aquaculture production in Southern Africa. *Aquaculture*. 535, Article ID: 736386.

Moyo, N.A.G. and Rapatsa-Malatji, M.M. 2023. A review and meta-analysis of selected plant protein sources as a replacement of fishmeal in the diet of tilapias. *Annals of Animal Science*. 23 (**3**): 681-690.

Moyo, S., Masika, P.J. and Muchenje, V. 2019.The potential of Imbrasia belina worm as a poultry and fish feed. A review. *Journal of Animal and Feed Sciences*. 28: 209-2019.

Mujinya, B.B., Adam, M., Mees, F., Bogaert, J., Vranken, I., Erens, H., Baert, G., Ngongo, M. and Van Ranst, E. 2014. Spatial patterns and morphology of termite (*Macrotermes falciger*) mounds in the Upper Katanga, D.R. Congo. Catena. 114:97-106.

Munro, J.L. 1697. The food of a community of East African freshwater fishes. *Journal of Zoology*. 151(1):389-415.

Mustapha, A.K. and Kolawole, A.A. 2019. Potentials of Fresh Housefly Maggot in the Diet of *O. niloticus* Fingerlings. *Journal of Applied Sciences Environmental Management*. 23 (**4**): 681-687.

Musundire, R., Ngonyama, D., Chemura, A., Ngadze, R.T., Jackson, J., Matanda, M.J. Tarakini, T., Langton, M. and Chiwona-Karltun, L. 2021. Stewardship of Wild and Farmed Edible Insects as Food and Feed in Sub-Saharan Africa: A Perspective. *Frontiers in Veterinary Science*. 8, Article ID: 601386.

Musundire, R., Osuga, I.M., Cheseto, X., Irungu, J. and Torto, B. 2016. Aflatoxin Contamination Detected in Nutrient and Anti-Oxidant Rich Edible Stink Bug Stored in Recycled Grain Containers. *PLoS ONE*. 11(**1**).

Mutungi, C., Irungu, F.G., Nduko, J., Mutua, F., Affognon, H., Nakimbugwe, D., Ekesi, S. and Fiaboe, K.K.M. 2017. Postharvest processes of edible insects in Africa: A review of processing methods, and the implications for nutrition, safety and new products development. *Critical Reviews in Food Science and Nutrition*. 1-23.

Mwimanzi, L. and Musuka, C.G. 2014. The Potential of Mopani Worm (*Gonimbrasia belina*) as an Alternative Protein Source in Fish Feed. *International Journal of Aquaculture*.4(12): 73-78.

Nemadodzi, L.E., Managa, G.M. and Prinsloo, G. 2023. The Use of Gonimbrasia belina (Westwood, 1849) and Cirina forda (Westwood, 1849) Caterpillars (Lepidoptera: Sarturniidae) as Food Sources and Income Generators in Africa. *Foods*, 12 (**11**), 2184.

Netshifhefhe, S.R. and Duncan, F.D. 2022. Nutrient composition of *Macrotermes* species consumed in the Vhembe District, Limpopo Province, South Africa. *Journal of Insect as food and feed*. 8:95-100.

Netshifhefhe, S.R., Kunjeku, E.C. and Duncan, F.D. 2018. Human uses and indigenous knowledge of edible termites in Vhembe District, Limpopo Province, South Africa. *South African Journal of Science*. 114:1-10.

Ng, W.K., Liew, F.L., Ang, L.P. and Wong, K.W. 2001. Potential of mealworm (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, *C. gariepinus*. *Aquaculture Research*. 32: 273-280.

Nobo, G., Moreki, J.C. and Nsoso, S.J. 2012. Feed intake, body weight, average daily gain, feed conversion ratio and carcass characteristics of helmeted guinea fowl fed varying levels of phane meal (*Imbrasia belina*) as replacement of fishmeal under intestive system. *International Journal of Poultry Science*. 11:378-384.

Nogales-Mérida, S., Gobbi, P., Józefiak, D., Mazurkiewick, J., Dudek, K., Rawski, M., Kierńczyk, B. and Józefiak, A. 2019. Insect meals in fish nutrition. *Reviews in Aquaculture*. 11:1080-1103.

Novriadi, R. 2017. A Meta-Analysis Approach toward Fish Meal Replacement with Fermented Soybean Meal: Effects on Fish Growth Performance and Feed Conversion Ratio. *Asian Fisheries Science*. 30:227-244.

NRC (National Research Council). 1993. Nutrient Requirements of Fish. National Academy Press, Washington, DC. pp105.

NRC (National Research Council). 2011. Nutrient requirements of fish and shrimp. Washington DC: The National Academies Press. pp392.

Ntukuyoh, A.I., Udiong, D.S., Ikpe, E. and Akpakpan, A.E. 2012. Evaluation of Nutritional Value of Termites (*Macrotermes bellicosus*): Soldiers, Workers, and Queen in the Niger Delta Region of Nigeria. *International Journal of Food Nutrition and Safety*. 1(**2**): 60-65.

Nyuliwe, T.C., Mlambo, V., Madibana, M.J., Mwanza, M. and Wokadala, O.C. 2022. Partial Substitution of Fishmeal with Mopane Worm Meal in Dusky Kob Fingerling (*Argyrosomus japonicus*) Diets: Feed Utilization, Digestive Enzyme Activity, Blood Parameters, and Growth Performance. *Aquaculture Journal*. 2(**2**): 59-71.

O'Brien R.W. and Slaytor, M. 1982. Role of microorganisms in the metabolism of termites. *Australian Journal Biological Science*. 35:239-262.

Ogunji, J.O., Kloas, W., Wirth, M., Neumann, N. and Pietsch, C. 2008. Effect of housefly maggot meal (magmeal) diets on the performance, concentration of plasma glucose, cortisol and blood characteristics of *O. niloticus* fingerlings. *Journal of Animal Physiology and Animal Nutrition*. 92(4): 511-518.

Okore, O.O., Ekedo, C.M., Ubiaru, P.C. and Uzodinma, K. 2016. Growth and Haematological Studies of African Catfish (*C. gariepinus*) Juveniles Fed with Housefly Larva (*Musca dometica*) as Feed Supplement*. International Journal of Agriculture and Earth Science*. 2 (**3**).

Olaniyi, C.O., Olabomi, F.T. and Ajayi, O.M. 2016. Performance characteristics and nutrients utilization of African catfish fed termite based diets*. International Journal of Science, Environment and Technology*. 5(**5**):2679-2688.

Oonincx, D.G.A.B. and de Boer, I.J.M. 2012. Environmental Impact of the Production of Mealworms as a Protein Source for Humans-A Life Cycle Assessment. *PLOS ONE*. 7(**12**).

Oonincx, DGAB, van Broekhoven, S, van Huis, A, and van Loon, J.J.A. 2019. Correction: Feed Conversion, Survival and Development, and Composition of Four Insect Species on Diets Composed of Food By-Products. *PLOS ONE*. 14(10).

Opuszynski, K. and Shireman, J.V. 1995. Systematics, distribution, diversity, and abundance of herbivorous fishes. Herbivorous fishes. Culture and use of weed management. 1st ed. CRC Press, pp36.

Oyegoke, O.O., Akintola, A.J. and Fasoranti, J.O. 2013. Dietary potentials of the edible larvae of *Cirina forda* (westwood) as a poultry feed. *African Journal of Biotechnology*. 5 (**19**), 1799-1802.

Oyelese, O.A. 2007. Utilization of processed snail meal and supplementation with conventional fishmeal in the diet of *C. gariepinus*. *Journal of Fisheries International*. 2 (**2**): 140-146.

Peńaflorida V. D. 1989. An evaluation of indigenous protein sources as potential component in the diet formulation for tiger prawn, Penaeus monodon, using essential amino acid index (EAAI). *Aquaculture*. 83, (**3-4**): 319–330.

Phelps, R.J., Struthers, J.K. and Moyo, S.J.L. 1975. Investigations into the nutritive value of *Macrotermes falciger* (Isoptera: Termitidae). *African Zoology*. 10:123-132.

Piccolo, G., Iaconisi, V., Marono, S., Gasco, L., Lopente, R., Nizza, S. Bovera, F. and Parisi, G. 2017. Effect of Tenebrio molitor larvae meal on growth performance, in vivo nutrients digestibility, somatic and marketable indexes of gilthead sea bream (*Sparus aurata*). *Animal Feed Science and Technology*. 226:12-20.

Picker, M. and Griffiths, C. 2011. Alien & invasive animals, a South African perspective. Struik Publishers, Cape Town

Pinto, W., Rønnestad, I., Dinis, M.T. and Aragão, C. 2013. Taurine and fish development: Insights for the aquaculture industry. *Advances in experimental medicine and biology*. 779:329-334

Prachom, N., Boonyoung, S., Hassaan, M.S., El-Haroun, E. and Davies, S.J. 2021. Preliminary evaluation of Superworm (*Zophobas morio*) larval meal as a partial protein source in experimental diets for juvenile Asian sea bass, *Lates calcarifer*. *Aquaculture Nutrition*. 27(**5**):1304-1314.

Priyadarshana, M.K.C., Chaminda, N.W., Naveenan, M. and Magamage, M.P.S., Ruwandeepika, H.A.H. 2022. Substitution of fishmeal with black soldier fly *Hermetia illucens* Linnaeus, 1758 larvae in finfish aquaculture - a review. *Asian Fisheries science*. 34:114-126.

Rahman, M.M., Han, H., Kim, K., Kim, K., Lee, B. and Lee, S. 2016. Apparent digestibility coefficients of the extruded pellet diets containing various fish meals for olive flounder, *Paralichthys olivaceus*. *Fisheries and Aquatic Sciences*. 19(**17**).

Rapatsa, M.M. and Moyo, N.A.G. 2017. Evaluation of Imbrasia belina meal as a fishmeal substitute in *O. mossambicus* diets: Growth performance, histological analysis and enzyme activity. *Aquaculture Reports*. 5, 18-26.

Rapatsa, M.M. and Moyo, N.A.G. 2019. Enzyme activity and histological analysis of *C. gariepinus* fed on *Imbrasia belina* meal used for partial replacement of fishmeal. *Fish Physiology and Biochemistry*. 45:1309-1320.

Rapatsa, M.M. and Moyo, N.A.G. 2022. A Review and Meta-analysis of the Effects of Replacing fishmeal with insect meals on growth of tilapias and sharptooth catfish. *Aquaculture nutrition*.1-10. Article ID:9367587.

Rašković, A., Stilinović, N., Kolarović, J., Vasović, V., Vukmirović, S. and Mikov, M. 2011. The protective effects of silymarin against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats. *Molecules*. 16: 8601-8613.

Rašković, B.S., Stanković, M.B., Marković, Z.Z. and Poleksić, V.D. 2011. Histological methods in the assessment of different feed effects on liver and intestine of fish. *Journal of Agricultural Sciences*. *56* (**1**): 87-100.

Renna, M., Rastello, L. and Gasco, L. 2022. Can insects be used in the nutrition of ruminants? *Journal of Insects Food and Feed*. 8(**10**):1041-1045.

Renna, M., Rastello, L., Veldkamp, T., Toral, P.G., Gonzalez-Ronquillo, M., Jimenez, L.E.R. and Gasco, L. 2023. Are insects a solution for feeding ruminants? Legislation, scientific evidence, and future challenges. *Animal Frontiers*. 13(**4**): 102-111.

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., Prearo, M., Capucchio, M.T., Biasato, I., Biasibetti, E., De Marco, M., Brugiapaglia, A., Zoccarato, I. and Gasco, L. 2017. Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* Walbaum) diets. *Journal of Animal Science and Biotechnology*. 1(**8**), Article ID:57.

Richter, N., Siddhuraju, P. and Becker, K. 2003. Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*O. niloticus* L.). *Aquaculture*. 1-4(**217**): 599-611.

Rumbos, C.I. and Athanassiou, C.G. 2021. The Superworm, *Zophobas morio* (Coleoptera: Tenebrionidae): A 'Sleeping Giant' in Nutrient Sources. *Journal of Insect Science*. 21(**2**): 1-11.

Rumpold, B.A. and Schlüter, O.K. 2013. Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies*. 17: 1-11.

Russell, D.J., Thuesen, P.A. and Thomson, F.E. 2012. A review of the biology, ecology, distribution and control of Mozambique tilapia, *O. mossambicus* (Peters 1852) (Pisces: Cichlidae) with particular emphasis on invasive Australian populations. *Reviews in Fish Biology and Fisheries*. 22:533-554.

Sagun, S., Collins, E., Martin, C., Nolan, E.J. and Horzempa, J. 2016. Alarm Odor Compounds of the Brown Marmorated Stink Bug Exhibit Antibacterial Activity. *Journal of Pharmacognosy and natural products*. *2* (**3**):119.

Sakhare, V.B. and Jetithor, S.G. 2016. Food and feeding behaviour of Mozambique tilapia (*O. mossambicus* Peters) from Borna Reservoir of Maharashtra, India. *Journal of Fisheries*. 4(**3**):431-434.

Saleh, H.H.E. 2020. Effect of Feeding on Fresh (wet) Housefly Maggots (*Musca domestica*) with or without Artificial Diet on Water Quality and Growth Rates of African Catfish (*C. gariepinus* Burchell, 1822) Fry under Laboratory Conditions. *Journal of Zoological Research*. 2 (**2**).

Sankar, H.S., Jose, J., Varadarajan, R., Bhanu, S.V., Joy, S. and Philip, B. 2014. Functional Zonation of Different Digestive Enzymes in *Etroplus suratensis* and *O. mossambicus. International Journal of Scientific and Research Publications*. 4 (**5**).

Sankara, F., Pousga, S., Dao, N.C.A., Gbemavo, D.S.J.C., Clottey, V.A., Coulibaly, K., Nacoulma, J.P., Ouedraogo, S. and Kenis, M. 2018. Indigenous knowledge and potential use of termites as poultry feed in Burkina Faso. *Journal of Insects as Food and Feed*. 4: 211-218.

Santiago, C.B. and Lovell, R.T. 1988. Amino acid requirements for growth of nile tilapia. *Journal of Nutrition*. 188 (**12**):1540-1546.

Schallmey, M., Singh, A. and Ward, O.P. 2004. Developments in the use of Bacillus species for industrial production. *Canadian Journal of Microbiology*. 50(**1**):1-17.

Selaledi, L., Mbajiorgu, C.A. and Mabelebele, M. 2020. The use of yellow mealworm (T. molitor) as alternative source of protein in poultry diets: a review*. Tropical animal health and production*. 52(1):7-16.

Shekarabi, S.P.H., Mehrgan, M.S. and Banavreh, A. 2021. Feasibility of superworm, *Zophobas morio*, meal as a partial fishmeal replacer in fingerling rainbow trout, *Oncorhynchus mykiss*, diet: growth performance, amino acid profile, proteolytic enzymes activity and pigmentation. *Aquaculture Nutrition*. 27(**4**) 1077-1088.

Shukla, A., Kumar, M., Gupta, G., Pathak, N. and Mishra, V. 2019. A review on replacing fishmeal in aqua feeds using plants and animal protein sources. *International Journal of chemical studies*. 7(**3**): 4732-4739.

Siddaiah, G.M., Kumar, R., Kumari, R., Chandan, N.K., Debbarma, J., Damle, D.K., Das, A. and Giri, S.S. 2023. Dietary fishmeal replacement with *Hermetia illucens* (Black soldier fly, BSF) larvae meal affected production performance, whole body composition, antioxidant status, and health of snakehead (*Channa striata*) juveniles. *Animal Feed Science and Technology*. 297, Article ID:115597.

Siulapwa, N., Mwambungu, A., Lungu, E. and Sichilima, W. 2012. Nutritional Value of Four Common Edible Insects in Zambia*. International Journal of Science and Research*. Article ID: 02014227.

Skelton, P. 2001. A complete guide to the freshwater fishes of southern Africa. Struik Publishers, Cape Town, South Africa, pp395.

Sogari, G., Amato, M., Palmieri, R., Saadoun, J.H., Formici, G., Verneau,F. and Mancini, S. 2023. The future is crawling: Evaluating the potential of insects for food and feed security. *Current Research in Food Science*. 6, 100504.

Sogari, G., M. Amato, I. Biasato, S. Chiesa, and L. Gasco. 2019. The potential role of insects as feed: a multi-perspective review. *Animals*. 9 (**4**):119.

Sogbesan, A.O. and Ugwumba, A.A.A. 2008. Nutritional Evaluation of Termite (*Macrotermes subhyalinus*) Meal as Animal Protein Supplements in the Diets of *Heterobranchus longifilis* (Valenciennes, 1840) Fingerlings. *Turkish Journal of Fisheries and Aquatic Sciences*. 8: 149-157.

Soltan, M.A., El-Bab, A.F.F. and Saudy, A.M. 2011. Effect of replacing dietary fish meal by cottonseed meal on growth performance and feed utilization of the Nile tilapia, (*O. niloticus*). *Egyptian Journal of Aquatic Biology and Fisheries*. 15(**2**):17-33.

Sonnekus, B., Slippers, B., Hurley, B.P., Joubert, E. Stiller, M. and Fourie, G. 2022. Diversity and Molecular Barcoding of Stink Bugs (Hemiptera: Pentatomidae) Associated with Macadamia in South Africa. *Insects*.13(**7**):601.

Ssepuuya, G., Namulawa, V., Mbabazi, D., Mugerwa, S., Fuuna, P., Nampijja, Z., Ekesi, S., Fiaboe, K.K.M. and Nakimbugwe, D. 2017. Use of insects for fish and poultry compound feed in sub-Saharan Africa – A systematic review. *Journal of Insects as food and feed*. 3(**4**): 289-302.

Stadtlander, T., Stamer, A., Buser, A., Wohlfahrt, J., Leiber, F. and Sandrock, C. 2017. *Hermetia illucens* meal as fish meal replacement for rainbow trout on farm. *Journal of Insects as Food and Feed*. 3(**3**): 165-175.

Tang, C., Yand, D., Liao, H., Sun, H., Liu, C., Wei, L. and Li, F. 2019. Edible insects as food source: A review. *Food production, processing and Nutrition*. 1(**8**).

Tang, C., Yang, D., Huaijian, L., Sun, H., Liu, C. Lanjun, W. and Li F. 2019. Edible insects as a food source: A review. *Food Production, Processing and Nutrition*.1(**1**).

Teffo, L.S., Toms, R.B. and Eloff, J.N. 2007. Preliminary data on the nutritional composition of the composition of the edible stink-bug, *Encosternum delegorguei* Spinola, consumed in Limpopo province, South Africa. *South African Journal of Science*. 103: 434-436.

Tekle-Giorgis, Y., Wagaw, S. and Dadebo, E. 2016. The food and feeding habits of the African catfish, *Clarias gariepinus* (Burchell, 1822) (Pisces: Clariidae) in Lake Hawassa and Shallo swamp, Ethiopia. *Journal of Biology Science*, 15(**1**), 1-18.

Tengjaroenkul, B., Smith, B.J. and Smith, S.A. 2000. Distribution of intestinal enzyme activities along the Intestinal tract of cultured Nile tilapia, *O. niloticus* L. *Aquaculture*. 182(**3**):317-327.

Tesfahun, A. 2018. Feeding biology of the African catfish *C. gariepinus* (Burchell) in some of Ethiopian Lakes: A review. *International Journal of Fauna and Biological Studies*. 5(**1**):19-23.

Tesfahun, A. and Alebachew, S. 2023. Diet composition and feeding habits of the African sharptooth catfish *Clarias gariepinus* (Burchell, 1822) from Ribb Reservoir, South Gondar, Ethiopia. Cogent food and agriculture, 9, 2284228.

Tiamiyu, L.O., Okomoda, V.T. and Iber, B. 2013. Growth response of *C. gariepinus* fingerlings fed diet substituted groundnut cake meal and cotton seed meal. *Livestock Research for Rural Development*. 25(**2**).

Tippayadara, N., Dawood, M.A.O., Krutmuang, P., Hoseinifar, S.H., Doan, H.V. and Paolucci, M. 2021. Replacement of fish meal by black soldier fly (*Hermetia illucens*) larvae meal: Effects on growth, haematology, and skin mucus immunity of Nile tilapia, *O. niloticus*. *Animals*. 11(**1**): 193.

Todd, M.C., Washington, R., Cheke, R.A. and Knivetons, D. 2002. Brown locust outbreaks and climate variability in southern Africa. *Journal of Applied Ecology*. 39:31-42.

Tran, G., Hauze, V. and Makkar, H.P.S. 2015. Insects in fish diets. *Animal Frontiers*. 5(**2**): 37-44.

Tran, H.Q., Prokešová, M., Zare, M., Matoušek, J., Ferrocino, I., Gasco, L. and Stejskal, V. 2022. Production performance, nutrient digestibility, serum biochemistry, fillet composition, intestinal microbiota and environmental impacts of European perch (*Perca fluviatilis*) fed defatted mealworm (*Tenebrio molitor*). *Aquaculture*. 547, Article ID: 737499.

Tsado, A.N., Okoli, R.N., Jiya, A.G., Gana, D., Zubairu, R., Alhassan, E.H. and Salihu, Z.I. 2021. Effect of Supplementing Fish Diet with Grasshopper Meal On Growth Parameters of African Catfish (*C. gariepinus). AROC in Agriculture*. 1 (**1**); 1-5.

van Dyk, J.C. and Pieterse, G.M. 2008. A histo-morphological study of the testis of the sharptooth catfish (*C. gariepinus*) as reference for future toxicological assessments. *Journal of Applied Ichthyology*. 24:415-422.

van Huis, A. 2013. Potential of insects as food and feed in assuring food security. *Annual Review of Entomology*. 58 (**1**): 563-583.

Van Huis, A. 2017. Cultural significance of termites in sub-Saharan Africa. Journal of ethnobiology and ethnomedicine. 13(**1**):8.

van Huis, A. 2022. Edible insects: challenges and prospects. *Entomological Research. 52*(**4**):161-177.

van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74 (**10**): 3583-3597.

Waiyamitra, P., Piewband, C., Techangamsuwan, S., Liew, W.C. and Surachetpong, W. 2021. Infection of Tilapia tilapinevirus in Mozambique Tilapia (*O. mossambicus*), a Globally Vulnerable Fish Species. *Viruses*: 13(**6**):1104.

Wang, L., Li, J., Jin, J.N., Zhu, F., Roffeis, M. and Zhang, X. Z. 2017. A comprehensive evaluation of replacing fishmeal with housefly (*Musca domestica*) maggot meal in the diet of Nile tilapia (*O. niloticus*): growth performance, flesh quality, innate immunity and water environment. *Aquaculture Nutrition*. 23(**5**), 983-993.

Watanabe, D., Gotoh, H., Miura, T. and Maekawa, K. 2014. Social interactions affecting caste development through physiological actions in termites. *Frontiers in Physiology*. 5, Article ID:127.

Xiao, X., Jin, P., Zheng, L., Cai, M., Yu, Z., Yu, J. and Zhang, J. 2018. Effects of black soldier fly (*Hermetia illucens*) larvae meal protein as a fishmeal replacement on the growth and immune index of yellow catfish (*Pelteobagrus fulvidraco*). *Aquaculture Research*. 1-9.

Yeboah, S.O. and Mitei, Y.C. 2009. Further lipid profiling of the oil from the mophane caterpillar, Imbrasia belina. *Journal of the American Oil Chemists' Society*. 86, 1047.

Zarantoniello, M., Randazzo, B., Secci, G., Notarstefano, V., Giorgini, E., Lock, E.J., Parisi, G. and Olivotto, I. 2021. Application of laboratory methods for understanding fish responses to black soldier fly (*Hermetia illucens*) based diets. *Journal of Insects as Food and Feed*. 8 (**11**):1173-1195.

Zhang, J., Dong, Y., Song, K., Li, X., Tan, B., Lu, K. and Zhang, C. 2022. Effects of the Replacement of Dietary Fish Meal with Defatted Yellow Mealworm *Tenebrio molitor*) on Juvenile Large Yellow Croakers (*Larimichthys crocea*) Growth and Gut Health. *Animals*.12, 2659.

APPENDIX A

Cogent Food & Agriculture

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/oafa20

Dietary full-fat Stinkbug (*Encosternum delegorguei*) meal effects on growth performance, blood chemistry, liver and intestinal histology of juvenile Mozambique tilapia (*Oreochromis mossambicus*)

Livhuwani E. Nephale, Ngonidzashe A.G. Moyo & Mmaditshaba M. Rapatsa

To cite this article: Livhuwani E. Nephale, Ngonidzashe A.G. Moyo & Mmaditshaba M. Rapatsa (2023) Dietary full-fat Stinkbug (*Encosternum delegorguei*) meal effects on growth performance, blood chemistry, liver and intestinal histology of juvenile Mozambique tilapia (*Oreochromis mossambicus*), Cogent Food & Agriculture, 9:1, 2253717, DOI: 10.1080/23311932.2023.2253717

To link to this article: https://doi.org/10.1080/23311932.2023.2253717

Published online: 18 Sep 2023.

Nephale et al., *Cogent Food & Agriculture* (2023), 9: 2253717
https://doi.org/10.1080/23311932.2023.2253717

cogent food & agriculture

ANIMAL HUSBANDRY & VETERINARY SCIENCE | RESEARCH ARTICLE

Dietary full-fat Stinkbug (*Encosternum delegorguei*) meal effects on growth performance, blood chemistry, liver and intestinal histology of juvenile Mozambique tilapia (*Oreochromis mossambicus*)

Livhuwani E. Nephale[1]*, Ngonidzashe A.G. Moyo[1] and Mmaditshaba M. Rapatsa[1]

Received: 17 May 2023
Accepted: 28 August 2023

*Corresponding author: Livhuwani E. Nephale, Aquaculture Research Unit, School of Agricultural and Environmental Sciences, Faculty of Science and Agriculture, University of Limpopo (Turfloop Campus), X1106, Polokwane, Sovenga 0727, South Africa
E-mail: nephale.eva@gmail.com

Reviewing editor:
Pedro González-Redondo, University of Seville, Spain

Additional information is available at the end of the article

Abstract: The main objective in this study was to evaluate full-fat Stinkbug as an alternative protein source in the diet of *Oreochromis mossambicus*. Five diets were formulated to partially replace fishmeal at 0, 10, 30, 50, and 70% levels and were denoted as D1, D2, D3, D4, and D5, respectively. The diets were formulated to be isonitrogenous, isocaloric, and isolipidic. Each diet was fed to triplicate groups of fish for 8 weeks. All growth performance indices declined with an increase in Stinkbug meal inclusion. The specific growth rate declined from 2.16% in the control to 1.28% in D5 ($P < 0.05$). The thermal-unit growth coefficient declined from 1.30 in the control to 0.72 in diet D5 ($P < 0.05$). The protein efficiency ratio declined from 2.95 in the control to 1.66 in D5 ($P < 0.05$). However, organosomatic indices did not differ across the fish diets. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities did not differ significantly ($P > 0.05$) across diets D1 to D4. No histological alterations were evident in the liver and intestine across diets. It was therefore concluded that Stinkbug meal may partially replace fishmeal at 30% replacement level in the diet of *O. mossambicus* without adverse effects on growth performance and nutrient utilisation indices.

Subjects: Agriculture & Environmental Sciences; Marine & Aquatic Science; Nutrition

Keywords: Encosternum delegorguei; Oreochromis mossambicus; alanine aminotransferase; aspartate aminotransferase; histology

ABOUT THE AUTHORS

Livhuwani Eva Nephale is a PhD candidate at the University of Limpopo, Aquaculture Research Unit. She holds an MSc in Aquaculture from the University of Limpopo. Her current research interest is fish nutrition.

Prof N. A. G Moyo is an established researcher with more than 30 years' experience in Aquatic ecosystems including Aquaculture. He holds a PhD from University of Waterloo in Canada and has supervised more than 10 PhD students.

Prof M. M Rapatsa is an upcoming researcher. She holds a PhD in aquaculture from the University of Limpopo and has published more than 15 papers in Aquaculture.

1. Introduction

The use of insect meals in aquafeed has recently become a research priority in the aquaculture sector (Abdel-Tawwab et al., 2020; Llagostera et al., 2019). This is mainly due to their nutritional composition and high feed conversion efficiency (Bruni et al., 2018; Fawole et al., 2020). Insects form part of the natural diet of both marine and freshwater fish species (Makkar et al., 2014; van Huis, 2013) and different orders exhibit different nutritional compositions (Hua, 2021). The insect meal protein content ranges between 50 and 82% on a dry matter basis, whilst the fat content ranges from 10 to 30% (Henry et al., 2015; Rumpold & Schlüter, 2013). The amino acid profile and the fatty acid composition of insect meal are highly dependent on the insect diet, larval stage, and size (Alfiko et al., 2022). Insect meals are also rich in minerals and vitamins (Terova et al., 2021). Insects that have been used in aquafeed include black soldier fly larvae (*Hermetia illucens*) (Fischer et al., 2022; Guerreiro et al., 2020; Mohan et al., 2022), mealworm larvae (*Tenebrio molitor*) (Coutinho et al., 2021; Jeong et al., 2020; Tran et al., 2022), housefly larvae (*Musca domestica*) (Akinwole et al., 2020; Gbai et al., 2019; Saleh, 2020), mopane worm (*Imbrasia belina*) (Nyuliwe et al., 2022; Rapatsa & Moyo, 2017, 2019), and superworm (*Zophobas morio*) (Henry et al., 2022; Jabir et al., 2012; Prachom et al., 2021). These insects have been used in different fish species (marine and freshwater) with varying degrees of success. In a recent review, Rapatsa and Moyo (2022) recommended the use of locally available insects in the replacement of fishmeal in the diets of *O. mossambicus*.

Some of these insects have been tested on Tilapia *sp*. such as *O. mossambicus* and *Oreochromis niloticus*. Rapatsa and Moyo (2017) showed that substituting fishmeal with *I. belina* meal increased the growth performance of *O. mossambicus* with an increase in *I. belina* meal inclusion. Additionally, studies showed that *H. illucens* can completely replace fishmeal in the diets of *O. niloticus* without any adverse effects on growth performance and nutrient utilisation (Kishawy et al., 2022; Tippayadara et al., 2021). However, Devic et al. (2017) showed that the best growth performance of *O. niloticus* fed diets with *H. illucens* was at 30% inclusion level. Furthermore, inclusion levels beyond 30% showed a compromised growth performance and poor nutrient utilisation. The varied results may be due to the processing method of insect meal, the insect larval stage, fish size, and fish species (Tschirner & Simon, 2015). The use of insect-based protein sources in aquafeed was prompted by the escalating cost of fishmeal, which is the main protein component in animal feed. Thus, there is a need to investigate the potential use of locally available insects in aquafeed.

In Southern Africa, one of the insects that can be used to replace fishmeal is the Stinkbug (*Encosternum delegorguei*). It is widely distributed in subtropical, open wood and bushveld of Southern regions of Zimbabwe and Northern Provinces of South Africa (Makore et al., 2015). The Stinkbug is mainly sold at the open market in Thohoyandou Town between May and October. Seasonal availability is one of the challenges associated with the replacement of fishmeal with insects (van Huis, 2022). Most insects are available during the rainy season. However, the Stinkbug is one of the insects that is harvested during the dry season, when there are no other insects available. The Stinkbug contains a high fat content (Teffo et al., 2007). However, its protein content and amino acid profile are poorly understood. Furthermore, the distribution of Stinkbugs coincides with the distribution of the Mozambique tilapia (*Oreochromis mossambicus*), which is one of the commonly cultured warmwater species in Southern Africa. This makes Stinkbug an ideal candidate for the replacement of fishmeal in Southern Africa.

The Mozambique tilapia (*Oreochromis mossambicus*) is one of the most widely cultured warm freshwater species in Southern Africa. It is a species of economic importance and plays a role in the gross domestic product of South Africa. However, its production has not yet reached its full potential. *Oreochromis mossambicus* is a good aquaculture candidate species (El-Sayed, 2020). Its capacity to utilize insect meals was recently reviewed and showed that it is pre-adapted to utilise insect-based diets (Rapatsa & Moyo, 2022). It is therefore imperative to carry out a detailed study on the effect of dietary full-fat Stinkbug meal as a fishmeal replacement on some aspects of the physiology of *O. mossambicus*. Therefore, the objective of this study was to determine the effect of dietary full-fat Stinkbug meal on growth performance, blood serum chemistry, liver and intestine histology of juvenile *O. mossambicus*.

2. Materials and methods

2.1. Proximate composition

Stinkbugs were purchased from Thohoyandou Town open market, in the Vhembe District of Limpopo Province, South Africa. Stinkbugs (dried) were ground using a miller. Stinkbug meal was analysed for proximate and nutrient composition (Table 1).

Dry matter was determined by drying samples using an oven at 105°C until a constant weight was reached. Moisture content was determined by obtaining the difference between the initial weight and the weight lost after samples were dried in the oven at 105°C for 24 h. Crude protein (AOAC #984.13) was determined using the Micro-Kjeldahl method, and the content was estimated by multiplying nitrogen by 6.25 (Foss 2400 Kjeltec analyser unit, Denmark) (Association of Official Analytical Chemists AOAC, 2003). Fat content (AOAC #954.02) was determined using solvent extraction with petroleum ether (Soxtec system, Foss Tecator lipid analyser (AOAC, 2003). Ash content (AOAC #942.05) was determined by incinerating samples at 550°C for 6 hours in a muffle furnace (Heraeus K1252, Germany) (AOAC, 2003). The crude fibre was determined by digesting dried lipid-free residues with 1.25% H_2SO_4 and 1.25% NaOH. The loss on ignition was recorded as crude fibre (Hlophe-Ginindza et al., 2015). Gross energy was analysed using DDS Isothermal CP 500 bomb calorimeter. Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) were determined by boiling sample with a neutral detergent solution and acid detergent solution, respectively (van Soest et al., 1991).

2.2. Mineral composition

Selected minerals (Iron, potassium, and sodium) were analysed in the Stinkbug meal. Samples were hydrolysed with 65% concentrated nitric acid and 37% hydrochloric acid. Hydrogen peroxide was used to remove nitrous vapours. The mineral content was then determined using Inductively

Table 1. Proximate composition, mineral content, and amino acid profile of the Stinkbug meal compared to fishmeal

Proximate composition	SBM %DM	FM %DM	Essential amino acid	SBM g/kg (DM)	FM* g/kg (DM)
Dry matter (%)	88.75	86.02[a]	Lysine	6.85	25.58
Moisture	11.25	13.98[a]	Methionine	1.76	2.23
Protein	29.53	65.5[b]	Valine	4.07	19.38
Fat	48.59	12.0[b]	Leucine	4.77	33.54
Ash	3.14	18.0[b]	Histidine	3.56	10.45
Fibre	8.70	1.8[b]	Isoleucine	2.50	19.04
NDF	27.11	0.0[b]	Threonine	2.87	16.74
ADF	50.84	0.0[b]	Tryptophan	0.08	5.70[c]
GE (KJ/100 g)	2 514	-	Phenylalanine	2.70	17.24
Minerals	**mg/l**		**Non-essential amino acid**		
Iron (Fe)	0.91	-	Tyrosine	4.04	7.92
Potassium (K)	21.7	-	Glycine	4.76	21.17
Sodium (Na)	69.2	-	Aspartic acid	6.70	41.48
			Serine	3.82	17.29
			Proline	3.80	19.90
			Glutamic acid	10.19	62.40
			Alanine	4.80	25.44
			Cystine	15.31	1.09

NDF: neutral detergent fibre; ADF: acid detergent fibre, DM: dry matter. GE: gross energy, [a]Muin and Taufek (2022), [b]Rapatsa and Moyo (2017). *Hu et al. (2020). SBM: Stinkbug meal; FM: Fishmeal. -: not available, [c]Djissou et al. (2016).

coupled plasma-optical emission spectrometry (ICP-OES) (Manditsera et al., 2019). The analysis was performed using standard solutions of known concentrations. All chemicals used were analytical reagent grade. The selected mineral content of Stinkbug meal is outlined in Table 1.

2.3. Amino acid profile

Stinkbug meal was analysed for amino acids at the Central Analytical Facilities (CAF) of the Stellenbosch University (South Africa). Samples were hydrolysed with 6 M HCI at 110°C for 22 h in sealed tubes (Ishida et al., 1981). Separation was performed on a gas chromatograph (6890N, Agilent Technologies Network) coupled to an Agilent technologies inert XL EI/CI Mass Selective Detector (MSD) (5975, Agilent Technologies Inc., Palo Alto, CA). Amino acid profile of the Stinkbug meal is outlined in Table 1. The amino acid profile of experimental diets was calculated based on the amino acid profile of the feed ingredients used in experimental diets formulation (Table 3).

2.4. Fatty acid profile

The fatty acid methyl esters (FAMEs) for Stinkbug meal were also analysed at the Central Analytical Facilities (CAF) of the Stellenbosch University (South Africa). This was done by adding 2 ml of 2:1 chloroform: methanol solution to 100 mg of the sample (Folch et al., 1957). The sample was vortexed and sonicated at room temperature for 30 min. The sample was then centrifuged at 3000 rpm for 1 min. Chloroform (200 µl) was dried completely with a gentle stream of nitrogen and reconstituted and vortexed with 170 µl of methyl tert-butyl ether (MTBE) and 30 µl of trimethylsulfonium hydroxide (TMSH). One µl of the derivitised sample was injected in a 5:1 split ratio onto the GC-FID. Separation was performed on a gas chromatograph (6890N, Agilent Technologies) coupled to flame ionization detector (FID). Separation of the FAMEs was performed on a polar RT-2560 (100 m, 0.25 mm ID, 0.20 µm film thickness) (Restek, USA) capillary column. Hydrogen was used as the carrier gas at a flow rate of 1.2 ml/min. The injector temperature was maintained at 240°C. One µl of the sample was injected in a 5:1 split ratio. The oven temperature was programmed as follows: 100°C for 4 min, ramped to 240°C at a rate of 3 °C/min for 10 min. The fatty acid profile of the Stinkbug meal is outlined in Table 4. The fatty acid profile of the experimental diets (D1, D2, D3, D4 & D5) is outlined in Table 5.

2.5. Feed formulation

Five diets were formulated to partially substitute fishmeal at 0, 10, 30, 50, and 70% (Table 2). The 0% diet contained 30% fishmeal and served as a control, with no Stinkbug meal. The diets were named D1, D2, D3, D4, and D5, denoting 0, 10, 30, 50, and 70% replacement levels, respectively (Table 2). The diets were formulated to be isonitrogenous (30% crude protein), isocaloric (15 MJ/Kg), and isolipidic (12% fat). The Winfeed 3, EFG (Natal) program was used to formulate diets. The Stinkbugs (dried) were purchased from Thohoyandou Town open market. The yellow maize, wheat bran, sunflower meal, and sunflower oil were purchased from a local market. Vitamin/Mineral premix, fishmeal, and binder were purchased from Irvines Africa (Pty) Ltd. Chromic oxide was purchased from Sigma Aldrich. Several studies that used insect meals as fishmeal substitutes have added methionine and lysine in the diets (Cho et al., 2022; Djissou et al., 2016; Gai et al., 2023). To make this study comparable, methionine and lysine were also added in the diets. Moreover, the proximate composition of the Stinkbug meal recorded extremely low levels of methionine (1.76 g/kg) and lysine (6.85 g/kg) that do not meet the requirement for *O. mossambicus* (methionine: 9.9 g/kg and lysine: 37.8 g/kg). Thus, experimental diets were supplemented with crystalline methionine and lysine which were purchased from Nutroteq Pty Ltd. Chromic oxide (Cr_2O_3) was used as an inert marker in each diet at 10 g/kg. The ingredients were ground and screened through a 60-mesh size sieve and mixed thoroughly using a planetary mixer (Hobart, Troy, OH, USA) until they were well mixed. During mixing, water was added (10–20% v/w) occasionally until the desired dough thickness was reached. The dough was pelleted through a meat grinder (SLP-45 Shang Hai Taiyi, China) and pushed through to produce extruded strands (2 mm diameter). Pelleted strands were sun-dried and then stored in polyethylene buckets with lids separately at 4°C. The proximate composition of experimental diets is outlined in Table 2.

Table 2. Ingredients (g/kg, DM) used to formulate experimental diets where Stinkbug meal replaced fishmeal at different replacement levels and the proximate composition of experimental diets

Ingredients	Experimental diets				
	D1	D2	D3	D4	D5
Fishmeal[a]	300	270	210	150	90
Stinkbug	0	30	90	150	210
Yellow Maize	221	222	233	230	228
Wheat bran	219	218	207	210	212
Sunflower meal	100	100	100	100	100
Sunflower oil	60	60	60	60	60
Methionine	20	20	20	20	20
Lysine	20	20	20	20	20
Vitamin/Mineral premix[b]	20	20	20	20	20
Binder	30	30	30	30	30
Chromic oxide	10	10	10	10	10
Total	1000	1000	1000	1000	1000
Proximate composition					
Dry matter (%)	92.24	92.40	92.97	92.78	92.10
Crude protein (%DM)	30.18	30.02	30.33	30.49	30.21
Fat (%DM)	12.06	12.91	12.25	12.11	12.65
Ash (%DM)	10.59	10.60	10.83	10.25	10.20
Gross Energy (MJ/Kg of DM)	15.78	15.85	15.11	15.52	15.22

[a]Fishmeal (crude protein: 65.5%, fat: 12.0%).

[b]Vitamin/Mineral premix: Vit. A, 12 000 IU; Vit. D3, 1 200 IU; Vit. E, 120 IU; Vit. B4, 10000 g; Vit. C, 120 g; Vit. B3, 25 g; Vit. B5, 15 g; Vit. B2, 6 g; Vit. B6, 5 g; Vit. B1, 4 g; Vit. K3, 2 g; Vit. B9, 1 g; Vit. H, 0.25 g; Vit. B12, 0.04 g. ZnO, 200 g; $FeSO_4$; $CuSO_4$, 7 g; MnO, 5 g; KI, 2 g; Na_2SeO_3, 0.15 g; $CoSO_4$, 0.05 g. D1 (0%), D2 (10%), D3 (30%), D4 (50%), D5 (70%).

Table 3. Calculated amino acid profile of experimental diets (% of dry matter)

Amino acids*	D1	D2	D3	D4	D5
Lysine	4.93	4.41	4.40	4.37	4.17
Methionine	1.01	1.11	1.26	1.41	1.33
Valine	2.56	2.33	2.24	2.69	2.41
Leucine	3.60	3.68	3.54	3.71	3.39
Histidine	1.20	1.12	1.36	1.05	1.01
Isoleucine	2.08	2.11	2.14	2.12	2.15
Threonine	3.00	3.02	3.01	3.04	3.05
Tryptophan	0.56	0.41	0.40	0.44	0.36
Phenylalanine	2.24	2.24	2.43	2.55	2.62

*Amino acids were calculated based on the amino acid profile of the feed ingredients that were used in experimental diets formulation

Table 4. Fatty acid profile of the Stinkbug meal

Fatty acids	g/kg of TFA
C8:0 (Caprylic acid)	0.01
C12:0 (Lauric acid)	0.02
C13:0 (Tridecanoic acid)	0.02
C14:0 (Myristic acid)	0.39
C16:0 (Palmitic acid)	6.62
C18:0 (Stearic acid)	0.40
C20:0 (Arachidic acid)	0.03
C14:1 (Myristoleic acid)	0.32
C16:1 (Palmitic acid)	2.82
C18:1n-9 (Oleic)	3.76
C20:1 (Gondoic)	0.55
C18:2n-6 (linoleic acid)	1.17
ΣSFA	7.46
ΣMUFA	7.45
ΣPUFA	1.17

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acid. TFA: total fatty acids.

2.6. Experimental design

Feeding trials were conducted at the Aquaculture Research Unit (ARU), University of Limpopo, South Africa. Mozambique tilapia (*Oreochromis mossambicus*) juveniles were bred at the Aquaculture Research Unit and kept in the hatchery tanks. Before the experiment commenced, fish were collected from the hatchery tanks and placed in a 100 L container with water that was mixed with 2 phenoxyethanol (1 ml/L) as an anaesthetic. Fifteen fish were randomly stocked into 500 L capacity fiberglass experimental tanks in a recirculating system (Filled to 400 L mark). The tanks were connected to a sump and pump that supplied the tanks with water at 10 L/min. The tanks were individually heated with a submersible aquarium heater (ViaAqua Glass heater, 200 W) and aerated with airstones. Fish were left to acclimatise in experimental tanks for 2 weeks while feeding on commercial pellets (Aqua-plus, Avi Products (Pty) Ltd). After the acclimatisation period, fish were anesthetised, and the initial body weight was recorded for each tank. Fish were then randomly allocated to tanks in triplicates and allocated their diets. A total of 15 tanks were used to stock the fish at 15 fish per tank (Stocking density). Fish were fed experimental diets to apparent satiation for 8 weeks twice a day (09:00 h and 15:00 h). Fish were considered satiated when two pellets were left uneaten for 5 min in a tank. Feed intake for each tank was recorded on daily basis. Fish tanks were fitted with the Guelph system, with mild modifications (Cho et al., 1982). Faecal matter samples were collected two weeks after the commencement of the experiment from the collection tube. The faecal matter samples were used for protein apparent digestibility coefficient analysis. Water temperature was kept between 27 and 29°C, dissolved oxygen (6.5–8.0 mg/l), ammonia (<1 mg/l), and pH (6.8–8.0). These parameters were monitored on weekly basis using a handheld multiparameter meter (Professional plus YSI 605,000). The experiment was conducted under natural photoperiod. All ethical protocols were observed and approved by the University of Limpopo Animal Research Ethical Committee (AREC/09/2022:PG).

2.7. Growth performance indices

Fish were weighed at the commencement of the trial, after every two weeks, and at the end of the feeding trial. At the end of the experiment, fish were starved for 24 h before growth performance indices were measured. Three fish from each replica (9 fish per treatment) were randomly selected for measurement of growth parameter indices.

Table 5. The fatty acid profile of experimental diets (g/kg)

Fatty acid	D1	D2	D3	D4	D5
C12	0.02	0.02	0.03	0.02	0.03
C13	0.02	0.02	0.02	0.02	0.03
C14	1.26	1.58	1.79	1.37	1.58
C14:1	0.02	0.11	0.39	0.48	0.76
C15	0.13	0.15	0.14	0.09	0.07
C16	12.84	18.07	32.46	33.30	48.47
C16:1	2.11	7.87	22.36	26.55	41.79
C17	0.16	0.17	0.13	0.08	0.07
C17:1	0.11	0.16	0.11	0.06	0.05
C18	4.57	5.57	8.73	8.32	11.48
C18:1 (cis)	17.77	21.22	38.27	40.56	59.11
C18:2 (cis)	38.15	41.38	68.85	67.44	94.92
C20	0.30	0.29	0.60	0.64	0.81
C20:1 & C18:3n3	2.25	1.81	1.60	1.20	1.17
C21	0.01	0.02	0.02	0.01	0.01
C20:2	0.05	0.05	0.05	0.02	0.02
C22	0.52	0.60	1.04	0.97	1.42
C20:3n6	0.01	0.01	0.01	0.01	0.03
C22:1	0.67	0.76	0.68	0.34	0.23
C20:4n6	0.05	0.06	0.05	0.02	0.01
C23	0.03	0.01	0.03	0.03	0.04
C20:5n3	2.73	2.95	2.67	1.36	0.88
C24	0.15	0.16	0.24	0.20	0.30
C24:1	0.16	0.17	0.15	0.07	0.04
C22:6n3	4.36	4.57	4.14	2.01	1.31

For growth performance, the following parameters were calculated:

$$\text{Specific growth rate (SGR)} : \text{SGR} = \left[\frac{\text{In}W_t - \text{In}W_0}{t}\right] \times 100\%$$

Where In, W_t, W_0, and t are the natural logarithm, the final body weight (g), initial body weight (g), and time feeding period (days), respectively.

$$\text{Thermal-unit growth coefficient} : \text{TGC} = 1000 \times \frac{\text{Final weight}^{\frac{1}{3}} - \text{Initial weight}^{\frac{1}{3}}}{\text{Temperature}(^{\circ}\text{C}) \times \text{days}}$$

Feed conversion ratio (FCR)= feed consumed (g)/weight gained (g)

Protein efficiency ratio (PER)= increase in body mass (g)/protein consumed (g)

Weight gain (g) = final weight (g)-initial weight(g)

Feed intake (g/fish/day) = weight of eaten feed (g)/number of fish/feeding period (day).

Fish survival rate (%) = (final number of fish/initial number of fish) × 100

Apparent protein digestibility coefficient (APDC) was determined according to Cho et al. (1982)

Chromic oxide in diets and faeces was determined by adding concentrated nitric acid (HNO_3) into the samples and then 70% perchloric acid ($HClO_4$) was added to oxidize chromic oxide until the colour orange appear. The samples were then read for absorbance at 350 nm, using ultrapure water as a blank. Chromic oxide was then calculated as follows:

Weight of chromic oxide in sample = Absorbance − 0.0032/0.2089

Chromic oxide (%) = (weight of chromic oxide/sample weight (mg)) ×100

$$\text{APDC } (\%) = 100\left[1 - \left(\frac{\text{Cr2O3 in diet}}{\text{Cr2O3 in faeces}}\right) \times \left(\frac{\text{protein in faeces}}{\text{protein in diet}}\right)\right]$$

2.8. Organosomatic indices

Fish were collected from experimental tanks and sedated with 2-phenoxyethanol (1 ml/L). Three fish from each dietary replica (9 fish/diet) were used for organosomatic indices. The condition factor, hepato-somatic index, and viscero-somatic index were calculated.

CF= final body weight(g)/fish length(cm)^3 ×100

Hepato-somatic index (HSI)= liver weight(g)/fish weight(g) × 100

Viscero-somatic index (VSI)= viscera weight(g)/fish weight(g) × 100

2.9. Blood chemistry

At the end of the feeding trial, fish were fasted for 24 h. Three fish samples from each dietary replica were randomly selected and sedated with 2-phenoxyethanol (1 ml/L). Blood samples (~3 ml) were drawn from the caudal vasculature into vials using heparinised syringes and centrifuged for 15 min at 3000 rpm (Kishawy et al., 2022). Blood samples from each replica were pooled. The serum was used for the analysis of triglyceride, cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). The analyses were conducted using a commercial automatic biochemical kit and according to the manufacturers protocol (Sigma-Aldrich, USA).

2.10. Liver and intestinal histological analysis

In each dietary replicate, three fish were randomly selected, anaesthetised, weighed, and sacrificed. Fish liver and intestines (distal) were removed and separately fixed in 10% formalin solution until analysis. During analysis, liver samples were cut into 1 cm^3 blocks, whilst intestine samples were cut into 1 cm length. All samples were washed for 2 h in distilled water. Samples were then dehydrated in ethanol solution of 70, 80, 96, and 100% levels concentrations. Liver and intestine samples were made transparent using xylene. Samples were infiltrated using Tissue-Tek® III wax in an oven at 60°C. Samples were further embedded in Tissue-Tek® III wax blocks, sectioned at 5 µm thickness (Leica: RM2125 RTS). The sectioned samples were placed in microscope slides and left to dry. After drying, slides were stained with haematoxylin and eosin (H&E) and covered with coverslips as described by van Dyk and Pieterse (2008).

2.11. Liver and intestinal histological assessment

Liver and intestine slides were assessed using a light microscope at different magnifications (Leica Microsystems model DM750, Leica, Bannockburn, IL, USA). The intestine histology was quantified using a modified scoring system from (Baeza-Ariño et al., 2014). For villi length and villi thickness, six measurements per slide were taken to establish mean values. Three slides were assessed per treatment. Goblet cells were quantified by counting the number present in each villus. Six villi per slide were quantified to establish mean values. Liver histology was quantified by analysing the liver hepatocyte condition according to McFadzen et al. (1997). The grading was from 1–3, where 1 describes a healthy liver, 2 as intermediate, and 3 as a degraded liver. Dietary replicates were allocated an average grade value based on the dominant histological alterations observed. Three slides were assessed for each replicate.

3. Statistical analysis

Shapiro-Wilk and Barlett's tests were used to test data for normality of distribution and homogeneity of variance, respectively. One-way analysis of variance (ANOVA) was used to test significant differences in growth parameter indices, organosomatic indices, blood serum chemistry, and histology. The data was significantly different when $P < 0.05$. Tukey's HSD post hoc test was used to determine mean differences. The data was analysed using SPSS version 28.0 software package (Statistical Package and Service Solutions, IBM, Chicago, IL, USA).

4. Results

4.1. Proximate composition

Stinkbug meal contained 29.53% protein and 48.59% fat content (Table 1). The protein content of the Stinkbug meal was relatively lower than fishmeal (65%). The ash content of the Stinkbug meal was 3.14%, whilst the ash content of fishmeal was 18%. The highest mineral recorded in the Stinkbug meal was sodium (69.2 mg/l).

The essential amino acids (EAA) composition of the Stinkbug meal showed that lysine (6.85 g/kg), leucine (4.77 g/kg), and valine (4.07 g/kg), were the most abundant (Table 1). On the other hand, cystine (15.31 g/kg), glutamic acid (10.19 g/kg), and aspartic acid (6.70 g/kg) showed the highest concentration of non-essential amino acids, respectively (Table 1). Fishmeal recorded relatively higher amino acid profile than the Stinkbug meal (Table 1). Experimental diets contained nine essential amino acids (Table 3).

The Stinkbug meal comprised high levels (7.46 mg/g) of saturated fatty acids (SFA) and monounsaturated fatty acid (MUFA) (7.45 mg/g), respectively (Table 4). Palmitic fatty acid dominated the SFA, whilst oleic fatty acid dominated the MUFA. Furthermore, Stinkbug meal contained PUFA, linoleic acid which is one of the most essential omega-6 fatty acids. The experimental diets showed that the fatty acid profile was dominated by C18:2 cis (linoleic acid), C18:1 cis (oleic acid), C16 (palmitic acid), and C16:1 (palmitoleic acid) (Table 5). Palmitic acid, oleic acid and palmitoleic acid were the fatty acids that increased in experimental diets with an increase in Stinkbug meal.

4.2. Growth performance parameters

There was a decrease in growth performance with increasing Stinkbug meal inclusion (Table 6). The highest WG (167.37 g), SGR (2.16%), TGC (1.30), and PER (2.95) were recorded in fish fed the control diet (D1). The fish fed the control diet (D1) also had the lowest FCR (1.42). The APDC of fish fed the control diet (79.67%) was higher ($P < 0.05$) than the rest of the diets. However, diets D2 to D5 showed no significant difference in APDC ($P > 0.05$). Diet D5 recorded the lowest growth performance and nutrient utilisation indices (Table 6). Organosomatic indices (CF, VSI, HSI) were not influenced by Stinkbug meal inclusion ($P > 0.05$).

4.3. Blood serum chemistry

The ALT values of fish fed the control diet (22.66 U/L) did not differ ($P > 0.05$) from diets D2, D3, and D4 (Table 7). The diet with the highest inclusion level (D5) recorded the highest ALT level (32.66 U/L), which differed significantly ($P < 0.05$) from the rest of the diets. The AST values showed no discernible pattern. However, the highest AST level was recorded in diet D5 (318.33 U/L) and differed ($P < 0.05$) from other diets. Stinkbug meal did not influence ($P > 0.05$) the cholesterol and triglyceride levels among the diets.

4.4. Liver and intestinal histology

There was no significant difference in the intestine villi height across the diets ($P > 0.05$) (Table 8). However, a numerically higher villi height was recorded in diet D1 (201 µm). Villi thickness also did not differ significantly ($P > 0.05$) across different diets. However, Diet D1 also recorded the thickest villi (64.66 µm). Substituting fishmeal with stinkbug meal had no influence on the number of goblet

Table 6. Growth parameters and organosomatic indices (mean ± SD) of *Oreochromis mossambicus* fed diets with Stinkbug meal as a fishmeal replacement at increasing substitution levels for 8 weeks. *n* = 9

Dietary groups	D1 (0%)	D2 (10%)	D3(30%)	D4(50%)	D5 (70%)
Indices					
IBW (g)	68.74 ± 4.73	75.47 ± 3.81	76.68 ± 6.29	64.04 ± 5.07	69.29 ± 4.07
FBW (g)	236.11 ± 6.08	218.01 ± 2.03	205.79 ± 3.90	166.16 ± 5.05	145.38 ± 2.30
WG (g)	167.37 ± 4.32^{a}	143.53 ± 3.93^{b}	128.32 ± 2.35^{c}	103.12 ± 2.33^{d}	75.09 ± 1.52^{e}
SGR (%)	2.16 ± 0.07^{a}	1.86 ± 0.17^{b}	1.73 ± 0.25^{c}	1.68 ± 0.33^{c}	1.28 ± 0.10^{d}
TGC	1.30 ± 0.03^{a}	1.12 ± 0.11^{b}	1.04 ± 0.18^{c}	0.95 ± 0.19^{d}	0.72 ± 0.06^{e}
FCR	1.42 ± 0.15^{a}	1.56 ± 0.30^{b}	1.72 ± 1.25^{c}	1.77 ± 0.54^{c}	2.15 ± 0.43^{d}
FI (g/fish/day)	0.74 ± 0.05^{a}	0.55 ± 0.09^{b}	0.42 ± 0.04^{c}	0.39 ± 0.11^{c}	0.36 ± 0.15^{c}
PER	2.95 ± 0.09^{a}	2.87 ± 0.46^{ab}	2.82 ± 0.65^{b}	2.28 ± 0.47^{c}	1.66 ± 0.14^{d}
APDC (%)	79.67 ± 2.55^{a}	70.89 ± 2.10^{b}	70.21 ± 2.25^{b}	69.14 ± 1.93^{b}	65.57 ± 1.48^{b}
Organosomatic Indices					
CF (g/cm3)	2.30 ± 0.13^{a}	2.02 ± 0.19^{a}	1.82 ± 0.38^{a}	1.74 ± 0.06^{a}	1.54 ± 0.25^{a}
VSI (%)	12.85 ± 0.79^{a}	12.33 ± 4.53^{a}	12.97 ± 2.01^{a}	12.85 ± 1.15^{a}	11.51 ± 5.00^{a}
HSI (%)	4.03 ± 0.24^{a}	3.64 ± 0.49^{a}	3.54 ± 1.82^{a}	3.36 ± 3.81^{a}	3.35 ± 0.41^{a}
Survival (%)	100	100	100	100	100

Means in a row with different superscripts were significantly different ($P < 0.05$). IBW: Initial body weight, FBW: Final body weight, WG: Weight gain, SGR: Specific growth rate, TGC: Thermal-unit growth coefficient, FCR: Food conversion ratio, FI: Feed intake, PER: Protein efficiency ratio, APDC: Apparent protein digestibility coefficient, CF: Condition factor, VSI: Viscero-somatic index, HSI: Hepatosomatic index

Table 7. Blood chemistry (mean ± SD) of *Oreochromis mossambicus* fed diets with Stinkbug meal at different replacement levels, *n* = 3

Diets	ALT (U/L)	AST (U/L)	Chol (mmol/l)	Trig (mmol/l)
D1	22.66 ± 3.51^{a}	221.33 ± 3.21^{a}	0.35 ± 0.00^{a}	0.02 ± 0.00^{a}
D2	20.63 ± 4.50^{a}	224.33 ± 3.51^{a}	0.32 ± 0.02^{a}	0.03 ± 0.00^{a}
D3	24.33 ± 4.93^{a}	213.00 ± 3.00^{b}	0.33 ± 0.01^{a}	0.02 ± 0.00^{a}
D4	23.33 ± 5.68^{a}	225.33 ± 2.30^{a}	0.31 ± 0.04^{a}	0.03 ± 0.00^{a}
D5	32.66 ± 2.51^{b}	318.33 ± 2.08^{c}	0.30 ± 0.01^{a}	0.02 ± 0.00^{a}

ALT: alanine aminotransferase; AST: aspartate aminotransferase; Chol: cholesterol; Trig: triglyceride; Different superscripts on each column are significantly different ($P < 0.05$, ANOVA).

Table 8. The score for the distal intestine of *O. mossambicus* fed diets with stinkbug meal at different replacement levels. Villi height and thickness are expressed as mean±SD. *n* = 9

Diets	Villi height (µm)	Villi thickness (µm)	Goblet cell (no)	Hepatocyte score
D1	201.00 ± 6.92^{a}	64.66 ± 5.20^{a}	45.00 ± 3.05^{a}	1
D2	193.00 ± 4.04^{a}	61.33 ± 0.88^{a}	50.00 ± 3.00^{a}	1
D3	177.33 ± 4.09^{a}	61.00 ± 1.52^{a}	50.00 ± 4.61^{a}	1
D4	182.66 ± 7.51^{a}	54.33 ± 3.84^{a}	46.00 ± 2.96^{a}	1
D5	179.66 ± 2.96^{a}	62.66 ± 0.88^{a}	48.00 ± 1.76^{a}	1

Different superscripts in a column are significantly different ($P < 0.05$, ANOVA).

cogent food & agriculture

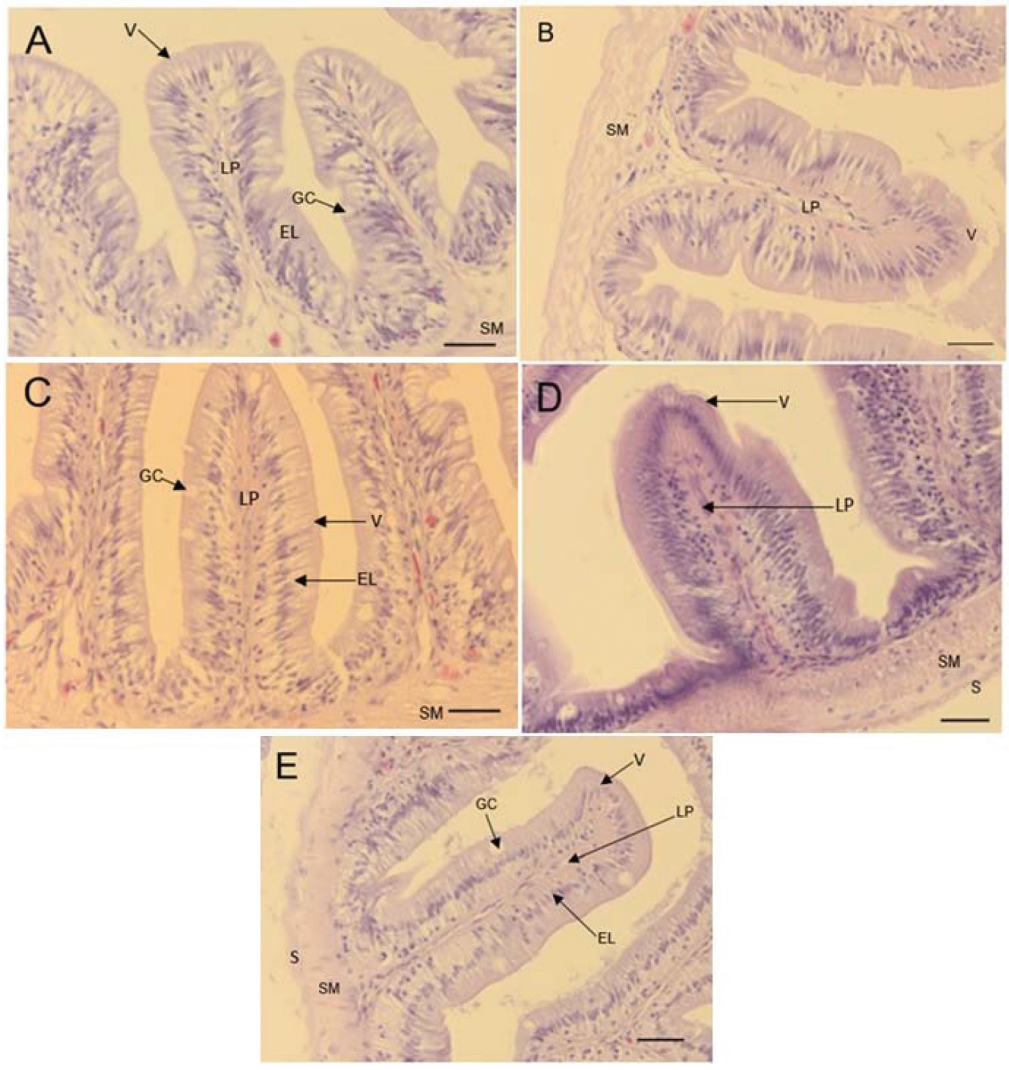

Figure 1. Micrographs of *O. mossambicus* fed Stinkbug meal as fishmeal replacement at different inclusion levels stained with H& E. A: diet D1 (0%), B: diet D2 (10%), C: diet D3 (30%), D: diets D4 (50%), E: diet D5 (70%). V: villi, LP: Lamina propria, GC: goblet cell, EL: epithelial layer, SM: Submucosa, S: Serosa. Scale bar: 20 µm.

cell in the intestine ($P > 0.05$). Furthermore, the micrographs showed normal intestine at different inclusion levels (Figure 1). The liver hepatocyte score was 1 across all diets (Table 8). This was confirmed by the normal liver micrographs of fish from all diets (Figure 2).

5. Discussion

There was a decrease in the growth performance of *O. mossambicus* with increasing Stinkbug meal inclusion. The observed decline in growth performance may be linked to an imbalance in the amino acid profile of the Stinkbug meal. Although the Stinkbug meal contained all the essential amino acids, the concentrations recorded did not meet the requirements of *O. mossambicus*. Methionine and lysine are known to be the most limiting amino acids in fish growth (Inje et al., 2018). The study recorded 1.76 g/kg methionine and 6.85 g/kg lysine levels in the Stinkbug meal. These values are relatively lower than the requirement for *O. mossambicus*. Methionine and lysine requirements for *O. mossambicus* are 9.9 g/kg and 37.8 g/kg, respectively (Santiago & Lovell, 1988). It was thus deemed necessary to supplement experimental diets with crystalline methionine and lysine. The decline in growth performance may also be attributed to an imbalance in the fatty acid profile of the Stinkbug meal. Although Stinkbug meal contains linoleic acid, the study showed that this insect lacks omega-3 fatty acids such as α-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential fatty acids for growth. Like most other terrestrial insects,

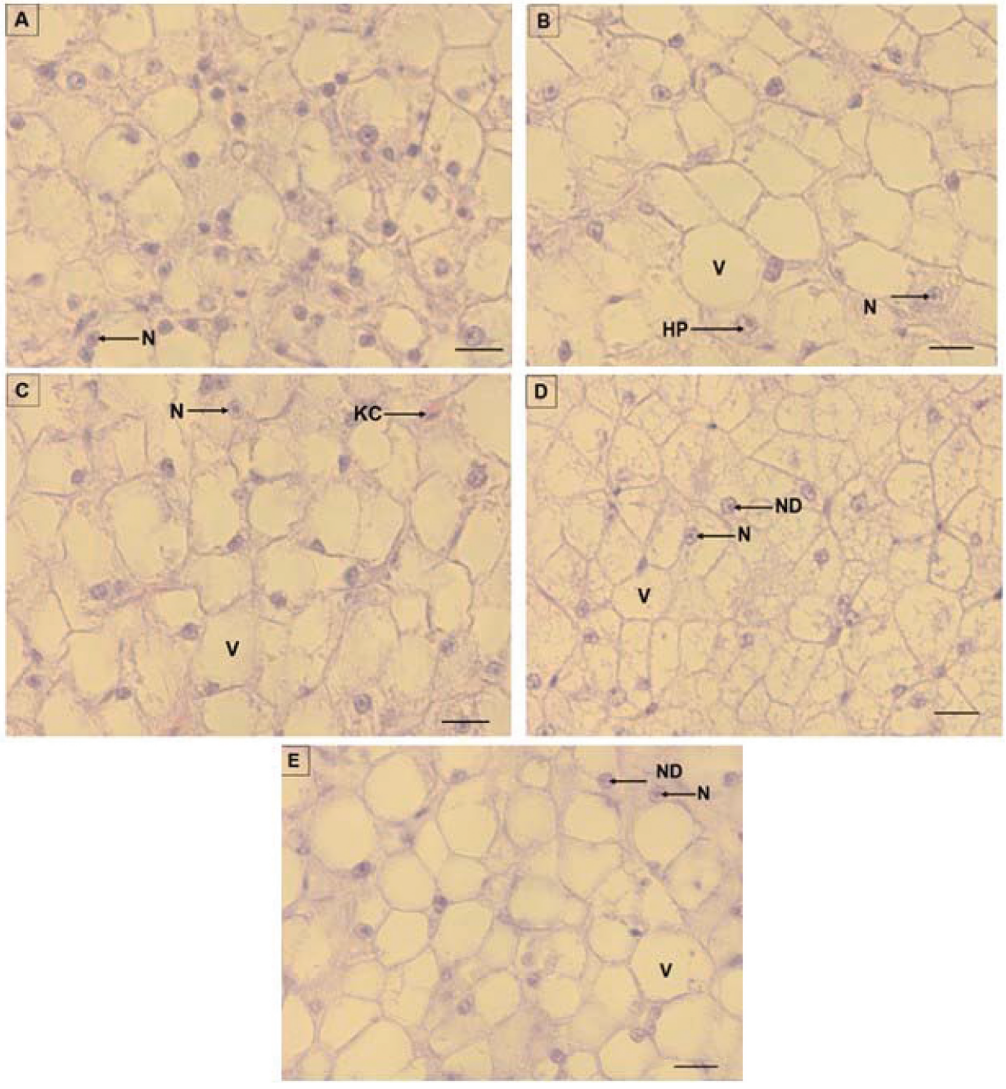

Figure 2. Liver micrographs of *O. mossambicus* fed diets with Stinkbug meal as a fishmeal replacement. A (control: 0%), B (diet D2; 25%), C (diet D3: 30%), D (diet D4: 50%), and E (diet D5; 75%). V: vacuolisation, N: nucleus, ND: nuclear displacement, HP: Hepatic plate, KC: Kupffer cell. Scale bar: 20 µm.

Stinkbug lacks EPA and DHA when compared to aquatic insects (Parmar et al., 2022; Twining et al., 2016). It is also possible that chitin might have contributed to the decrease in growth performance. Although chitin was not determined, NDF and ADF are taken as surrogate measures of chitin (Finke, 2007). Furthermore, chitin interferes with the digestion and absorption of nutrients in fish species, thus compromising fish growth (Gu et al., 2022; Tran et al., 2022). This phenomenon was also reported in studies where insect meal replaced fishmeal in aquafeed (Anvo et al., 2017; Fawole et al., 2020; Zarantoniello et al., 2021).

There was a noticeable reduction in feed intake with an increase in Stinkbug meal inclusion. This may also have contributed to the reduced growth performance that was observed in fish species. The reduced feed intake may be attributed to trans-2-decenal and trans-2-octenal compounds that the Stinkbug releases as a defence mechanism (Sagun et al., 2016). These compounds are known to cause an unpleasant odour that repels predators. The pungent smell was also observed in diets with Stinkbug meal. It is speculated that the Stinkbug odour reduced voluntary feed intake. Although there was a decline in the growth performance, organosomatic indices (CF, HSI, VSI) were not significantly different across all diets. The condition factor (CF) explains the well-being of fish species. In this study, Stinkbug meal did not influence the condition factor. This shows that fish from all experimental diets were in good health. The hepatosomatic index followed a similar trend.

This index reveals the health status of the liver. Thus, utilising diets with Stinkbug meal as a fishmeal replacement did not cause any metabolic or liver deficiencies in *O. mossambicus*. The Viscero-somatic index also did not differ significantly among diets. This confirms that no excessive hepatic fat was accumulated in fish from all diets. Similar findings were observed in fish that have been fed insect meal as a fishmeal substitute (Renna et al., 2017; Tippayadara et al., 2021; Xiao et al., 2018). This suggests that Stinkbug meal had no negative effects on the health status of *O. mossambicus*.

The Stinkbug meal had no influence on the ALT and AST levels. The ALT and AST are liver enzymes that often leak into the bloodstream when there is hepatic damage or increased stress level (Adeoye et al., 2020). Belghit et al. (2018) indicated that feeding an Atlantic salmon (*Salmo salar*) a diet with *H. illucens* as a fishmeal substitute did not affect the ALT and AST levels. *Salmo salar* is one of the fish species that feed on aquatic insects in nature, and it is reportedly pre-adapted to utilising insect-based diets (Henry et al., 2015). In the wild, *O. mossambicus* also feeds on insects (El-Sayed, 2020). It is probably also pre-adapted to utilising insect-based diets. The Stinkbug meal had no effect on the cholesterol and triglyceride levels across diets. Cholesterol levels in fish fed insect-based diets are known to be reduced due to chitin level (Belghit et al., 2018). However, in this study the levels of cholesterol may have not been affected by the chitin level. Similar observations were recorded when *Argyrosomus japonicus* was fed diets with *H. illucens* as a fishmeal replacement (Madibana et al., 2020).

Substituting fishmeal with Stinkbug meal did not affect the liver and intestine histology of *O. mossambicus* from all experimental diets. The liver is an organ involved in nutrient metabolism (Rašković et al., 2011). It is also a good indicator of the nutritional status of the fish. Changes in its morphological structure is paramount in determining the capacity of a species to digest and effectively utilise a diet. Lack of liver histological alterations shows that *O. mossambicus* can utilise an insect-based diet. The intestine villi and thickness across diets did not differ significantly. The goblet cell prevalence was not influenced by the Stinkbug meal in all experimental diets. Goblet cells are responsible for secreting mucus as a protective compound (Kim & Ho, 2010). This shows that the Stinkbug meal did not have detrimental effects on the intestine histology of *O. mossambicus*. The intestine is an organ where feed digestion and nutrient absorption take place (Elia et al., 2018). This further shows that *O. mossambicus* utilised an insect-based diet without adverse effects. This is probably because this species has requisites digestive enzymes to handle an insect-based diet. This agrees with a study where *O. mossambicus* successfully utilised a diet where mopane worm replaced fishmeal (Rapatsa & Moyo, 2017). The study shows that the Stinkbug meal may partially replace fishmeal at 30% inclusion level. This is because at inclusion levels beyond 30%, the growth performance and nutrient utilisation declined significantly. Moreover, at 70% inclusion level, the AST and ALT levels increased significantly. High levels of these liver enzymes may have moderate metabolic effects on juvenile *O. mossambicus*. Furthermore, the FCR recorded at 30% inclusion level (1.72) showed that fish fed this diet were able to convert feed intake into body weight gain. To the best of the authors knowledge, there is no study that evaluated using Stinkbug meal as fishmeal replacement in aqua feeds. Thus, there are no comparable results for this study. The limitation associated with feeding *O. mossambicus* diets with Stinkbug meal is its poor growth performance at higher inclusion levels. Another limitation of using Stinkbug meal is its limited availability and seasonal variation. Stinkbug meal also contain high fat content which may cause oxidation in the diets.

6. Conclusion

This study evaluated the effects of dietary full-fat Stinkbug meal on the growth performance, blood serum chemistry, and liver and intestinal histology of juvenile *O. mossambicus*. The study showed that growth performance declined with an increase in Stinkbug meal. However, the organosomatic indices were not influenced by the inclusion of Stinkbug meal in *O. mossambicus* diets. The AST and ALT values were also not influenced by the Stinkbug meal inclusion. However, at the highest inclusion level, these enzymes were elevated. The liver and intestine were also not influenced by the Stinkbug meal

inclusion. The main limitation with using Stinkbug meal as a fishmeal replacement is its high fat content and an imbalance in fatty acid profile. A cost benefit analysis to determine the economic viability of using Stinkbug meal as a fishmeal replacement is recommended.

Acknowledgments
The National Research Foundation (NRF) and the University of Limpopo, Aquaculture Research Unit are recognised for financial support. Mr. Gavin Geldenhuys is appreciated for his technical assistance.

Funding
This work was supported by the National Research Foundation (NRF) and the University of Limpopo (Aquaculture Research Unit).

Author details
Livhuwani E. Nephale[1]
E-mail: nephale.eva@gmail.com
Ngonidzashe A.G. Moyo[1]
Mmaditshaba M. Rapatsa[1]
[1] Aquaculture Research Unit, School of Agricultural and Environmental Sciences, Faculty of Science and Agriculture, University of Limpopo (Turfloop Campus), Polokwane, South Africa.

Disclosure statement
No potential conflict of interest was reported by the author(s).

Data availability statement
Data is available upon request from the corresponding author(s).

Authors' contributions
Moyo N.A.G. and Rapatsa M.M. conceptualized the study. Nephale L.E. conducted experimental trials, analysed and interpreted the data. The manuscript was drafted by Nephale L.E. and all authors read and revised the manuscript. All authors approved the final manuscript.

Citation information
Cite this article as: Dietary full-fat Stinkbug (*Encosternum delegorguei*) meal effects on growth performance, blood chemistry, liver and intestinal histology of juvenile Mozambique tilapia (*Oreochromis mossambicus*), Livhuwani E. Nephale, Ngonidzashe A.G. Moyo & Mmaditshaba M. Rapatsa, *Cogent Food & Agriculture* (2023), 9: 2253717.

References

Abdel-Tawwab, M., Khalil, R. H., Metwally, A. A., Shakweer, M. S., Khallaf, M. A., & Abdel-Latif, H. M. R. (2020). Effects of black soldier fly (*Hermetia illucens* L.) larvae meal on growth performance, organs-somatic indices, body composition, and hematobiochemical variables of European sea bass, *Dicentrarchus labrax*. *Aquaculture 522*, 735136. Article 735136. https://doi.org/10.1016/j.aquaculture.2020.735136

Adeoye, A. A., Akegbejo-Samsons, Y., Fawole, F. J., & Davies, S. J. (2020). Preliminary assessment of black soldier fly (*Hermetia illucens*) larval meal in the diet of African catfish (*Clarias gariepinus*): Impact on growth, body index, and hematological parameters. *Journal of the World Aquaculture Society*, *51*(4), 1024–1033. https://doi.org/10.1111/jwas.12691

Akinwole, A. O., Dauda, A. B., & Ogunkunle, V. O. (2020). Growth performance, nutrient utilization, survival and body indices of African catfish (*Clarias gariepinus*, Burchell 1822) reared on maggot meal based diet. *FUDMA Journal of Sciences*, *4*(2), 53–59. https://doi.org/10.33003/fjs-2020-0402-109

Alfiko, Y., Xie, D., Astuti, R. T., Wong, J., & Wang, L. (2022). Insects as a feed ingredient for fish culture: Status and trends. *Aquaculture and Fisheries*, *7*(2), 166–178. https://doi.org/10.1016/j.aaf.2021.10.004

Anvo, M. P. M., Aboua, B. R. D., Compaoré, I., Siaaao, R., Zoungrana-Kaboré, C. Y., Kouamelan, E. P., & Toguyéni, A. (2017). Fish meal replacement by *Cirina butyrospermi* caterpillar's meal in practical diets for *Clarias gariepinus* fingerlings. *Aquaculture Research*, *48*(10), 5243–5250. https://doi.org/10.1111/are.13337

AOAC International. (2003). *Official methods of analysis of AOAC International* (17th ed.). Gaithersburg, MD, USA, Association of Analytical Communities.

Baeza-Ariño, R., Martīnez-Llorens, S., Nogales-Merida, S., Jover-Cerda, M., & Tomas-Vidal, A. (2014). Study of liver and gut alterations in sea bream, *Sparus aurata* L., fed a mixture of vegetable protein concentrate. *Aquaculture Research*, *47*(2), 460–471. https://doi.org/10.1111/are.12507

Belghit, I., Liland, N. S., Gjesdal, P., Biancarosa, I., Menchetti, E., Li, Y., Waagobo, R., & Krogdahl, E. L. (2018). Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). *Aquaculture*, *503*, 609–619. https://doi.org/10.1016/j.aquaculture.2018.12.032

Bruni, L., Pastorelli, R., Viti, C., Gasco, L., & Parisi, G. (2018). Characterisation of the intestinal microbial communities of rainbow trout (*Oncorhynchus mykiss*) fed with *Hermetia illucens* (black soldier fly) partially defatted larva meal as partial dietary protein source. *Aquaculture*, *487*, 56–63. https://doi.org/10.1016/j.aquaculture.2018.01.006

Cho, J., Bae, J., & Hwang, I. (2022). Effects of black soldier fly (*Hermetia illucens*) pre-pupae meal on the growth, stress, and immune responses of juvenile rainbow trout (*Oncorhynchus mykiss*) reared at different stocking densities. *Aquaculture Reports*, 25, Article ID 101202.

Cho, C. Y., Slinger, S. J., & Bayley, H. S. (1982). Bioenergetics of salmonid fishes energy intake, expenditure and productivity. *Comparative Biochemistry and Physiology*, *73*(1), 25–41. https://doi.org/10.1016/0305-0491(82)90198-5

Coutinho, F., Castro, C., Guerreiro, I., Rangel, F., Couto, A., Serra, C. R., Peres, H., Pousão-Ferreira, P., Rawski, M., Oliva-Teles, A., & Enes, P. (2021). Mealworm larvae meal in diets for meagre juveniles: Growth, nutrient digestibility and digestive enzymes activity. *Aquaculture 535*, 736362. Article 736362. https://doi.org/10.1016/j.aquaculture.2021.736362

Devic, E., Leschen, W., Murray, F., & Little, D. C. (2017). Growth performance, feed utilization and body composition of advanced nursing Nile tilapia (*Oreochromis niloticus*) fed diets containing black soldier fly (*Hermetia illucens*) larvae meal. *Aquaculture Nutrition*, *24*(1), 416–423. https://doi.org/10.1111/anu.12573

Djissou, A. S. M., Adjahouinou, D. C., Koshio, S., & Fiogbe, E. D. (2016). Complete replacement of fish meal by other animal protein sources on growth performance of *Clarias gariepinus* fingerlings. *International Aquatic Research*, *8*(4), 333–341. https://doi.org/10.1007/s40071-016-0146-x

Elia, A. C., Capucchio, M. T., Caldaroni, B., Magara, G., Dörr, A. J. M., Biasato, I., Biasibetti, E., Righetti, M.,

Pastorino, P., Prearo, M., Gai, F., Schiavone, A., & Gasco, L. (2018). Influence of *Hermetia illucens* meal dietary inclusion on the histological traits, gut mucin composition and the oxidative stress biomarkers in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *496*, 50–57. https://doi.org/10.1016/j.aquaculture.2018.07.009

El-Sayed, A. F. M. (2020). *Tilapia culture* (2nd ed.). Academic Press.

Fawole, F. J., Adeoye, A. A., Tiamiyu, L. O., Ajala, K. I., Obadara, S. O., & Ganiyu, I. O. (2020). Substituting fishmeal with *Hermetia illucens* in the diets of African catfish (*Clarias gariepinus*): Effects on growth, nutrient utilization, haemato-physiological response, and oxidative stress biomarker. *Aquaculture 518*, 734849. Article 734849. https://doi.org/10.1016/j.aquaculture.2019.734849

Finke, M. D. (2007). Estimate of chitin in raw whole insects. *Zoo Biology*, *26*(2), 105–115. https://doi.org/10.1002/zoo.20123

Fischer, H., Romano, N., Renukdas, N., Kumar, V., & Sinha, A. K. (2022). Comparing black soldier fly (*Hermetia illucens*) larvae versus prepupae in the diets of largemouth bass, *Micropterus salmoides*: Effects on their growth, biochemical composition, histopathology, and gene expression. *Aquaculture 546*, 737323. Article 737323. https://doi.org/10.1016/j.aquaculture.2021.737323

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*(1), 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5

Gai, F., Cusimano, G. M., Maricchiolo, G., Caccamo, L., Caimi, C., Macchi, E., Meola, M., Perdichizzi, A., Tartarisco, G., & Gasco, L. (2023). Defatted black soldier fly meal in diet for grow-out gilthead seabream (*Sparus aurata* L. 1758): Effects on growth performance, Gill Cortisol level, digestive enzyme activities, and intestinal histological structure. *Aquaculture Research*, *ID*, 3465335. https://doi.org/10.1155/2023/3465335

Gbai, M., Ouattara, N., Bamba, Y., Ouattara, M., Ouattara, A., & Yao, K. (2019). Substitution of the fishmeal by maggot meal in the feed of Nile tilapia *Oreochromis niloticus* at different stages of growth. *International Journal of Fisheries and Aquaculture Research*, *5*(4), 25–39. 2397-7507. www.eajournals.org

Guerreiro, I., Serra, C. R., Coutinho, F., Couto, A., Castro, C., Rangel, F., Pres, H., Pousão-Ferreira, P., Matos, E., Gasco, L., Gai, F., Olivia-Teles, A., & Enes, P. (2020). Digestive enzyme activity and nutrient digestibility in meagre (*Argyrosomus regius*) fed increasing levels of black soldier fly meal (*Hermetia illucens*). *Aquaculture Nutrition*, *27*(1), 142–152. https://doi.org/10.1111/anu.13172

Gu, J., Liang, H., Ge, X., Xia, D., Pan, L., Mi, H., & Ren, M. (2022). A study of the potential effect of yellow mealworm (*Tenebrio molitor*) substitution for fish meal on growth, immune and antioxidant capacity in juvenile largemouth bass (*Micropterus salmoides*). *Fish and Shellfish Immunology*, *120*, 214–221. https://doi.org/10.1016/j.fsi.2021.11.024

Henry, M., Gasco, L., Piccolo, G., & Fountoulaki, E. (2015). Review on the use of insects in the diet of farmed fish: Past and future. *Animal Feed Science and Technology*, *203*, 1–22. https://doi.org/10.1016/j.anifeedsci.2015.03.001

Henry, M. A., Golomazou, E., Asimaki, A., Psofakis, P., Fountoulaki, E., Mente, E., Rumbos, C. I., Athanassiou, C. G., & Karapanagiotidis, I. T. (2022). Partial dietary fishmeal replacement with full-fat or defatted superworm (*Zophobas morio*) larvae meals modulates the innate immune system of gilthead seabream, *Sparus aurata*. *Aquaculture Reports*, *27*, Article 101347.

Hlophe-Ginindza, S. N., Moyo, N. A. G., & Ngambi, J. W. (2015). The effect of exogenous enzyme supplementation on growth and digestive enzyme activities in *Oreochromis mossambicus* fed kikuyu-based diets. *Aquaculture Research*, *47*(12), 3777–3787. https://doi.org/10.1111/are.12828

Hua, K. (2021). A meta-analysis of the effects of replacing fish meals with insect meals on growth performance of fish. *Aquaculture 530*, 735732. Article 735732. https://doi.org/10.1016/j.aquaculture.2020.735732

Hu, Y., Huang, Y., Tang, T., Zhong, L., Chu, W., Dai, Z., Chen, Hu, Y., & Chen, K. (2020). Effect of partial black soldier fly (*Hermetia illucens* L.) larvae meal replacement of fish meal in practical diets on the growth, digestive enzyme and related gene expression for rice field eel (*Monopterus albus*). *Aquaculture Reports 17*, 100345. Article 100345. https://doi.org/10.1016/j.aqrep.2020.100345

Inje, O. F., Olufunmilayo, A. H., Audu, J. A., Ndaman, S. A., & Chidi, E. E. (2018). Protein quality of four indigenous edible insect species in Nigeria. *Food Science and Human Wellness*, *7*(2), 175–183. https://doi.org/10.1016/j.fshw.2018.05.003

Ishida, Y., Fujita, T., & Asai, K. (1981). New detection and separation method for amino acids by high-performance liquid chromatography. *Journal of Chromatography A*, *204*, 143–148. https://doi.org/10.1016/S0021-9673(00)81650-7

Jabir, A. R., Razak, S. A., & Vikineswary, S. (2012). Nutritive potential and utilization of super worm (*Zophobas morio*) meal in the diet of Nile tilapia (*Oreochromis niloticus*) juvenile. *African Journal of Biotechnology*, *11*(24), 6592–6598. https://doi.org/10.5897/AJB11.1084

Jeong, S. M., Khosravi, S., Mauliasari, I. R., & Lee, S. M. (2020). Dietary inclusion of mealworm (*Tenebrio molitor*) meal as an alternative protein source in practical diets for rainbow trout (*Oncorhynchus mykiss*) fry. *Fisheries and Aquatic Sciences*, *23*(1), 12. https://doi.org/10.1186/s41240-020-00158-7

Kim, Y. S., & Ho, S. B. (2010). Intestinal goblet cells and mucins in health and disease: Recent insights and progress. *Current Gastroenterology Reports*, *12*(5), 319–330. https://doi.org/10.1007/s11894-010-0131-2

Kishawy, A. T. Y., Mohammed, H. A., Zaglool, A. W., Attia, M. S., Hassan, F. A. M., Roushdy, E. M., Ismail, T. A., & Ibrahim, D. (2022). Partial defatted black solider larvae meal as a promising strategy to replace fish meal protein in diet for Nile tilapia (*Oreochromis niloticus*): Performance, expression of protein and fat transporters, and cytokines related genes and economic efficiency. *Aquaculture 555*, 738195. Article 738195. https://doi.org/10.1016/j.aquaculture.2022.738195

Llagostera, P. F., Kallas, Z., Reig, L., & Amores de Gea, D. (2019). The use of insect meal as a sustainable feeding alternative in aquaculture: Current situation, Spanish consumers' perceptions and willingness to pay. *Journal of Cleaner Production*, *229*, 10–21. https://doi.org/10.1016/j.jclepro.2019.05.012

Madibana, M. J., Mwanza, M., Lewis, B. R., Fouché, C. H., Toefy, R., & Mlambo, V. (2020). Black soldier fly larvae meal as a fishmeal substitute in juvenile dusky kob diets: Effect on feed utilization, growth performance, and Blood parameters. *Sustainability*, *12*(22), 9460. https://doi.org/10.3390/su12229460

Makkar, H. P. S., Tran, G., Heuze, V., & Ankers, P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*, *197*, 1–33. https://doi.org/10.1016/j.anifeedsci.2014.07.008

Makore, T. A., Garamumhango, P., Chirikure, T., & Chikambi, S. D. (2015). Determination of nutritional composition of *Encosternum delegorguei* caught in Nerumedzo community of Bikita, Zimbabwe. *International Journal of Biology*, *7*(4), 4. https://doi.org/10.5539/ijb.v7n4p13

Manditsera, F. A., Luning, P. A., Fogliano, V., & Lakemond, C. M. M. (2019). The contribution of wild harvested edible insects (*Eulepida mashona* and *Henicus whellani*) to nutrition security in Zimbabwe. *Journal of Food Composition and Analysis*, *75*, 17–25. https://doi.org/10.1016/j.jfca.2018.09.013

McFadzen, I. R. B., Coombs, S. H., & Halliday, N. C. (1997). Histological indices of the nutritional condition of sardine, *Sardina pilchardus* (walbaum) larvae of the north coast of Spain. *Journal of Experimental Marine Biology and Ecology*, *212*(2), 239–258. https://doi.org/10.1016/S0022-0981(96)02755-4

Mohan, K., Rajan, D. K., Muralisankar, T., Ganesan, A. R., Sathishkumar, P., & Revathi, N. (2022). Use of black soldier fly (*Hermetia illucens* L.) larvae meal in aquafeeds for a sustainable aquaculture industry: A review of past and future needs. *Aquaculture 553*, 738095. Article 738095. https://doi.org/10.1016/j.aquaculture.2022.738095

Muin, H., & Taufek, N. M. (2022). Evaluation of growth performance, feed efficiency and nutrient digestibility of red hybrid tilapia fed dietary inclusion of black soldier fly larvae (*Hermetia illucens*). *Aquaculture and Fisheries*. https://doi.org/10.1016/j.aaf.2022.09.006

Nyuliwe, T. C., Mlambo, V., Madibana, M. J., Mwanza, M., & Wokadala, O. C. (2022). Partial substitution of fishmeal with mopane worm meal in dusky kob fingerling (*Argyrosomus japonicus*) diets: Feed utilization, digestive enzyme activity, Blood parameters, and growth performance. *Aquaculture Journal*, *2*(2), 59–71. https://doi.org/10.3390/aquacj2020006

Parmar, T. P., Kindinger, A. L., Mathieu-Resuge, M., Twining, C. W., Shipley, J. R., Kainz, M. J., & Martin-Creuzburg, D. (2022). Fatty acid composition differs between emergent aquatic and terrestrial insects-A detailed single system approach. *Frontiers in Ecology and Evolution*, *10*. Article 952292. https://doi.org/10.3389/fevo.2022.952292

Prachom, N., Boonyoung, S., Hassaan, M. S., El-Haroun, E., & Davies, S. J. (2021). Preliminary evaluation of superworm (*Zophobas morio*) larval meal as a partial protein source in experimental diets for juvenile Asian sea bass, lates calcarifer. *Aquaculture Nutrition*, *27*(5), 1-1304–1314. https://doi.org/10.1111/anu.13269

Rapatsa, M. M., & Moyo, N. A. G. (2017). Evaluation of Imbrasia belina meal as a fishmeal substitute in *Oreochromis mossambicus* diets: Growth performance, histological analysis and enzyme activity. *Aquaculture Reports*, *5*, 18–26. https://doi.org/10.1016/j.aqrep.2016.11.004

Rapatsa, M. M., & Moyo, N. A. G. (2019). Enzyme activity and histological analysis of *Clarias gariepinus* fed on *Imbrasia belina* meal used for partial replacement of fishmeal. *Fish Physiology and Biochemistry*, *45*(4), 1309–1320. https://doi.org/10.1007/s10695-019-00652-3

Rapatsa, M. M., Moyo, N. A. G. (2022). A review and meta-analysis of the effects of Replacing fishmeal with insect meals on growth of tilapias and sharptooth catfish. *Aquaculture Nutrition 2022*, 1–10. Article 9367587. https://doi.org/10.1155/2022/9367587

Rašković, B. S., Stanković, M. B., Marković, Z. Z., & Poleksić, V. D. (2011). Histological methods in the assessment of different feed effects on liver and intestine of fish. *Journal of Agricultural Sciences*, *56*(1), 87–100. https://doi.org/10.2298/JAS1101087R

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., Prearo, M., Capucchio, M. T., Biasato, I., Biasibetti, E., De Marco, M., Brugiapaglia, A., Zoccarato, I., & Gasco, L. (2017). Evaluation of the suitability of a partially defatted black soldier fly (*Hermetia illucens* L.) larvae meal as ingredient for rainbow trout (*Oncorhynchus mykiss* walbaum) diets. *Journal of Animal Science and Biotechnology*, *8*(1). Article 57. https://doi.org/10.1186/s40104-017-0191-3

Rumpold, B. A., & Schlüter, O. K. (2013). Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science and Emerging Technologies*, *17*, 1–11. https://doi.org/10.1016/j.ifset.2012.11.005

Sagun, S., Collins, E., Martin, C., Nolan, E. J., & Horzempa, J. (2016). Alarm odor compounds of the brown marmorated stink bug exhibit antibacterial activity. *Journal of Pharmacognosy and Natural Products*, *2*(3), 119. https://doi.org/10.4172/2472-0992.1000119

Saleh, H. H. E. (2020). Effect of feeding on fresh (wet) housefly maggots (*Musca domestica*) with or without Artificial diet on water Quality and growth Rates of African catfish (*Clarias gariepinus* Burchell, 1822) fry under laboratory conditions. *Journal of Zoological Research*, *2*(2). https://doi.org/10.30564/jzr.v2i2.2053

Santiago, C. B., & Lovell, R. T. (1988). Amino acid requirements for growth of Nile tilapia. *Journal of Nutrition*, *188*(12), 1540–1546. https://doi.org/10.1093/jn/118.12.1540

Teffo, L. S., Toms, R. B., & Eloff, J. N. (2007). Preliminary data on the nutritional composition of the composition of the edible stink-bug, *ncosternum delegorguei* Spinola, consumed in Limpopo province, South Africa. *South African Journal of Science*, *103*, 434–436. https://hdl.handle.net/10520/EJC96630

Terova, G., Gini, E., Gasco, L., Moroni, F., Antonini, M., & Rimoldi, S. (2021). Effects of full replacement of dietary fishmeal with insect meal from *Tenebrio molitor* on rainbow trout gut and skin microbiota. *Journal of Animal Science and Biotechnology*, *12*(1). Article 30. https://doi.org/10.1186/s40104-021-00551-9

Tippayadara, N., Dawood, M. A. O., Krutmuang, P., Hoseinifar, S. H., Doan, H. V., & Paolucci, M. (2021). Replacement of fish meal by black soldier fly (*Hermetia illucens*) larvae meal: Effects on growth, haematology, and skin mucus immunity of Nile tilapia, *Oreochromis niloticus*. *Animals*, *11*(1), 193. https://doi.org/10.3390/ani11010193

Tran, H. Q., Prokešová, M., Zare, M., Matoušek, J., Ferrocino, I., Gasco, L., & Stejskal, V. (2022). Production performance, nutrient digestibility, serum biochemistry, fillet composition, intestinal microbiota and environmental impacts of European perch (*Perca fluviatilis*) fed defatted mealworm (*Tenebrio molitor*). *Aquaculture 547*, 737499. Article 737499. https://doi.org/10.1016/j.aquaculture.2021.737499

Tschirner, M., & Simon, A. (2015). Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. *Journal of Insects as Food and Feed*, *1*(4), 249–259. https://doi.org/10.3920/JIFF2014.0008

Twining, C. W., Brenna, J. T., Lawrence, P., Shipley, J. R., Tollefson, T. N., & Winkler, D. W. (2016). Omega-3 long-chain polyunsaturated fatty acids support aerial insectivore performance more than food quantity. *Proceedings of the National Academy of Sciences*, *113* (39), 10920–10925. https://doi.org/10.1073/pnas.1603998113

van Dyk, J. C., & Pieterse, G. M. (2008). A histo-morphological study of the testis of the sharp-tooth catfish (*Clarias gariepinus*) as reference for future toxicological assessments. *Journal of Applied Ichthyology*, *24*(4), 415–422. https://doi.org/10.1111/j.1439-0426.2008.01127.x

van Huis, A. (2013). Potential of insects as food and feed in assuring food security. *Annual Review of Entomology*, *58*(1), 563–583. https://doi.org/10.1146/annurev-ento-120811-153704

van Huis, A. (2022). Edible insects: Challenges and prospects. *Entomological Research*, *52*(4), 161–177. https://doi.org/10.1111/1748-5967.12582

van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*(10), 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Xiao, X., Jin, P., Zheng, L., Cai, M., Yu, Z., Yu, J., & Zhang, J. (2018). Effects of black soldier fly (*Hermetia illucens*) larvae meal protein as a fishmeal replacement on the growth and immune index of yellow catfish (*Pelteobagrus fulvidraco*). *Aquaculture Research*, *49*(4), 1–9. https://doi.org/10.1111/are.13611

Zarantoniello, M., Randazzo, B., Secci, G., Notarstefano, V., Giorgini, E., Lock, E. J., Parisi, G., & Olivotto, I. (2021). Application of laboratory methods for understanding fish responses to black soldier fly (*Hermetia illucens*) based diets. *Journal of Insects as Food and Feed*, *8*(11), 1173–1195. https://doi.org/10.3920/JIFF2020.0135

APPENDIX B

Journal of Animal and Feed Sciences, 33, 2, 2024, 243–252, https://doi.org/10.22358/jafs/175919/2024
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

Partial replacement of fish meal with soldier termite in juvenile Mozambique tilapia: Effects on growth performance, blood serum chemistry and histomorphology

L.E. Nephale*, N.A.G. Moyo and M.M. Rapatsa-Malatji

University of Limpopo, School of Agricultural and Environmental Sciences, Faculty of Science and Agriculture, Aquaculture Research Unit, Sovenga, 0727, South Africa

KEY WORDS: ALT, AST, histomorphology, *Macrotermes falciger*, *Oreochromis mossambicus*

Received: 18 September 2023
Revised: 24 November 2023
Accepted: 27 November 2023

* Corresponding author:
e-mail: nephale.eva@gmail.com

ABSTRACT. Insect-based meals have emerged as a viable global scale alternative to fish meal in aquafeed. This is mainly due to their high protein content, balanced amino acid composition, and fatty acid profile, which closely resembles that of fish meal. An 8-week trial was conducted to evaluate the growth performance, blood serum chemistry, and histomorphology of Mozambique tilapia (*Oreochromis mossambicus*) fed diets containing soldier termite meal as a partial substitute for fish meal. Five isonitrogenous, isolipidic, and isocaloric diets were formulated to partially replace fish meal with soldier termite meal at 0, 10, 30, 50, and 70%, labelled as D1, D2, D3, D4, and D5, respectively. The study showed that soldier termite meal could replace fish meal up to a 50% inclusion level. The growth performance and nutrient utilisation of fish fed diet D4 (50%) were comparable to fish fed the control diet. Fish fed the diet with the highest proportion of soldier termite meal (70%) showed significantly higher alanine aminotransferase and aspartate aminotransferase levels ($P < 0.05$). Cholesterol, triglyceride, and glucose levels were not influenced by the inclusion of soldier termite meal in the diet of Mozambique tilapia ($P > 0.05$). The histomorphological examination of the intestines revealed no discernible alterations. The current study has demonstrated that soldier termite meal can replace fish meal up to 50% of the feed content without inducing adverse effects on growth performance and health status of *O. mossambicus*. The cost-benefit analysis showed that substituting fish meal with soldier termite meal was economically sustainable.

Introduction

The Mozambique tilapia (*Oreochromis mossambicus*) is one of the species of economic importance in southern Africa. It is a valuable source of animal protein, especially in rural communities. *O. mossambicus* also plays a role in generating income for local fish farmers. This species is well-suited for aquaculture due to its adaptability to adverse environmental conditions, rapid growth rate, stress and disease resistance, reproductive capability in captivity, and versatile dietary preferences (El-Sayed, 2006). It is therefore one of the commonly cultured warm freshwater species in southern Africa. In South Africa, *O. mossambicus* culture is mainly practiced in the economically disadvantaged rural communities of Limpopo and Mpumalanga Provinces. *O. mossambicus* production in South Africa has not yet reached its full potential. Nonetheless, the aquaculture sector in the country achieved a noteworthy total production of 5 418 tons in 2015, valued at R 696 million (Adeleke et al., 2021).

Moyo and Rapatsa (2021) have identified the cost and feed quality as one of the major factors affecting tilapia production in southern Africa. Fish feed accounts for over 70% of the total costs of aquaculture operations. Thus, cheap alternatives can significantly improve aquaculture production.

Fish meal has been recognised as the main protein source in the aquaculture industry. This is primarily due to its high protein content, balanced amino acid and fatty acid profiles, high digestibility, and palatability (Abdel-Tawwab et al., 2020). However, the depletion of wild fish catch heightened the demand for fish meal, and its rising cost (1851.82 USD/ Metric ton) and limited availability have rendered fish meal an unsustainable protein source (Adeoye et al., 2019). This has led to escalating commercial pellet prices, placing a financial burden on many rural fish farmers. This pressing challenge necessitates the exploration of fish meal substitutes to provide cost-effective and nutritionally reach diets for rural fish farmers. Insect meals have gained recognition globally as a promising alternative for fish meal (Alfiko et al., 2022; Rapatsa and Moyo, 2022; Gebremichael et al., 2023). Most insect meals have a rich nutritional composition, balanced fatty acid composition, and favourable amino acid profile (Hua et al., 2021). Moreover, insects have a low carbon footprint compared to other meat industries (Gasco et al., 2021). Diptera is one of the orders known to contain protein and amino acid profiles comparable to fish meal. Therefore, it is imperative to search for insects with the potential to partially replace fish meal without compromising fish growth performance and their nutrient utilisation.

Rapatsa and Moyo (2017) evaluated the Mopane worm (*Imbrasia belina*) as a potential fish meal substitute in the diet of *O. mossambicus*. The Mopane worm is a commonly found insect widely distributed in South Africa and neighbouring countries. The study assessed the growth performance, histology, and enzyme activity of *O. mossambicus* fed diets containing Mopane worms. The latter study showed that specific growth rate (SGR), thermal unit growth coefficient (TGC), protein efficiency ratio (PER), and apparent digestibility coefficient of protein (ADCP) increased with raising levels of Mopane worm meal supplementation. Importantly, the liver and intestine histology of *O. mossambicus* showed no alterations across all diets tested. It was concluded that Mopane worm meal could be a viable candidate for fish meal replacement in the diet of *O. mossambicus*. Additionally, it highlighted the potential of *O. mossambicus* to effectively utilise an insect-based diet. Despite the positive nutritional index, the challenge associated with Mopane worm meal as a fish meal substitute is its high cost, limiting its overall feed value. Consequently, there is a paramount need to explore locally available insects that can serve as partial replacements for fish meal in the diet of *O. mossambicus*.

The Southern African Development Community (SADC) is abundantly endowed with a diverse range of insects (Mariod, 2020). These insects are primarily valued as a rich protein source, and extensive exploration has been conducted on their nutritional composition. Among them, the soldier termite (*Macrotermes falciger*) is one of the commonly found and consumed insects in Africa (Netshifhefhe and Duncan, 2022). There is a noticeable scarcity of proximate and nutritional composition data on soldier termite in the published literature. The soldier termite is an ideal candidate for incorporation into aquafeed due to its high protein content, vitamins, minerals, and beneficial amino acid profile (Netshifhefhe and Duncan, 2022). Although the fatty acid profile of *M. falciger* is not extensively documented, it is noteworthy that this insect contains elevated levels of linoleic acid – an essential fatty acid crucial for fish growth. Unlike most insects, which are only harvested during the rainy season, soldier termites are collected throughout the year. This significantly increases their availability for use in the animal feed industry. In contrast to most terrestrial insects, such as the Mopane worm (*I. belina*) and stink bug (*Encosternum delegorguei*), which are confined to specific regions, soldier termites are widely distributed across Africa. This makes them readily accessible to farmers in both urban and rural communities. Amongst the local terrestrial insects available on the market, soldier termites stand out as a cost-effective and affordable option. While there have been reports of the use of soldier termites as poultry feed in Burkina Faso and Ghana (Boafo et al., 2019), their potential to replace fish meal in aquafeed remains unexplored. Therefore, the null hypothesis of the study posited that replacing fish meal with soldier termite meal would have no discernible effect on the growth performance, blood serum chemistry, and histomorphology of juvenile *O. mossambicus*.

Material and methods

Ethical approval

The study has received ethical approval from the University of Limpopo Animal Research Ethical Committee (AREC/09/2022:PG).

Experimental diets

Soldier termites (sun-dried) were purchased from the Thohoyandou Open market in South Africa. Soldier termite meal was ground to powder and subsequently used to substitute fish meal at varying proportions: 0, 10, 30, 50, and 70%. These dietary formulations were designated as D1, D2, D3, D4, and D5, respectively (Table 1). The diets were formulated to be isonitrogenous (30% protein), isolipidic (12% fat), and isocaloric (15 MJ/kg) using Winfeed 3, EFG (Natal) software. The control diet (D1) contained 30% fishmeal and no insect meal. Feed ingredients were weighed and mixed using a planetary mixer (Hobart, Troy, OH, USA). During mixing, water (10–20% v/w) was added as required until the desired dough thickness was reached. The mixture was granulated into a 3-mm pellets using a meat mincer connected to a planetary mixer. The pellets were separately collected on trays labelled with the respective diet names and sun-dried. After drying, the pellets were stored in polyethylene containers, which were also marked with the corresponding names of the diets.

Table 1. Ingredients (g/kg) and proximate composition of experimental diets replacing fish meal with soldier termite meal at different inclusion levels

Fish meal replacement	0% (Control)	10%	30%	50%	70%
Diets	D1	D2	D3	D4	D5
Fish meal[1]	300	270	210	150	90
Soldier termites	0	30	90	150	210
Maize	211.8	204.8	219	224.8	200
Wheat bran	196	185.9	166.1	180.1	160.1
Sunflower meal	78.5	96	102.3	82.8	126
Soybean meal	60	60	60	60	60
Sunflower oil	63.7	63.3	62.6	62.3	63.9
Methionine[2]	20	20	20	20	20
Lysine[2]	20	20	20	20	20
Vit/min premix[3]	20	20	20	20	20
Binder[4]	30	30	30	30	30
Total	1000	1000	1000	1000	1000
Proximate composition					
crude protein, %DM	30.56	30.87	30.08	30.19	30.39
fat, %DM	12.32	12.07	12.02	12.03	12.39
GE, MJ/kg	15.12	15.08	15.41	15.03	15.33
DM, %	91.60	91.86	92.33	92.56	92.80

D1 – diet 1 (0% of soldier termite), D2 – diet 2 (10%), D3 – diet 3 (30%), D4 – diet 4 (50%), D5 – diet 5 (70%), GE – gross energy, DM – dry matter; [1] fish meal: 65.5% crude protein, 12% fat, 18% ash (Irvine's Africa Pty Ltd., South Africa); [2] methionine and lysine (Nutroteq; South Africa); [3] vitamin/mineral premix composed of: IU: vit. A 12 000, vit. D_3 1 200, vit. E 120; g: vit. B_4 1 000, vit. C 120, vit. B_3 25, vit. B_5 15, vit. B_2 6, vit. B_6 5, vit. B_1 4, vit. K_3 2, vit. B_9 1, vit. H 0.25, vit. B_{12} 0.04, ZnO 200, $FeSO_4$ 65, $CuSO_4$ 7, MnO 5, KI 2, Na_2SeO_3 0.15, $CoSO_4$ 0.05 (Irvine's Africa (Pty) Ltd., South Africa); [4] Binder (Irvine's Africa (Pty) Ltd., South Africa)

Proximate composition

Soldier termite meal and experimental diets were analysed for the proximate composition according to AOAC (2012). Dry matter and moisture contents were analysed by drying the samples for 24 h at 105 °C until constant weight was reached. Ash content was determined by weight loss after incinerating the samples in a muffle furnace for 6 h at 550 °C (Heraeus Instruments K1252, Hanau, Germany). Fat content was determined by petroleum ether extraction using a Soxhlet apparatus for 16 h (Soxtec system, Foss Tecator Lipid Analyser, FOSS, Hillerød, Denmark). Crude protein was determined using a Micro-Kjeldahl apparatus (Foss 2400, Kjeltec Analyser Unit, FOSS, Hillerød, Denmark), and protein content was calculated as N × 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by boiling the samples with a neutral detergent solution and an acid detergent solution, respectively (van Soest et al.,1991). Gross energy was analysed using a Digital Data Systems (DDS) Isothermal CP 500 bomb calorimeter (DDS Calorimeters, Digital Data Systems (Pty) Ltd., Randburg, South Africa). The proximate composition of experimental diets is outlined in Table 1, while the proximate composition of soldier termite meal is listed in Table 2.

Mineral content

Concentrations of selected minerals (iron, potassium, and sodium) in soldier termite meal were analysed by hydrolysing the samples with 65% concentrated nitric acid and 37% hydrochloric acid. Hydrogen peroxide was used to remove nitrous vapour. The mineral content was subsequently determined using Inductively coupled plasma-optical emission spectrometry (ICP-OES, ICPE-9800, Shimadzu® Europa GmbH, Duisburg, Germany) (Manditsera et al., 2019). The analysis was performed using standard solutions of predetermined concentrations. All chemicals used were of analytical reagent grade. The mineral content of soldier termite meal is shown in Table 2.

Amino acid and fatty acid analysis

Soldier termite meal was analysed for amino acids and fatty acid profiles at the Central Analytical Facility (CAF) of Stellenbosch University (South Africa). For amino acid analysis: samples (20 mg) were hydrolysed with 6 M HCl at 110 °C for 4 h in sealed tubes (Ishida et al., 1981). Approximately 130 µl was transferred into a 2-ml tube and dried under a gentle stream of nitrogen. The samples were reconstituted and derivatised with 30 µl of

Table 2. Proximate composition, mineral content, and amino acid profile of soldier termite meal compared to fish meal, and *Oreochromis mossambicus* requirements

Proximate composition, %DM				Amino acid profile, g/kg	
Soldier termite meal		Soldier termite meal		*Fishmeal	**Requirement for *O. mossambicus*
DM, %	85.47	Lysine	14.06	42.2	37.8
Ash, %DM	5.05	Methionine	5.50	19.4	09.9
Fat, %DM	7.29	Phenylalanine	7.39	37.4	25.0
Protein, %DM	57.58	Valine	6.43	27.7	22.0
Carbohydrates	16.25	Tryptophan	0.31	5.7	0.43
NDF, %DM	61.99	Threonine	7.00	23.1	29.3
ADF, %DM	20.27	Isoleucine	6.45	24.5	20.1
Energy, KJ/100g	1 581	Histidine	9.57	17.5	10.5
Minerals, mg/l		Leucine	8.11	37.9	34.0
Fe	3.4	Tyrosine	5.89		
P	46.3	Glycine	6.79		
Na	23.3	Aspartic acid	8.98		
		Serine	9.24		
		Proline	6.24		
		Glutamic acid	24.88		
		Alanine	7.68		
		Cystine	17.77		

DM – dry matter, NDF – neutral detergent fibre, ADF – acid detergent fibre; * Djssou et al. (2016); ** Santiago and Lovell (1988)

N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) and 100 µl of acetonitrile at 100 °C for 1 h, cooled down to room temperature, and injected into a 6890N gas chromatograph (Agilent technologies network). Separation was performed using a gas chromatograph coupled to an inert XL EI/CI Mass Selective Detector (MSD) (5975, Agilent Technologies Inc., Santa Clara, CA, USA). The amino acid profile of soldier termite meal is outlined in Table 2.

For the analysis of fatty acids, a methodology of Folch et al. (1957) was employed, where 2 ml of a 2:1 chloroform:methanol mixture was combined with 100 mg of the sample (Folch et al., 1957). The sample was vortexed and sonicated at room temperature for 30 min, and then centrifuged at 3000 rpm for 1 min. Chloroform (200 µl) was completely evaporated with a gentle stream of nitrogen, reconstituted with 170 µl of methyl tert-butyl ether (MTBE) and 30 µl of trimethylsulfonium hydroxide (TMSH) and vortexed. The derivatised sample (1 µl) was injected in a 5:1 split ratio into a gas chromatography-flame ionization detector (GC-FID). Separation was performed using a 6890N gas chromatograph (Agilent Technologies, Santa Clara, United States) coupled to a flame ionization detector (FID). Separation of fatty acid methyl esters (FAME) was performed using a polar RT-2560 capillary column (100 m, 0.25 mm ID, 0.20 µm film thickness) (Restek, Bellefonte, PA, USA). Hydrogen was used as the carrier gas at a flow rate of 1.2 ml/min, and the injector temperature was maintained at 240 °C. One microlitre of the sample was injected at a 5:1 split ratio. The oven temperature was programmed as follows: 100 °C for 4 min, ramped up to 240 °C at a rate of 3 °C/min for 10 min. The fatty acid profile of soldier termite meal is presented in Table 3.

Fish stocking

The experiment was conducted at the Aquaculture Research Unit (ARU) of the University of Limpopo, Limpopo Province, South Africa. Male Mozambique tilapia (*O. mossambicus*) juveniles were obtained from the ARU hatchery. During the acclimatisation period, fish were fed a commercial diet (Aqua-plus, Avi Products (Pty) Ltd.) for two weeks. During stocking, fish were sedated using 2-phenoxyethanol (1 ml/5 l) solution prior to stocking in the experimental tanks. Fish were weighed prior to the commencement of the experimental feeding trial.

Experimental design

A total of 150 juvenile male *O. mossambicus* of uniform size (average initial weight 9.70 ± 1.2 g) were randomly assigned to 5 dietary groups in triplicate at a stocking density of 10 fish per tank. The weight of 10 fish in each tank was recorded (bulk weight). The experiment was conducted in a recirculating aquaculture system (RAS) using 500 l fiberglass tanks filled to a level of 400 l. The tanks were connected to a sump and a pump that supplied the tanks with water at a rate of 10 l/min.

Each tank was heated with a submersible aquarium heater (ViaAqua Glass heater, 200W, Commodity Axis Inc., Camarillo, CA, USA) and aerated with an air stone. Fish were fed their assigned diets to apparent satiation for 8 weeks at 09:00 and 15:00. Fish were considered satiated when 2 sinking pellets were left uneaten for 3 min. The experiment was conducted under a natural photoperiod. Feed intake was recorded daily for each tank by weighing the pellets in a Petri dish and feeding the fish in a specific tank to apparent satiation twice daily. Feed intake per day was calculated by subtracting the final feed weight from its initial weight.

Water quality management

Water quality parameters were monitored daily using a Professional plus YSI 605000 handheld multiparameter meter (YSI Inc., Yellow Springs, OH, USA). Water temperature was maintained within the range of 27–29 °C, dissolved oxygen levels between 6.5 and 8.0 mg/l, ammonia <1 mg/l, and pH at 6.8–8.0.

Growth performance parameters

At the end of the feeding trial, fish were starved for 24 h before final measurement of growth parameters. Fish from each tank were collected, anesthetised (2-phenoxyethanol, 1 ml/5 l), and weighed in bulk. The following parameters were calculated:

$$\text{specific growth rate (SGR)} = \left[\frac{\ln W_t - \ln W_0}{t}\right] \times 100\%,$$

where: In, W_t, W_0, and t are natural logarithm, final body weight (g), initial body weight (g), and feeding time (days), respectively;

$$\text{thermal-unit growth coefficient (TGC)} = 1000 \times \frac{\text{final weight (g)}^{\frac{1}{3}} - \text{initial weight (g)}^{\frac{1}{3}}}{\text{temperature (°C)} \times \text{days}};$$

where: feed conversion ratio (FCR) = feed consumed (g) / weight gain (g);
protein efficiency ratio (PER) = weight gain (g) / protein consumed (g);
weight gain (WG) = final weight (g) − initial weight (g);
feed intake (FI) = weight of feed consumed (g) / fish/day;
fish survival = (final number of fish / initial number of fish) × 100.

Organosomatic indices

Three fish from each dietary replicate (9 fish/diet) were used to measure the following organosomatic indices: condition factor (CF), hepatosomatic index (HSI), and viscero-somatic index (VSI), which were calculated as follows:

CF = body weight (g) / fish length (cm)^3 × 100;
HSI = liver weight (g) / fish weight (g) × 100;
VSI = visceral weight (g) / fish weight (g) × 100, visceral weight includes the liver.

Blood serum chemistry

Three fish from each dietary replicate were randomly selected and sedated with 2-phenoxyethanol (1 ml/5 l). Blood samples (± 3 ml) were drawn from the caudal vasculature into vials using heparinised syringes and centrifuged for 10 min at 3500 rpm. Blood samples from each replica were labelled based on their respective dietary groups. Serum was used for the analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides, cholesterol, and glucose. The analyses were conducted using a commercial automatic biochemical kit (Sigma-Aldrich, Burlington, MA, USA).

Intestinal histomorphology analysis

For the analysis of intestinal histomorphology, three fish were selected from each dietary replicate (9 fish/diet). The distal part of the intestine of each fish was individually preserved in 10% formalin solution in sampling bottles. During analysis, the intestine samples were cut into 1-cm-long sections. All samples were washed for 2 h in distilled water, and subsequently dehydrated in a graded ethanol series (70–96%) and cleared using xylene. Tissue-Tek® III wax (Sakura Finetek, Torrance, CA, USA) was used to infiltrate the samples in an oven at 60 °C. The samples were then embedded in Tissue-Tek® III wax blocks, and cut into 5-µm-thick sections using a microtome (Leica: RM2155, Madrid, Spain). The sectioned samples were placed onto microscope slides and allowed to dry. Following this step, the slides were stained with haematoxylin and eosin (H&E) and covered with coverslips as described by van Dyk and Pieterse (2008).

Intestinal histomorphology assessment

Intestine slides were assessed using a light microscope (Leica Microsystems model DM750, Leica, Bannockburn, IL, USA) equipped with a digital camera (Digital Video Camera Company, Austin, TX, USA). Slide micrographs and intestinal villus height and thickness were captured using ANALYSIS™ digital image analysis software (Soft Imaging Systems GmbH, Münster, Germany). Intestinal histology was assessed by measuring villus length and thickness; six measurements per slide were

taken to establish mean values (n = 6). Slides from each replicate were labelled according to their respective diets.

Cost-benefit analysis

The cost-benefit analysis was determined following the methodology outlined by Bahnasawy et al. (2003):

$$\text{incidence cost} = \frac{\text{cost of feed}}{\text{quantity of fish produced (kg)}};$$

$$\text{profit index} = \frac{\text{local market value of fish}}{\text{cost of feed}}.$$

The underlying assumption in this analysis is the constancy of all operating costs, with the cost of ingredients being the sole variable cost.

Statistical analysis

Prior to statistical analyses, the Shapiro-Wilk and Levene tests were used to examine data for normality of distribution and homogeneity of variance, respectively. One-way analysis of variance (ANOVA) was used to test for significant differences in growth parameter indices, blood serum chemistry, and histomorphology. Significant differences between means were determined using the Tukey HSD post hoc test. All statistical data was considered significant at $P < 0.05$. The data were statistically analysed using the SPSS software package version 28.0 (Statistical Package and Service Solutions, IBM, Chicago, IL, USA).

Results

Proximate composition

Soldier termite meal contained approx. 58% protein and 5% ash (Table 2), while NDF and ADF at approx. 62% and 20%, respectively. Potassium was the dominant mineral in soldier termite meal.

Soldier termite meal also contained both essential and non-essential amino acids (Table 2). Lysine (14.06 g/kg), histidine (9.57 g/kg), and leucine (8.11 g/kg) dominated the essential amino acid profile, but the meal also contained 5.50 g/kg methionine. The amino acid profile of fish meal was higher compared to soldier termite meal and amino acid requirement for *O. mossambicus*.

The fatty acid profile analysis revealed that soldier termite meal was rich in saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids (Table 3). Key components of the fatty acid profile included oleic acid (0.47 g/kg), stearic acid (0.28 g/kg), and linoleic acid (0.22 g/kg), which dominated the composition (Table 3). Although in small quantities, soldier termite meal contained α-linolenic acid (0.02 g/kg), which is one of the limiting fatty acids in fish diets.

Table 3. Fatty acid profile of soldier termite meal (*Macrotermes falciger*)

Fatty acids	g/kg
C14:0 myristic acid	0.01
C16:0 palmitic acid	0.17
C17:0 margaric acid	0.04
C18:0 stearic acid	0.28
C20:0 arachidic acid	0.02
C17:1n-10 heptadecenoic acid	0.02
C18:1n-9(cis) oleic acid	0.47
C18:2n-6 (cis) linoleic acid	0.22
C18:3n-3 α-linolenic acid	0.02
Σ SFA	0.54
Σ MUFA	0.49
Σ PUFA	0.24

SFA – saturated fatty acid, MUFA – monounsaturated fatty acid, PUFA – polyunsaturated fatty acid

Growth performance indices

The best results concerning the growth performance (SGR, TGC, FI, PER) were obtained for fish fed the control diet (Table 4); the lowest FCR values were also recorded for this group of fish (1.58). Of the diets with soldier termite meal as a substitute for fish meal, diet D4 showed the highest SGR, TGC, and PER, and these values were significantly different from the other diets ($P < 0.05$) (Table 4). All growth performance indices declined at inclusion levels above 50% ($P < 0.05$). The addition of soldier termite meal had no effect on somatic indices ($P > 0.05$). No mortality was recorded in any dietary groups.

Blood serum chemistry

The lowest ALT values, not significantly different from each other ($P > 0.05$), were obtained for the control diet (D1) and diet D4 (Table 5). A similar trend was observed for AST values. However, at inclusion levels exceeding 50%, both ALT and AST values showed a significant increase ($P < 0.05$). Cholesterol, triglyceride, and glucose levels were not affected by soldier termite meal supplementation ($P > 0.05$) in any of the dietary groups (Table 5).

Intestinal histomorphology

The analysis of villus height showed no discernible pattern, while villus thickness was not influenced by the incorporation of soldier termite meal in the diet of *O. mossambicus* (Table 6).

The inclusion of soldier termite meal in the diet of *O. mossambicus* exerted no marked effect on intestinal histomorphology (Figure 1). Fish across all

Table 4. Growth performance and somatic indices (mean ± standard error) of *Oreochromis mossambicus* fed soldier termite meal as a fish meal replacement at different inclusion levels for 8 weeks; n = 9

Parameters	Diets				
	D1 (0%)	D2 (10%)	D3 (30%)	D4 (50%)	D5 (70%)
IBW, g	97.83 ± 2.23	98.00 ± 3.80	98.73 ± 2.32	97.5 ± 3.58	98.4 ± 1.91
FBW, g	310.50 ± 3.99	207.9 ± 4.57	239.00 ± 2.88	268.7 ± 2.66	173.00 ± 3.76
WG, g	212.67 ± 5.24^{a}	109.9 ± 2.52^{d}	140.27 ± 2.44^{c}	171.2 ± 3.5^{b}	74.6 ± 1.53^{e}
SGR, %/day	2.02 ± 0.09^{a}	1.31 ± 0.05^{d}	1.55 ± 0.11^{c}	1.77 ± 0.07^{b}	0.98 ± 0.03^{e}
FCR	1.58 ± 0.09^{e}	2.93 ± 0.05^{b}	2.22 ± 0.11^{c}	1.96 ± 0.07^{d}	3.84 ± 0.03^{a}
TGC	1.35 ± 0.10^{a}	0.82 ± 0.04^{d}	0.99 ± 0.05^{c}	1.15 ± 0.11^{b}	0.59 ± 0.04^{e}
FI, g/fish/day	0.79 ± 0.03^{a}	0.68 ± 0.06^{b}	0.73 ± 0.02^{a}	0.75 ± 0.06^{a}	0.59 ± 0.01^{c}
PER	1.53 ± 0.11	1.31 ± 0.05	1.34 ± 0.27	1.43 ± 0.21	1.24 ± 0.03
Somatic indices					
CF, g/cm^{3}	2.31 ± 0.96	1.48 ± 0.02	1.32 ± 0.35	1.63 ± 0.04	1.37 ± 0.11
VSI, %	11.69 ± 1.43	12.26 ± 1.78	10.51 ± 1.70	12.30 ± 2.35	7.66 ± 1.62
HIS, %	1.79 ± 0.24	1.80 ± 0.19	3.00 ± 0.81	2.61 ± 0.26	2.47 ± 1.04
survival, %	100	100	100	100	100

D1 – diet 1 (0% of soldier termite), D2 – diet 2 (10%), D3 – diet 3 (30%), D4 – diet 4 (50%), D5 – diet 5 (70%), IBW – initial body weight, FBW – final body weight, WG – weight gain, SGR – specific growth rate, FCR – food conversion ratio, TGC – thermal growth coefficient, FI – feed intake, PER – protein efficiency ratio, CF – condition factor, VSI – viscero-somatic index, HIS – hepato-somatic index; $^{a–e}$ – means within a row with different superscripts are significantly different at $P < 0.05$

Table 5. Blood serum chemistry (mean ± standard error) of *Oreochromis mossambicus* fed diets with soldier termite meal as a fish meal replacement at different inclusion levels; n =9

Diets	ALT, U/l	AST, U/l	Chol, mmol/l	Trig, mmol/l	Glu, mmol/l
D1	30.00 ± 2.33^{a}	101.14 ± 3.24^{a}	1.32 ± 0.02	2.05 ± 0.01	4.37 ± 0.14
D2	81.30 ± 3.17^{b}	131.11 ± 4.65^{b}	1.29 ± 0.02	2.04 ± 0.02	5.35 ± 0.12
D3	54.10 ± 2.40^{c}	122.64 ± 1.76^{c}	1.40 ± 0.01	2.10 ± 0.02	3.32 ± 0.11
D4	35.21 ± 5.56^{a}	107.51 ± 3.28^{a}	1.41 ± 0.02	2.04 ± 0.01	4.39 ± 0.12
D5	100.41 ± 3.47^{d}	141.5 ± 2.33^{d}	1.29 ± 0.03	2.26 ± 0.01	4.74 ± 0.12

D1 – diet 1 (0% of soldier termite), D2 – diet 2 (10%), D3 – diet 3 (30%), D4 – diet 4 (50%), D5 – diet 5 (70%), ALT – alanine transaminase, AST – aspartate transaminase, Chol – cholesterol, Trig – triglyceride, Glu – glucose; $^{a–d}$ – means within a column with different superscripts are significantly different at $P < 0.05$

Table 6. Intestine histomorphology of *Oreochromis mossambicus* fed diets with soldier termite meal as a fish meal replacement at increasing inclusion levels; n = 9

Villi, µm	Diets				
	D1 (0%)	D2 (10%)	D3 (30%)	D4 (50%)	D5 (70%)
Height	98.12 ± 1.62^{a}	100.25 ± 3.11^{a}	104.63 ± 2.69^{a}	144.85 ± 1.25^{b}	116.25 ± 1.30^{c}
Thickness	56.36 ± 1.20	54.66 ± 1.36	50.10 ± 1.55	52.41 ± 1.62	55.15 ± 1.45

D1 – diet 1 (0% of soldier termite), D2 – diet 2 (10%), D3 – diet 3 (30%), D4 – diet 4 (50%), D5 – diet 5 (70%); values are expressed as mean ± standard error; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

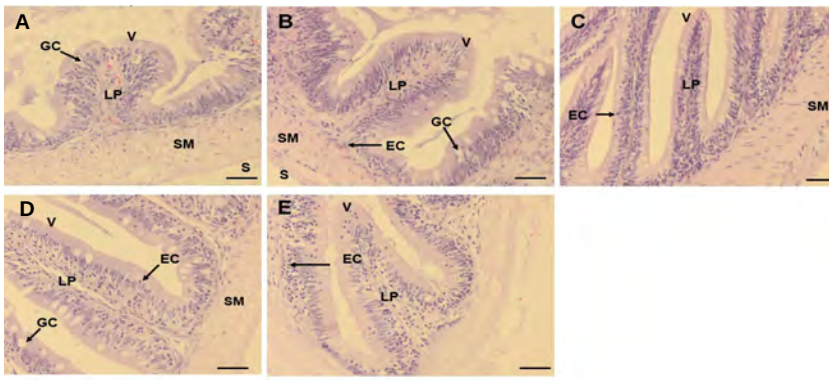

Figure 1. Micrograph of *Oreochromis mossambicus* fed diets with soldier termite meal as a fish meal replacement (A) diet D1 (0% of soldier termite), (B) diet D2 (10%), (C) diet D3 (30%), (D) diet D4 (50%), (E) diet D5 (70%)
V – villi, LP – lamina propria, GC – goblet cell, SM – submucosa, S – serosa, EC – epithelial cells; scale bar: 20 µm

dietary treatments showed normal intestinal histomorphology with distinguishable villi, lamina propria, submucosa, and epithelial cells.

Cost-benefit analysis

The cost-benefit analysis showed that increasing the proportion of soldier termite meal resulted in an increase in the profit index, with a decrease in incidental costs (Table 7).

Table 7. Cost-benefit analysis of substituting fish meal with soldier termite meal in the diet of *Oreochromis mossambicus*

Cost-benefit indices	Diets				
	D1 (0%)	D2 (10%)	D3 (30%)	D4 (50%)	D5 (70%)
Incidence cost	0.15	0.11	0.09	0.07	0.06
Profit index	1.18	1.21	1.26	1.32	1.38

D1 – diet 1 (0% of soldier termite), D2 – diet 2 (10%), D3 – diet 3 (30%), D4 – diet 4 (50%), D5 – diet 5 (70%)

Discussion

The study demonstrated that the optimal inclusion level of soldier termite meal in the diet of *O. mossambicus* was 50%. Fish fed a diet with a 50% inclusion level exhibited growth performance parameters and nutrient utilisation comparable to fish fed the control diet (fish meal-based). Soldier termite meal contained 57% protein, which met the requirements for *O. mossambicus* (30%). Moreover, the growth performance recorded at the 50% inclusion level could be attributed to the favourable amino acid profile of soldier termite meal, containing all essential amino acids, including methionine and lysine, which are the most limiting amino acids in fish growth (Lovel, 1989). The growth performance and nutrient utilisation recorded in the 50% diet could be also influenced by the fatty acid profile of soldier termite meal, which contained PUFAs (linoleic acid and α-linolenic acid), i.e., essential fatty acids. Linoleic acid and α-linolenic acid play an important role in growth, development, and energy production of fish species. The fatty acid requirements of *Tilapia* sp. are poorly understood. Li et al. (2013) reported that linoleic acid alone could meet the essential fatty acid requirements of juvenile tilapia hybrids. On the other hand, Chou et al. (2001) demonstrated that tilapia species required both omega-6 and omega-3 fatty acids in their diets. Currently, the comprehensive fatty acid requirements for *Tilapia* sp. are not well-established. According to the authors' knowledge, there are no existing studies that have assessed the utilisation of soldier termite meal as a fish meal replacement in fish species. However, farmers in Ghana and Burkina Faso have been reported to use soldier termites as feed for poultry (Boafo et al., 2019).

Supplementation of soldier termite meal above 50% resulted in a decline in growth performance parameters. This phenomenon is common in fish fed insect-based diets (Fawole et al., 2020). It is well established that the decrease in growth performance at higher inclusion levels of insect-based diets is mainly caused by the presence of chitin (Shekarabi et al., 2021; Weththasinghe et al., 2022; Zhao et al., 2023). Insects contain close to 10% chitin (Belluco et al., 2013; Kinyuru et al., 2015;), and this compound has been reported to affect digestibility in fish species (Shekarabi et al., 2021). Most fish species lack chitinhydrolysing enzymes (chitinases). However, chitin has also been reported to exert potential positive effects such as improving the fish immune system or their performance (Saavedra et al., 2023). In the current study, the observed decline in growth performance parameters at a higher termite meal supplementation (70%) may not be entirely due to the increased chitin content. Furthermore, Rapatsa and Moyo (2017) observed the presence of chitinase activity in the gastrointestinal tract of *O. mossambicus*, suggesting a potential mechanism for chitin breakdown and utilisation.

The blood serum chemistry indicated that incorporating soldier termite meal as a fish meal replacement had no influence on cholesterol, triglyceride, and glucose levels. This showed that fish fed diets with soldier termite meal were not exposed to stress. Glucose levels are known to increase when organisms are under stressful conditions, probably due to the action of catecholamine on stored glycogen in fish tissues. Similar observations were made in a study involving fish fed an insect-based diet (Ogunji et al., 2008). Serum triglycerides and total cholesterol are the predominant free lipids that are distributed in fish and serve as indicators of fat metabolism (Hu et al., 2020). This shows that the incorporation of soldier termite meal in the diet of *O. mossambicus* did not affect its fat metabolism. However, the meal examined did influence AST and ALT levels in *O. mossambicus*, which were significantly elevated at higher inclusion levels. AST and ALT are the main indices used to evaluate liver injury. Liver damage can lead to an increase in cell membrane permeability, causing enzymes like AST and ALT to be released from cells into the bloodstream. Consequently, elevated levels of these two enzymes signal liver damage (Belghit et al., 2018). This further demonstrates that the inclusion of soldier termite meal can have a signifi-

cant impact on fish liver health. On the other hand, the histomorphology of the fish intestine remained unaffected across all dietary groups, indicating that the incorporation of soldier termite meal did not influence the structural characteristics of the gut. The intestine is a region where nutrient digestion and assimilation occur, and it also reflects the nutritional status of the fish when a novel ingredient is introduced into the fish diet. The normal intestinal histomorphology observed in the present study may be attributed to the ability of *O. mossambicus* to digest an insect-based diet (Rapatsa and Moyo, 2017). In the wild, *O. mossambicus* includes insects as a significant part of its diet at various growth stages. Furthermore, *O. mossambicus* possesses the enzymes necessary to digest a feed containing up to 50% of insect-based meal. The cost-benefit analysis showed that replacing fish meal with soldier termite meal could yield higher profit margins than using fish meal-based diets. This shift could contribute to increased aquaculture production, improved livelihood of fish farmers, and positively impact the country's economy.

Conclusions

The study has demonstrated that soldier termite meal can replace fish meal up to a 50% inclusion level, without adversely affecting the growth performance and nutrient utilisation of juvenile *Oreochromis mossambicus*. The addition of termite meal in proportions higher than 50% reduced growth performance and deteriorated the health status of juvenile *O. mossambicus*. Substituting fish meal with soldier termite meal is economically sustainable. It is recommended to evaluate the potential for mass rearing and commercial use of soldier termites. The study rejected the null hypothesis.

Funding

This work was supported by the National Research Foundation (NRF) and the University of Limpopo (Aquaculture Research Unit).

Acknowledgments

The National Research Foundation (NRF) and the University of Limpopo, Aquaculture Research Unit are recognised for financial support. Mr. Gavin Geldenhuys is appreciated for his technical assistance.

Conflict of interest

The Authors declare that there is no conflict of interest.

References

Abdel-Tawwab M., Khalil R.H., Metwally A.A., Shakweer M.S., Khallaf M.A., Abdel-Latif H.M.R., 2020. Effects of black soldier fly (*Hermetia illucens* L.) larvae meal on growth performance, organs-somatic indices, body composition, and hematobiochemical variables of European sea bass, *Dicentrarchus labrax*. Aquaculture 522, 735136, https://doi.org/10.1016/j.aquaculture.2020.735136

Adeleke B., Robertson-Andersson D., Moodley G., Taylor S., 2021. Aquaculture in Africa: A comparative review of Egypt, Nigeria, and Uganda Vis-Á-Vis South Africa. Rev. Fish. Sci. Aquac. 29, 167–197, https://doi.org/10.1080/23308249.2020.1795615

Adeoye A.A., Akegbejo-Samsons Y., Fawole F.J., Davies S.J., 2019. Preliminary assessment of black soldier fly (*Hermetia illucens*) larval meal in the diet of African catfish (*Clarias gariepinus*): impact on growth, body index, and hematological parameters. J. World Aquac. Soc. 51, 1024–1033, https://doi.org/10.1111/jwas.12691

Alfiko Y., Xie D., Astuti R.T., Wong J., Wang L., 2022. Insects as a feed ingredient for fish culture: status and trends. Aquac. Fish. 7, 166–178, https://doi.org/10.1016/j.aaf.2021.10.004

AOAC, 2012. Official Methods of Analysis. Association of Official Analytical Chemist. 19th Edition. Washington DC (USA)

Bahnasawy M.H., Abdel-Baky T.E., Abd-Allah G.A., 2003. Growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings raised in an earthen pond. Arch. Polish Fish. 11, 277–285

Belghit I., Liland N.S., Gjesdal P., Biancarosa I., Menchetti E., Li Y., Waagobo R., Krogdahl E.L., Lock E.-J., 2018. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). Aquaculture 503, 609–619, https://doi.org/10.1016/j.aquaculture.2018.12.032

Belluco S., Losasso C., Maggioletti M.G., Alonzi C.C., Paoletti M.G., Ricci A., 2013. Edible insects in a food safety and nutritional perspective: a critical review. Compr. Rev. Food Sci. Food Saf. 12, 296–313, https://doi.org/10.1111/1541-4337.12014

Boafo H.A., Affedzie-Obresi S., Gbemavo D.S.J.C., Clottey V.A., Nkegbe E., Adu-Aboagye G,. Kenis M., 2019. Use of termites by farmers as poultry feed in Ghana. Insects 10, 69, https://doi.org/10.3390/insects10030069

Chou B.S., Shiau S.Y., Hung S.S.O., 2001. Effect of dietary cod liver oil on growth and fatty acids of juvenile hybrid tilapia. N. Am. J. Aquac. 63, 277–284, https://doi.org/10.1577/1548-8454(2001)063<0277:EODCLO>2.0.CO;2

Djissou A.S.M., Adjahouinou D.C., Koshio S., Fiogbe E.D., 2016. Complete replacement of fish meal by other animal protein sources on growth performance of *Clarias gariepinus* fingerlings. Int. Aquat. Res. 8, 333–341, https://doi.org/10.1007/s40071-016-0146-x

El-Sayed A.F.M. (Editor), 2006. Tilapia Culture. CABI Publishers. Oxfordshire (UK)

Fawole F.J., Adeoye A.A., Tiamiyu L.O., Ajala K.I., Obadara S.O., Ganiyu I.O., 2020. Substituting fishmeal with *Hermetia illucens* in the diets of African catfish (*Clarias gariepinus*): effects on growth, nutrient utilization, haematophysiological response, and oxidative stress biomarker. Aquaculture 518, 734849, https://doi.org/10.1016/j.aquaculture.2019.734849

Folch J., Lees M., Stanley G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509, https://doi.org/10.1016/S0021-9258(18)64849-5

Gasco L., Józefiak A., Henry M., 2021. Beyond the protein concept: health aspects of using edible insects on animals. J. Insects Food Feed 7, 715–741, https://doi.org/10.3920/JIFF2020.0077

Gebremichael A., Kucska B., Ardó L., et al., 2023. Physiological response of grower African catfish to dietary black soldier fly and mealworm meal. Animals 13, 968 https://doi.org/10.3390/ani13060968

Hu Y., Huang Y., Tang T., Zhong L., Chu W., Dai Z., Chen K., Hu Y., 2020. Effect of partial black soldier fly (*Hermetia illucens* L.) larvae meal replacement of fish meal in practical diets on the growth, digestive enzyme and related gene expression for rice field eel (*Monopterus albus*). Aquac. Rep. 17, 100345, https://doi.org/10.1016/j.aqrep.2020.100345

Hua K., 2021. A meta-analysis of the effects of replacing fish meals with insect meals on growth performance of fish. Aquaculture 530, 735732, https://doi.org/10.1016/j.aquaculture.2020.735732

Ishida Y., Fujita T., Asai K., 1981. New detection and separation method for amino acids by high-performance liquid chromatography. J. Chromatogr. A 204, 143–148, https://doi.org/10.1016/S0021-9673(00)81650-7

Kinyuru J.N., Mogendi J.B., Riwa C.A., Ndung'u N.W., 2015. Edible insects – a novel source of essential nutrients for human diet: learning from traditional knowledge. Anim. Front. 5, 14–19

Li E., Lim C., Klesius P.H., Welker T.L., 2013. Growth, body fatty acid composition, immuneresponse, and resistance to *Streptococcus iniae* of hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus*, fed diets containing various levels of linoleic and linolenic acids. J. World Aquac. Soc. 44, 42–55, https://doi.org/10.1111/jwas.12014

Lovell T. (Editor), 1989. Nutrition and Feeding of Fish. Springer. New York, NY (USA), pp. 260, https://doi.org/10.1007/978-1-4757-1174-5

Manditsera F.A., Luning P.A., Fogliano V., Lakemond C.M.M., 2019. The contribution of wild harvested edible insects (*Eulepida mashona* and *Henicus whellani*) to nutrition security in Zimbabwe. J. Food Compos. Anal. 75, 17–25, https://doi.org/10.1016/j.jfca.2018.09.013

Mariod A.A. (Editor), 2020. African Edible Insects as Alternative Source of Food, Oil, Protein and Bioactive Components. Springer. New York, NY (USA), pp. 314, https://doi.org/10.1007/978-3-030-32952-5

Moyo N.A.G., Rapatsa M.M., 2021. A review of the factors affecting tilapia aquaculture production in Southern Africa. Aquaculture 535, 736386, https://doi.org/10.1016/j.aquaculture.2021.736386

Netshifhefhe S.R., Duncan F.D., 2022. Nutrient composition of *Macrotermes* species consumed in the Vhembe District, Limpopo Province, South Africa. J. Insects Food Feed 8, 95–100, https://doi.org/10.3920/JIFF2020.0071

Ogunji J.O., Kloas W., Wirth M., Schulz C., Rennert B., 2008. Housefly Maggot meal (Magmeal) as a protein source for *Oreochromis niloticus* (Linn.). Asian Fish. Sci. 21, 319–331, https://doi.org/10.33997/j.afs.2008.21.3.006

Rapatsa M.M., Moyo N.A.G. 2022. A review and meta-analysis of the effects of replacing fishmeal with insect meals on growth of tilapias and sharptooth catfish. Aquac. Nutr. 2022, 9367587, https://doi.org/10.1155/2022/9367587

Rapatsa M.M., Moyo N.A.G., 2017. Evaluation of Imbrasia belina meal as a fishmeal substitute in *Oreochromis mossambicus* diets: growth performance, histological analysis and enzyme activity. Aquac. Rep. 5, 18–26, http://doi.org/10.1016/j.aqrep.2016.11.004

Saavedra M., Barata M., Matias A.C. et al., 2023. Effect of dietary incorporation of yellow mealworm as a partial fishmeal replacer on growth, metabolism, and intestinal histomorphology in juvenile meagre (*Argyrosomus regius*). Aquac. Nutr. 2023, 6572421, https://doi.org/10.1155/2023/6572421

Santiago C.B., Lovell R.T., 1988. Amino acid requirements for growth of Nile tilapia. J. Nutr. 188, 1540–1546, https://doi.org/10.1093/jn/118.12.1540

Shekarabi S.P.H., Mehrgan M.S., Banavreh A., 2021. Feasibility of superworm, *Zophobas morio*, meal as a partial fishmeal replacer in fingerling rainbow trout, *Oncorhynchus mykiss*, diet: growth performance, amino acid profile, proteolytic enzymes activity and pigmentation. Aquac. Nutr. 27, 1077–1088, https://doi.org/10.1111/anu.13249

van Dyk J.C., Pieterse G.M., 2008. A histo-morphological study of the testis of the sharptooth catfish (*Clarias gariepinus*) as reference for future toxicological assessments. J. Appl. Ichthyol. 24, 415–422, https://doi.org/10.1111/j.1439-0426.2008.01127.x

van Soest P.J., Robertson J.B., Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597, https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Weththasinghe P., Hansen J.Ø., Mydland L.T., Øverland M., 2022. A systematic meta-analysis based review on black soldier fly (*Hermetia illucens*) as a novel protein source for salmonids. Rev. Aquac. 14, 938–956, https://doi.org/10.1111/raq.12635

Zhao J., Pan J., Zhang Z., Chen Z., Mai K., Zhang Y., 2023. Fishmeal protein replacement by defatted and full-fat black soldier fly larvae meal in juvenile turbot diet: effects on the growth performance and intestinal microbiota. Aquac. Nutr. 2023, 8128141, https://doi.org/10.1155/2023/8128141

APPENDIX C

Scientific African 24 (2024) e02125

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Utilization of an insect-based diet by a herbivorous fish (*Oreochromis mossambicus*) and an opportunistic predator (*Clarias gariepinus*)

Livhuwani E. Nephale *, Ngonidzashe A.G. Moyo, Mmaditshaba M. Rapatsa-Malatji

Aquaculture Research Unit, School of Agricultural and Environmental Sciences, Faculty of Science and Agriculture, University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga 0727, South Africa

ARTICLE INFO

Editor: DR B Gyampoh

Keywords:
Oreochromis mossambicus
Clarias gariepinus
Macrotermes falciger
Growth indices
Organosomatic indices
Biochemistry

ABSTRACT

An 8-week feeding trial was conducted to evaluate the effect of Alates termite (*Macrotermes falciger*) based diet in a herbivorous (*Oreochromis mossambicus*) and an opportunistic predator (*Clarias gariepinus*). Five diets were formulated to replace fishmeal at 0, 10, 30, 50, and 70 % and were denoted D1, D2, D3, D4, and D5, respectively. *Clarias gariepinus* sub-adults (209.24 ± 2.41 g/fish) were stocked at 5 fish per tank in recirculating fibreglass tanks (400 L) in triplicates. *Oreochromis mossambicus* juveniles (7.4 ± 4.78 g/fish) were randomly stocked at 10 fish per tank in recirculating fibreglass tanks (400 L) in triplicates. Fish were fed their allocated diets, twice a day to apparent satiation. Growth performance indices (specific growth rate, thermal-unit growth coefficient) were lowest at inclusion level above 50 % in both species ($P < 0.05$). The nutrient utilization indices (feed intake, protein efficiency ratio) were lowest at inclusion level above 50 % in both species. The organosomatic indices (condition factor, hepato-somatic index, viscero-somatic index) were not influenced by the inclusion of Alates termite meal in both species. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were not influenced by Alates termite meal in *O. mossambicus* ($P > 0.05$). However, AST and ALT levels significantly increased at inclusion levels above 50 % in *C. gariepinus* ($P < 0.05$). The triglyceride, cholesterol, and glucose levels showed no significant difference across diets in both fish species ($P > 0.05$). The study showed that both species may utilize an insect-based diet up to 50 % inclusion levels without adverse effects on growth performance and nutrient utilization.

Introduction

Insects have been described as a promising alternative protein source in aqua feed globally [1]. This is mainly due to their high feed conversion efficiency, lower environmental impact, and high nutritional composition [2,3]. The use of insect meals in different fish species has been extensively reviewed [4–6] with different findings. When a black soldier fly larvae (*Hermetia illucens*) replaced fishmeal at different inclusion levels, a rainbow trout (*Oncorhynchus mykiss*) recorded the best growth performance and nutrient utilization at 15 % [7], an African catfish (*Clarias gariepinus*) at 50 % [8,9] whilst Nile Tilapia (*Oreochromis niloticus*) showed that *H. illucens* can completely replace fishmeal without adverse effects on the growth performance and health status [10,11]. These results may be due to the different feeding habits of these species. Furthermore, these studies are not comparable because researchers used

* Corresponding author.

https://doi.org/10.1016/j.sciaf.2024.e02125
Received 4 July 2023; Received in revised form 31 January 2024; Accepted 21 February 2024
Available online 22 February 2024

different processing methods of the insect meal and fish were fed different ration levels. The rearing conditions were also not the same across the studies.

Utilization of Alates termite meal by *O. mossambicus* and *C. gariepinus* fish species is not adequately documented. These species have different feeding habits, and it is important to determine their potential to utilize Alates termite meal-based diets. Rapatsa and Moyo [12] reported an increase in the growth performance of a herbivorous *Oreochromis mossambicus* with an increase in Mopane worm (*Imbrasia bellina*) meal inclusion. However, a decline in growth performance was observed in an opportunistic predator *Clarias gariepinus* when fed the same diet and reared under the same experimental conditions [13]. These studies showed that a herbivorous species can effectively utilize an insect-based diet better than an opportunistic predator. Thus, it is paramount to evaluate the response of two species with different feeding habits under the same experimental conditions. The research question to be addressed in this study is: how does an insect-based diet affect the growth performance and blood serum chemistry of a herbivorous fish (*O. mossambicus*) and an opportunistic predator (*C. gariepinus*)?

The Mozambique tilapia (*Oreochromis mossambicus*) and the African catfish (*Clarias gariepinus*) are some of the warm freshwater species of economic importance in southern Africa [14]. *Oreochromis mossambicus* is a herbivore, whilst *Clarias gariepinus* is an opportunistic predator. Feeding fish with different feeding habits an insect-based diet has resulted in different growth performance. Thus, the current study will assess the growth performance of *O. mossambicus* and *C. gariepinus* fed a diet with a locally available insect as a fishmeal substitute. Furthermore, insect-based diets may influence the health and nutritional status of the fish. These parameters may be evaluated through an assessment of the blood serum chemistry. Thus, the current study will evaluate the growth performance and blood serum chemistry of *O. mossambicus* and *C. gariepinus* fed an insect-based diet.

In southern Africa, *Macrotermes falciger* Alates (winged termites) are one of the dominant edible insects, especially in rural communities [15,16]. Alates termites swarm seasonally at the onset of the rainy season, usually between October and April. The nutritional composition of Alates termite of the *Macrotermes* sp. is well documented [17–19]. These studies have reported that *Macrotermes* sp. contains protein content between 20.4 % and 42.25 %, fat content above 46.5 %, rich minerals and vitamins, and a balanced fatty acid profile [18,20,21]. Netshifhefhe and Duncan [15] reported that *Macrotermes falciger* are the most preferred termite species, mainly due to their larger size and nutritional composition. The *Macrotermes falciger* Alates contain 41.8 % crude protein, 44.3 % fat content, and a balanced amino acid profile [22]. Although *Macrotermes falciger* Alates contain a good nutritional composition, their use as feed and as a fishmeal replacement in the animal feed industry is not documented. Thus, Alates of the *Macrotermes falciger* may be used as a novel ingredient in the diet of *O. mossambicus* and *C. gariepinus*. The main objective of this study was to investigate the utilization of an insect-based diet by a herbivorous and an opportunistic predator under the same experimental conditions. The inclusion of the insect meal may lower feed costs in the culturing of *O. mossambicus* and *C. gariepinus*.

Materials and methods

Diet formulation

The Alates termite (de-winged) were purchased from the Thohoyandou Open Market in the Vhembe District of Limpopo Province, South Africa. The Alates termite was ground to powder and replaced fishmeal at 0, 10, 30, 50, and 70 %, which were designated as D1, D2, D3, D4, and D5, respectively (Table 1). The diets were formulated to be isonitrogenous (30 % protein), isolipidic (12 % fat), and

Table 1
Feed ingredients (g/kg) mixed to formulate experimental diets where Alates termite meal substituted fishmeal at increasing levels.

Inclusion levels	0 %	10 %	30 %	50 %	70 %
Ingredients	**D1**	**D2**	**D3**	**D4**	**D5**
Fishmeal	300	270	210	150	90
Alates meal	0	30	90	150	210
Yellow maize	205	205	205	185	155
Wheat bran	242.7	234.0	212.7	202.9	229.4
Sunflower meal	102.0	110.7	131.8	161.3	164.9
Sunflower oil	60.3	60.3	60.5	60.8	60.7
Methionine	20	20	20	20	20
Lysine	20	20	20	20	20
Vitamin/Mineral premix[a]	20	20	20	20	20
Binder	30	30	30	30	30
Total	1000	1000	1000	1000	1000

Fishmeal: 65.5 % CP; 12.0 % fat; 18.9 GE (MJ/kg); 92.1 % DM; 18.0 % ash.
Alates meal: 40.46 % CP; 40.40 % fat; 2 301 GE (KJ/100 g); 93.51 % DM; 6.14 % ash.
Yellow maize: 12.82 % CP; 2.24 % fat; 16.1 GE (MJ/kg); 87.55 % DM; 1.93 % ash.
Wheat bran: 15.3 % CP; 3.3 % fat; 16.4 GE (MJ/kg); 86.9 % DM; 4.8 % ash.
Sunflower: 20.01 % CP; 24.98 % fat; 19.7 GE (MJ/kg); 97.65 % DM; 3.87 % ash.
CP: crude protein; GE: gross energy; DM: dry matter.
[a] Vitamin/Mineral premix: Vit. A, 12 000 IU; Vit. D3, 1 200 IU; Vit. E, 120 IU; Vit. B4, 10,000 g; Vit. C, 120 g; Vit. B3, 25 g; Vit. B5, 15 g; Vit. B2, 6 g; Vit. B6, 5 g; Vit. B1, 4 g; Vit. K3, 2 g; Vit. B9, 1 g; Vit. H, 0.25 g; Vit. B12, 0.04 g. ZnO, 200 g; $FeSO_4$; $CuSO_4$, 7 g; MnO, 5 g; KI, 2 g; Na_2SeO_3, 0.15 g; $CoSO_4$, 0.05 g.

isocaloric (15 MJ/kg), this was done using Winfeed 3, EFG (Natal) software. The control diet contained 30 % fishmeal and no insect meal. The feed ingredients were weighed and mixed using a planetary mixer (Hobart, Troy, OH, USA). During mixing, water (10–20 % v/w) was added as required until the desired dough thickness was attained. The mixture was pelleted into 3 mm diameter size using a meat mincer connected to the planetary mixer. The pellets were labelled and separately sun-dried and stored in polyethylene buckets at 4 °C.

Proximate composition of the Alates termite meal and experimental diets

The experimental diets (Table 2) and Alates termite meal (Table 3) were analysed for the proximate composition according to AOAC [23]. Crude protein was determined using Micro-Kjeldahl apparatus (Foss 2400 Kjeltec analyser unit, Denmark). Protein content was calculated as $N \times 6.25$. Fat content was determined by petroleum ether extraction using Soxhlet apparatus for 16 h (Soxtec system, Foss Tecator lipid analyser). Gross energy was analysed using DDS Isothermal CP 500 bomb calorimeter. Dry matter and moisture content were analysed by drying the sample for 24 h at 105 °C until a constant weight was reached. Ash content was determined by the weight loss after incinerating samples in a muffle furnace for 6 h at 550 °C. Dietary fibre was determined by digesting dried lipid-free residues with 1.25 % H_2SO_4 and 1.25 % NaOH. The loss on ignition was recorded as dietary fibre [24]. Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) were determined by boiling the sample with a neutral detergent solution and acid detergent solution, respectively [25].

Alates termite meal contained 40.46 % protein content, 40.40 % fat content, and 6.14 % ash content (Table 3). The NDF and ADF of the Alates termite meal were 56.19 % and 22.09 %, respectively. The highest concentration of essential amino acids recorded in the Alate termite meal were lysine (12.99 g/kg), leucine (6.98 g/kg), and histidine (5.15 g/kg), respectively (Table 3). The non-essential amino acids were dominated by cystine (22.82 g/kg), glutamic acid (12.48 g/kg), and aspartic acid (8.17). Sodium constituted the highest value for minerals (41.6 mg/l), followed by potassium (33.8 mg/l), and iron (1.93 mg/l). The fatty acids that dominated the Alates termite meal were palmitic (6.5781 mg/g), oleic (6.5714 mg/g), and stearic (3.1547 mg/g), which are all SFA. This insect also contained linoleic acid and α-linolenic acid, which are polyunsaturated fatty acids (PUFA).

Mineral content

The mineral content was determined using Inductively coupled plasma-optical emission spectrometry (ICP-OES) as described by Manditsera et al. [26]. Samples were hydrolyzed with 65 % concentrated nitric acid and 37 % hydrochloric acid. Hydrogen peroxide was used to remove nitrous vapours. The analysis was performed using standard solutions of known concentrations. All chemicals used were analytical reagent grade.

Amino acid analysis

Alates termite meal was analysed for amino acids at the Central Analytical Facilities (CAF) of Stellenbosch University (South Africa). Samples (20 mg) were hydrolysed with 6 M HCI at 110 °C for 4 h in sealed tubes [27]. About 130 μL was transferred into a 2 ml tube and dried under a gentle stream of nitrogen. The samples were reconstituted and derivitized with 30 μL MTBSTFA and 100 μL acetonitrile at 100 °C for 1 hour, cooled down to room temperature and injected into the gas chromatograph (6890 N, Agilent technologies network). Separation was performed on gas chromatograph coupled to an Agilent technologies inert XL EI/CI Mass Selective Detector (MSD) (5975, Agilent technologies Inc., Palo Alto, CA).

Fatty acid analysis

Fatty acid analysis for Alates termite meal was also analysed at the Central Analytical Facilities (CAF) of Stellenbosch University (South Africa). The analysis was performed by adding 2 ml of 2:1 chloroform: methanol to 100 mg of the sample [28]. The fatty acid profile of the Alate termite meal is presented in Table 3.

Fish stocking and experimental design

The experiment was undertaken at the University of Limpopo, Aquaculture Research Unit (ARU), Limpopo Province, South Africa. The fish species used in the study were obtained from the ARU. The study was approved for ethical clearance by the University of

Table 2
The proximate composition of experimental diets.

Proximate composition	D1	D2	D3	D4	D5
Crude protein (%DM)	30.00	30.00	30.00	30.00	30.00
Fat (%DM)	12.49	12.13	12.68	12.32	12.27
Gross Energy (MJ/Kg)	15.02	15.08	15.00	15.01	15.03
Dry matter (%DM)	92.89	93.01	93.49	93.81	94.05
Ash (%DM)	10.53	10.20	9.62	9.00	8.71

Table 3
Proximate composition, mineral content, fatty acids profile and amino acid profile of the Alates termite meal.

Proximate composition	%DM	Fatty acids	mg/g	Essential AA	g/kg (DM)
Dry matter (%)	93.51	C12:0	0.02	Lysine	12.99
Ash (%DM)	6.14	C14:0	0.27	Methionine	3.76
Fat (%DM)	40.40	C16:0	6.57	Phenylalanine	4.79
Protein (%DM)	40.46	C18:0	3.15	Valine	4.69
Carbohydrates (%DM)	6.54	C16:1	0.34	Tryptophan	0.37
Crude fibre (%DM)	8.70	C17:1n-10	0.07	Threonine	4.94
NDF (%DM)	56.19	C18:1n-9(*cis*)	6.57	Isoleucine	4.25
ADF (%DM)	22.09	C18:2n-6 (*cis*)	2.73	Histidine	5.15
Energy (KJ/100 g)	2301	C18:3n-3	0.32	Leucine	6.98
Minerals	**(mg/l)**	Σ SFA	10.03	**Non-essential AA**	
Iron	1.93	Σ MUFA	6.99	Tyrosine	3.86
Potassium	33.8	Σ PUFA	3.05	Glycine	4.99
Sodium	41.6			Aspartic acid	8.17
				Serine	6.04
				Proline	6.29
				Glutamic acid	12.48
				Alanine	6.09
				Cystine	22.82

NDF: Neutral detergent fibre; ADF: Acid detergent fibre; DM: dry matter; SFA: saturated fatty acids, MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. AA: Amino acids.

Limpopo Animal Research Ethical Committee (AREC/09/2022:PG).

The experiment was conducted in a Recirculating aquaculture system (RAS) using 500 L size fibreglass tanks filled to the 400 L mark. The tanks were connected to a sump and a pump that supplied the tanks with water at 10 L/min. Each tank was heated with a submersible aquarium heater (ViaAqua Glass heater, 200 W) and aerated with airstones. Fish species were sedated with 2-phenoxyethanol (1 ml/L) prior to stocking in experimental tanks as stipulated in the ethical clearance certificate (AREC/09/2022:PG). This was done by mixing water from the experimental system with 2-phenoxyethanol at 1 ml/L in a separate tank. This was also done to minimise stress levels and physical damage during the handling of fish species. A completely randomised design was used to stock the fish in experimental tanks. *Clarias gariepinus* (209.24 ± 2.41 g/fish) were stocked at 5 fish per tank in triplicates, whilst *O. mossambicus* (7.4 ± 4.78 g/fish) were stocked at 10 fish per tank in triplicates. The stocking densities used do not affect the growth performance of both fish species used in the study. Higher stocking densities than the one used in the current study have been previously used without affecting the growth performance of *C. gariepinus* [13,29] and *Oreochromis* species [10,30].

Feeding procedure

Both fish species were fed a commercial diet (Aqua-plus, Avi Products (pty) ltd for two weeks during the acclimatization period. Fish were then weighed (Initial weight) per tank and re-stocked back to experimental tanks and fed allocated diets to apparent satiation for 8 weeks at 09h:00 and 15h:00. Feed intake was recorded on daily basis for each tank.

Water quality management

Water quality parameters were monitored on a weekly basis using a handheld multiparameter meter (Professional plus YSI 605,000). The water temperature was kept between 27 and 29 °C, dissolved oxygen (6.5–8.0 mg/l), ammonia (<1 mg/l), and pH (6.8–8.0). The experiment was conducted under natural photoperiod. Water quality parameters were kept the same for both *O. mossambicus* and *C. gariepinus* experimental tanks.

Growth performance parameters

Fish were weighed at the commencement of the experiment, after every two weeks, and at the end of the feeding trial. Fish samples were sedated (2-phenoxyethanol (1 ml/L) at each weighing interval. Fish were starved for 24 h before final growth parameters were taken. The 24 h starvation gives accurate fish weight without feed in the gut.

The following parameters were measured for both *O. mossambicus* and *C. gariepinus*:

Specific growth rate (SGR) :

$$SGR = \left[\frac{\text{In } W_{t-} \text{ In}W_0}{t}\right] \times 100\%$$

Where In, W_t, W_0, and t are the natural logarithm, the final body weight (g), initial body weight (g), and time feeding period (days), respectively [31].

Thermal-unit growth coefficient (TGC) [32]:

$$TGC = 1000 \times \frac{\text{Final weight(g)}^{\frac{1}{3}} - \text{Initial weight(g)}^{\frac{1}{3}}}{\text{Temperature(°C)} \times \text{Days}}$$

Feed conversion ratio (FCR) = feed eaten (g) / weight gained (g) [33]
Protein efficiency ratio (PER) = weight gain (g) / protein consumed (g) [33]
Weight gain (WG) = final weight (g) - initial weight (g)
Feed intake (FI) = weight of eaten feed (g) / fish / day
Fish survival = (final number of fish / initial number of fish) x 100

Organosomatic indices

Three fish from each dietary replicate (9 fish/diet) were used to measure organosomatic indices. The condition factor (CF), Hepato-somatic index (HSI), and Viscero-somatic index (VSI) were calculated as follows:

CF = body weight (g) / fish length $(cm)^3$ x 100 [34]
HSI = liver weight (g) / fish weight (g) x 100 [8]
VSI = visceral weight (g) / fish weight (g) x 100, viscera weight includes the liver [8].

Blood serum chemistry

Three fish samples from each dietary replicate were randomly selected and sedated with 2-phenoxyethanol (1 ml/L). Blood samples were drawn from the caudal vasculature into vials using heparinised syringes and centrifuged for 10 min at 3500 rpm. Blood samples from each replica were pooled ($n = 3$). The serum was used for the analysis of aspartate aminotransferase (AST), alanine amino-transferase (ALT), triglyceride, cholesterol, and glucose. The analyses were conducted using a commercial automatic biochemical kit (Sigma-Aldrich, St. Louis, USA). The same procedure was employed for both experiments (*O. mossambicus* and *C. gariepinus*).

Statistical analysis

Shapiro-Wilk and Barlett's tests were used to test data for normality of distribution and homogeneity of variance, respectively. One-way analysis of variance (ANOVA) was used to test significant differences in growth parameter indices (SGR, TGC), nutrient utilization indices (FCR, PER, FI), organosomatic indices (CF, VSI, HSI) and blood serum chemistry (AST, ALT, triglyceride, cholesterol, glucose). The data was significant when $P < 0.05$. Significant differences between means were determined by Tukey's HSD post hoc test. The data was analysed using SPSS version 28.0 software package (Statistical Package and Service Solutions, IBM, Chicago, IL, USA). The statistical analysis was similar for both *O. mossambicus* and *C. gariepinus*.

Results

Growth performance indices

The WG increased with an increase in inclusion levels in *O. mossambicus* (Table 4A). However, the WG significantly dropped at the highest inclusion level ($P < 0.05$). The SGR and TGC were highest at the 50 % inclusion level in *O. mossambicus* (Table 4A).

The FI and PER were lowest at the inclusion level above 50 % ($P < 0.05$) in *O. mossambicus*. The highest FCR was also recorded at inclusion level above 50 % ($P < 0.05$).

The organosomatic indices (CF, VSI, HSI) were not influenced by inclusion of Alates termite meal in *O. mossambicus* ($P > 0.05$). No mortalities were recorded throughout the feeding trial.

Table 4A
Growth performance indices of *O. mossambicus* fed diets with Alates termite meal as a fishmeal replacement. Values are expressed as mean ± SD. Different superscripts in a row show a significant difference ($P < 0.05$, ANOVA). $n = 9$.

Dietary groups	D1 (0 %)	D2 (10 %)	D3(30 %)	D4(50 %)	D5 (70 %)	P value
IBW (g)	61.18±5.53	73.84±6.62	76.06±8.26	71.43±5.02	74.04±2.64	0.075
FBW (g)	137.31±5.69^a	165.29±3.91^a	165.49±1.93^a	174.12±5.65^a	133.15±7.61^b	<0.001
WG (g)	76.13±0.88^a	91.45±2.94^b	93.16±2.82^b	102.69±2.76^b	59.11±4.02^c	<0.001
SGR (%/day)	1.45±0.11^a	1.44±0.06^a	1.40±0.04^a	1.60±0.06^b	1.06±0.02^c	<0.001
TGC	0.73±0.06	0.79±0.21	0.75±0.5	0.85±0.29	0.53±0.15	0.340
FI (g/fish/day)	0.42±0.01^a	0.49±0.02^b	0.53±0.04^b	0.53±0.01^b	0.30±0.02^c	<0.001
PER	2.53±0.11^a	3.04±0.24^a	2.97±0.17^a	3.43±0.19^b	1.96±0.17^c	<0.001
FCR	1.65±0.82^a	1.72±0.32^b	1.72±0.37^b	1.66±0.45^a	1.77±0.12^b	<0.001
Organosomatic indices						
CF	2.26±0.24	1.43±0.18	1.69±0.02	1.66±0.22	1.50±0.8	0.411
VSI (%)	11.92±1.90	9.82±3.31	9.02±1.97	10.34±2.93	9.25±0.07	0.588
HSI (%)	1.28±0.40	2.53±1.25	1.96±1.30	1.60±0.96	2.43±0.33	0.470
Survival (%)	100	100	100	100	100	–

In *C. gariepinus*, WG was lowest at the inclusion level above 50 % (Table 4B). The highest SGR was recorded at the 50 % inclusion level. The TGC in fish fed the control diet and 50 % inclusion level diet did not differ significantly ($P > 0.05$) in *C. gariepinus*.

There was no significant difference in FI across diets in *C. gariepinus* ($P > 0.05$). However, fish fed diet D5 recorded the lowest FI. The PER dropped significantly at the highest inclusion level ($P < 0.05$).

The organosomatic indices were also not influenced by the inclusion of Alates termite meal in the diet of *C. gariepinus*. No mortalities were recorded throughout the feeding trial.

Blood serum chemistry

The AST level in *O. mossambicus* was not influenced by the inclusion of Alates termite meal in fish diets ($P > 0.05$, Fig. 1A). However, the AST level in *C. gariepinus* increased at inclusion level higher than 50 %.

In *O. mossambicus*, the ALT level did not change with higher inclusion level ($P > 0.05$, Fig. 1B). However, *C. gariepinus* ALT level significantly increased at inclusion level above 50 % ($P < 0.05$).

Triglyceride level did not change with an increase in inclusion levels in both species ($P > 0.05$, Fig. 1C). Cholesterol followed a similar trend (Fig. 1D).

Glucose level in *O. mossambicus* showed no discernible pattern (Fig. 1E). Similar findings were observed in *C. gariepinus*.

Discussion

The 50 % inclusion level recorded the highest growth performance indices (WG, SGR, TGC) and nutrient utilization (PER) in *O. mossambicus*. However, at inclusion level of 70 %, growth performance and nutrient utilization declined significantly. A decline in growth performance and nutrient utilization can be attributed to intrinsic and extrinsic factors. It is possible that chitin content affected the growth performance and nutrient utilization of *O. mossambicus*. Chitin forms part of the exoskeleton of insects and has been reported to induce growth depression by reducing nutrient bioavailability in fish species [35]. Several studies have shown that insect meal inclusion levels of more than 30 % lead to a decline in growth performance due to higher chitin levels [36–38]. However, it has also been shown that chitin may have beneficial activity on the fish immune system and improve gut health due to its immunostimulant characteristics [39–41]. The decline in growth performance and nutrient utilization may also be attributed to the increase in Alates termite meal inclusion levels. Higher insect meal inclusion levels are often associated with a decline in growth performance and nutrient utilization. Feed intake was significantly different between the control and the 70 % inclusion level in *O. mossambicus*. It is speculated that the lower feed intake in fish fed D5 may have contributed to the poor growth performance and nutrient utilization reported in diet D5. The lowest PER was also recorded in diet D5 (70 %). This may be due to the anti-protease activity reported in insect meals [42]. The decline in growth performance and nutrient utilization in fish fed diet D5 was also evident in *C. gariepinus*. The decline in growth performance and nutrient utilization in *C. gariepinus* may also be attributed to the same factors in *O. mossambicus*. The combined effect of chitin and anti-protease action may have led to a high FCR registered in both species.

The organosomatic indices (CF, VSI, and HSI) were not influenced by the substitution of fishmeal with Alates termite meal in *O. mossambicus* and *C. gariepinus*. This shows that the health status of both fish species was not affected by substitution of fishmeal with Alates termite meal. This gives further credence that chitin played a positive role in these two fish species. The organosomatic indices suggest that both species were not stressed, and these assertions were supported by the blood serum chemistry.

The AST and ALT levels in *O. mossambicus* were not influenced by Alates termite meal inclusion. The AST and ALT enzymes are biomarkers of liver damage in fish species [43]. This implies that *O. mossambicus* may have maintained a healthy liver during the feeding trial. *Oreochromis mossambicus* is one of the tilapia species that has been reported to be pre-adapted to utilizing insect-based diets [44,45]. Furthermore, all inclusion levels did not affect the health status of *O. mossambicus*. On the other hand, the AST and ALT levels in *C. gariepinus* increased significantly at the 70 % inclusion level. This entails that replacing fishmeal with 70 % Alates termite meal may have a mild effect on the health status of *C. gariepinus*. A significant increase in the activities of these enzymes could imply

Table 4B
Growth performance indices of *C. gariepinus* fed diets with Alates termite meal as a fishmeal replacement. Values are expressed as means ± SD. Different superscripts in a row show a significant difference ($P < 0.05$, ANOVA). $n = 9$.

Dietary groups	D1 (0 %)	D2 (10 %)	D3(30 %)	D4(50 %)	D5 (70 %)	P value
IBW (g)	1060.50 ± 2.88^{a}	1060.10 ± 2.55^{a}	1000.24 ± 1.24^{b}	1020.13 ± 5.67^{c}	997.19 ± 2.85^{d}	<0.001
FBW (g)	2083.61 ± 3.22^{a}	1810.39 ± 2.12^{b}	1830.85 ± 5.38^{c}	2010.59 ± 4.18^{d}	1581.04 ± 6.58^{e}	<0.001
WG (g)	1023.11 ± 5.79^{a}	750.29 ± 4.10^{b}	830.61 ± 3.40^{c}	990.46 ± 5.57^{d}	584.85 ± 2.94^{e}	<0.001
SGR (%/day)	1.20 ± 0.14^{a}	1.03 ± 0.09^{b}	1.07 ± 0.02^{b}	1.22 ± 0.19^{a}	0.86 ± 0.02^{c}	0.039
TGC	1.61 ± 0.15^{a}	1.24 ± 0.02^{b}	1.39 ± 0.13^{c}	1.60 ± 0.14^{a}	$1.03\pm0.0.08^{d}$	0.018
FI (g/fish/day)	6.14±1.38	5.18±1.32	5.39±1.36	6.18±1.36	4.9 ± 1.14	0.771
PER	3.41 ± 0.04^{a}	2.50 ± 0.05^{a}	2.76 ± 0.02^{a}	3.30 ± 0.11^{a}	1.94 ± 0.05^{b}	<0.001
FCR	1.68 ± 0.03^{a}	1.96 ± 0.03^{b}	1.85 ± 0.09^{c}	1.78 ± 0.09^{d}	2.40 ± 0.12^{e}	0.004
Organosomatic indices						
CF	0.67±0.01	0.74±0.07	0.75±0.03	0.77±0.0	0.66±0.02	0.101
VSI (%)	7.08±0.62	8.02±1.73	7.37±2.43	7.74±1.46	7.20±0.68	0.966
HSI (%)	1.82±0.04	2.14±0.38	2.07±0.55	1.99±0.12	1.75±0.30	0.754
Survival (%)	100	100	100	100	100	–

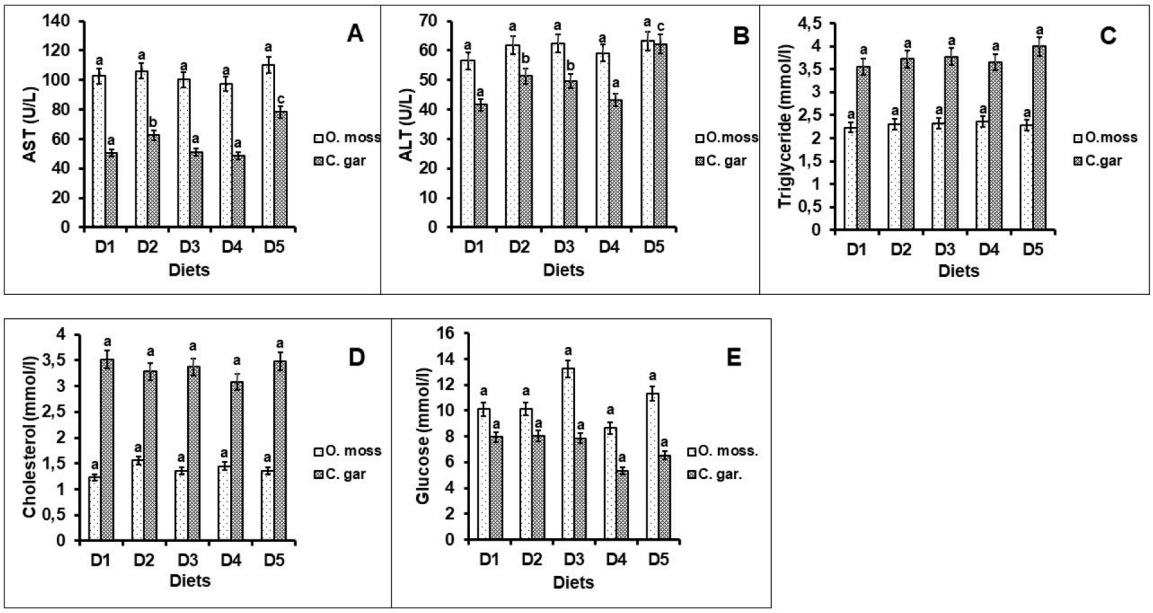

Fig. 1. Blood serum chemistry of *Oreochromis mossambicus* (O. moss) and *Clarias gariepinus* (C. gar) fed diets with Alates termite meal as a fishmeal replacement at increasing levels. $n = 3$. Different letters on each species bar represent a significant difference ($P < 0.05$, ANOVA). AST: alanine aminotransferase; ALT: aspartate aminotransferase. Bars represent standard error.

stress-induced tissue damage [46]. The AST and ALT enzyme levels further show that *O. mossambicus* was more tolerant to Alates termite meal inclusion than *C. gariepinus*. However, for both *O. mossambicus* and *C. gariepinus*, the higher AST and ALT levels were recorded in D5. High AST and ALT levels have been recorded in fish fed the highest inclusion level of an insect-based diet [47]. The study attributed these findings to an increase in chitin content with an increase in insect meal inclusion.

The triglyceride, cholesterol, and glucose levels in both species were not influenced by the inclusion of Alates termite meal. This further confirms that *O. mossambicus* and *C. gariepinus* were not exposed to stressful conditions. When fish species are under stressful conditions, glucose levels are known to increase significantly [48]. The current study showed that Alates termite meal inclusion levels up to 70 % has no substantial changes on glucose levels. These findings correspond with Ogunji et al. [49], where glucose levels did not differ significantly across diets in fish fed *Musca domestica* (magmeal) based diets. The cholesterol levels in both species were not influenced by replacing fishmeal with insect meal. Madibana et al. [50] also observed similar results when *Argyrosomus japonicus* was fed *Hermetia illucens* based diets. The triglyceride levels showed that substitution of fishmeal with Alates termite meal did not affect fat metabolism in *O. mossambicus* and *C. gariepinus*. Serum triglyceride serves as a fat metabolism biomarker [51]. The study showed that Alates termite meal can replace fishmeal up to 50 % inclusion level without negatively affecting the growth performance and nutrient utilization of *O. mossambicus* and *C. gariepinus*. However, inclusion level of 70 % may have a mild effect on the health status of *Clarias gariepinus*. One of the major drawbacks of using Alates termite meal as an alternative protein source is its seasonal variability.

Conclusion

Alates termite meal can replace fishmeal up to 50 % in both *O. mossambicus* and *C. gariepinus*. An inclusion level of 70 % elicited adverse effects on growth performance and nutrient utilization. Blood serum chemistry showed that replacing fishmeal with Alates termite meal has no negative effect on the health status of *O. mossambicus*. However, higher inclusion levels may cause stress in *C. gariepinus*. It is recommended that cost-benefit analysis of replacing fishmeal with Alates termite meal be undertaken to determine its economic viability. More studies on cultivation of termites have to be undertaken although several trials have failed.

Data availability

The data used in this study is available upon request from the corresponding author.

Funding

The study was funded by the National Research Foundation (NRF) and the University of Limpopo, Aquaculture Research Unit.

Ethics approval statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received (AREC/09/2022:PG). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

Author contributions

The study was conceptualised by Moyo, N.A.G. and Rapatsa, M.M. Nephale L.E. carried out experimental trials, analysed and interpreted the data and prepared the manuscript. All authors have read and approved the final version of the manuscript.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgement

The National Research Foundation (NRF) and the University of Limpopo, Aquaculture Research Unit are recognised for their financial support. Mr. Gavin Geldenhuys is appreciated for his technical expertise.

References

[1] A.A. Shah, P. Totakul, M. Matra, A. Cherdthong, Y. Hanboonsong, M. Wanapat, Nutritional composition of various insects and potential uses as alternative protein sources in animal diets, Anim. Biosci. 35 (2022) 317–331.
[2] M.Y. Hanan, M.A. Amatul-Samahah, M.Z. Jaapar, S.N. Mohamad, The effects of field cricket (Gryllus bimaculatus) meal substitution on growth performance and feed utilization of hybrid red tilapia (Oreochromis spp.), Applied Food Research 2 (2022). Article ID: 100070.
[3] H.P.S. Makkar, G. Tran, V. Heuze, P. Ankers, State-of-the-art on use of insects as animal feed, Anim. Feed. Sci. Technol. 197 (2014) 1–33.
[4] Y. Alfiko, D. Xie, R.T. Astuti, J. Wong, L. Wang, Insects as feed ingredient for fish culture: Status and trends, Aquaculture and Fisheries 7 (2022) 166–178.
[5] K. Hua, A meta-analysis of the effects of replacing fish meals with insect meals on growth performance of fish, Aquaculture 530 (2021). Article ID: 735732.
[6] M. Henry, L. Gasco, G. Piccolo, E. Fountoulaki, Review on the use of insects in the diet of farmed fish: past and future, Anim. Feed. Sci. Technol. 203 (2015) 1–22.
[7] C. Caimi, I. Biasato, G. Chemello, S.B. Oddon, C. Lussiana, V.M. Malfatto, M.T. Capucchino, E. Colombino, A. Schiavone, F. Gai, A. Trocino, A. Brugiapaglia, M. Renna, L. Gasco, Dietary inclusion of a partially defatted black soldier fly (Hermetia illucens) larva meal in low fishmeal-based diets for rainbow trout (Oncorhynchus mykiss), J. Anim. Sci. Biotechnol. 12 (2021) 50.
[8] A.A. Adeoye, Y. Akegbejo-Samsons, F.J. Fawole, S.J. Davies, Preliminary assessment of black soldier fly (Hermetia illucens) larval meal in the diet of African catfish (Clarias gariepinus): Impact on growth, body index, and hematological parameters, J. World Aquac. Soc. (2019) 1–10.
[9] F.J. Fawole, A.A. Adeoye, L.O. Tiamiyu, K.I. Ajala, S.O. Obadara, I.O. Ganiyu, Substituting fishmeal with Hermetia illucens in the diets of African catfish (Clarias gariepinus): Effects on growth, nutrient utilization, haematophysiological response, and oxidative stress biomarker, Aquaculture 518 (2020) 734849.
[10] N. Tippayadara, M.A.O. Dawood, P. Krutmuang, S.H. Hoseinifar, H.V. Doan, M. Paolucci, Replacement of fish meal by black soldier fly (Hermetia illucens) larvae meal: effects on growth, haematology, and skin mucus immunity of Nile tilapia, Oreochromis niloticus, Animals 11 (1) (2021) 193.
[11] A.T.Y. Kishawy, H.A. Mohammed, A.W. Zaglool, M.S. Attia, F.A.M. Hassan, E.M. Roushdy, T.A. Ismail, D. Ibrahim, Partial defatted black solider larvae meal as a promising strategy to replace fish meal protein in diet for Nile tilapia (Oreochromis niloticus): Performance, expression of protein and fat transporters, and cytokines related genes and economic efficiency, Aquaculture 555 (2022). Article 738195.
[12] M.M. Rapatsa, N.A.G. Moyo, Evaluation of Imbrasia belina meal as a fishmeal substitute in Oreochromis mossambicus diets: Growth performance, histological analysis and enzyme activity, Aquac. Rep. 5 (2017) 18–26.
[13] M.M. Rapatsa, N.A.G. Moyo, Enzyme activity and histological analysis of Clarias gariepinus on Imbrasia belina meal used for partial replacement of fishmeal, Fish. Physiol. Biochem. 45 (2019) 1309–1320.
[14] DAFF (Department of Agriculture, Forestry and Fisheries), Aquaculture Yearbook, Department of Agriculture, Forestry and Fisheries, South Africa, 2019, p. 214.
[15] S.R. Netshifhefhe, F.D. Duncan, Nutrient composition of Macrotermes species consumed in the Vhembe District, Limpopo Province, South Africa, J. Insect Food Feed 8 (2022) 95–100.
[16] S.R. Netshifhefhe, E.C. Kunjeku, F.D. Duncan, Human uses and indigenous knowledge of edible termites in Vhembe District, Limpopo Province, South Africa, S. Afr. J. Sci. 114 (2018) 1–10.
[17] A.D. Banjo, O.A. Lawal, E.A. Songonuga, The nutritional value of fourteen species of edible insects in southwestern Nigeria, Afr. J. Biotechnol. 5 (2006) 298–301.
[18] C.U. Igwe, C.O. Ujowundu, L.A. Nwaogu, G.N. Okwu, Chemical Analysis of an Edible African Termite, Macrotermes nigeriensis; a Potential Antidote to Food Security Problem, Biochem. Anal. Biochem. 1 (2011) 105.
[19] C.E. Mbah, G.O.V. Elekima, Nutrient composition of some terrestrial insects in Ahmadu bello University, Samaru Zaria Nigeria, Sci. World J. 2 (2007) 17–20.
[20] M.C. Chulu, Nutrient Composition of the Termite Macrotermes Falciger, Collected from Lusaka district, a Potential Agent Against Malnutrition, The University of Zambia, Lusaka, Zambia, 2015, p. 25. Msc. Dissertation.
[21] Z.T. Hlongwane, M. Siwela, R. Slotow, T.C. Munyai, Effect of geographical location, insect type and cooking method on the nutritional composition of insects consumed in South Africa, J. Insect Food Feed 8 (5) (2022) 537–556.
[22] R.J. Phelps, J.K. Struthers, S.J.L. Moyo, Investigations into the nutritive value of Macrotermes falciger (Isoptera: Termitidae), African Zoology 10 (1975) 123–132.
[23] Association of Official Analytical Chemists AOAC (2012) Official methods of analysis. association of analytical communities 19thEdition, Gaithersburg, MD, USA.
[24] S.N. Hlophe-Ginindza, N.A.G. Moyo, J.W. Ngambi, The effect of exogenous enzyme supplementation on growth and digestive enzyme activities in Oreochromis mossambicus fed kikuyu-based diets, Aquac. Res. 47 (2015) 3777–3787.
[25] P.J. van Soest, J.B. Robertson, B.A. Lewis, Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition, J. Dairy. Sci. 74 (1991) 3583–3597.
[26] F.A. Manditsera, P.A. Luning, V. Fogliano, C.M.M. Lakemond, The contribution of wild harvested edible insects (Eulepida mashona and Henicus whellani) to nutrition security in Zimbabwe, J. Food Compos. Anal. 75 (2019) 17–25.
[27] Y. Ishida, T. Fujita, K. Asai, New detection and separation method for amino acids by high-performance liquid chromatography, J. Chromatogr. 204 (1981) 143–148.

[28] J. Folch, M. Lees, G.H.S. Stanley, A simple method for the isolation and purification of total lipids from animal tissues, J. Biol. Chem. 226 (1) (1957) 497–509.
[29] J.O. Ogunji, S.C. Iheanacho, G.A. Abe, O.R. Ikeh, Assessing effects of substituting dietary fish meal with boiled donkey and cow blood meal on growth performance and digestive enzyme activities of Clarias gariepinus juvenile, J. World Aquac. Soc. (2020) 1–14.
[30] E.M. Mbokane, L.M. Mbokane, C.H. Fouche, The effect of fishmeal replacement with acid-fermented chicken silage on growth, digestive enzyme activity and histology of the intestine and liver of juvenile Mozambique tilapia (Oreochromis mossambicus), Aquaculture International 30 (2022) 491–512.
[31] G.G. Winberg, Rate of metabolism and food requirements of fishes, J. Fish. Res. Board Can. Transl. Series 194 (1956) 1–202.
[32] G.K. Iwama, A.F. Tautz, A simple growth model for salmonids in hatcheries, Can. J. Fish. Aqua. Sci. 38 (1981) 649–656.
[33] J. Ogunji, S. Iheanacho, Alternative protein source: Acceptability of cow blood meal in place of fish meal assessed via growth, antioxidant enzymes functions and haematological response in Clarias gariepinus (Burchell, 1822), Aquac. Res. 00 (2021) 1–11.
[34] D Pauly, Some simple methods for the assessment of tropical fish stocks, FAO Fish. Tech. Pap. FAO Rome 234 (1983) 52.
[35] M.P.M. Anvo, B.R.D. Aboua, I. Compaoré, R. Siaaao, C.Y. Zoungrana-Kaboré, E.P. Kouamelan, A. Toguyéni, Fish meal replacement by Cirina butyrospermi caterpillar's meal in practical diets for Clarias gariepinus fingerlings, Aquac. Res. (2017) 1–8.
[36] A.C. Elia, M.T. Capucchio, B. Caldaroni, G. Magara, A.J.M. Dörr, I. Biasato, E. Biasibetti, M. Righetti, P. Pastorino, M. Prearo, F. Gai, A. Schiavone, L. Gasco, Influence of Hermetia illucens meal dietary inclusion on the histological traits, gut mucin composition and the oxidative stress biomarkers in rainbow trout (Oncorhynchus mykiss), Aquaculture 496 (2018) 50–57.
[37] S.P.H. Shekarabi, M.S. Mehrgan, A. Banavreh, Feasibility of superworm, Zophobas morio, meal as a partial fishmeal replacer in fingerling rainbow trout, Oncorhynchus mykiss, diet: growth performance, amino acid profile, proteolytic enzymes activity and pigmentation, Aquac. Nutr. 27 (2021) 1077–1088.
[38] H.Q. Tran, M. Prokešová, M. Zare, J. Matoušek, I. Ferrocino, L. Gasco, V. Stejskal, Production performance, nutrient digestibility, serum biochemistry, fillet composition, intestinal microbiota and environmental impacts of European perch (Perca fluviatilis) fed defatted mealworm (Tenebrio molitor), Aquaculture 547 (2022). Article ID: 737499.
[39] A. Józefiak, S. Nogales-Mérida, Z. Mikołajczak, M. Rawski, B. Kierończyk, J. Mazurkiewicz, The utilization of fullfat insect meal in rainbow trout (Oncorhynchus mykiss) nutrition: the effects on growth performance, intestinal microbiota and gastrointestinal tract histomorphology, Annals of Animal Science 19 (2019) 747–765.
[40] S. Nogales-Mérida, P. Gobbi, D. Józefiak, J. Mazurkiewick, K. Dudek, M. Rawski, B. Kierńczyk, A. Józefiak, Insect meals in fish nutrition, Rev. Aquac. 11 (2019) 1080–1103.
[41] E. Ringø, Z. Zhou, R.E. Olsen, S.K. Song, Use of chitin and krill in aquaculture – the effect on gut microbiota and the immune system: a review, Aquac. Nutr. 18 (2012) 117–131.
[42] M.A. Henry, L. Gasco, S. Chatzifotis, G. Piccolo, Does dietary insect meal affect the fish immune system? The case of mealworm, Tenebrio molitor on European sea bass, Dicentrarchus labrax, Dev. Comp. Immunol. 81 (2018) 204–209.
[43] I. Belghit, N.S. Liland, P. Gjesdal, I. Biancarosa, E. Menchetti, Y. Li, R. Waagobo, E.L. Krogdahl, Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (Salmo salar), Aquaculture (2018) 503–1973.
[44] A.F.M. El-Sayed, Tilapia Culture, second ed, Academic Press, 2020.
[45] M.M. Rapatsa, N.A.G. Moyo, A Review and Meta-analysis of the Effects of Replacing fishmeal with insect meals on growth of tilapias and sharptooth catfish, Aquac. Nutr. (2022) 1–10. Article 9367587.
[46] J. Velíšek, V. Stejskal, J. Kouřil, Z. Svobodová, Comparison of the effects of four anaesthetics on biochemical blood profiles of perch, Aquac. Res. 40 (2009) 354–361.
[47] J. Gu, H. Liang, X. Ge, D. Xia, L. Pan, H. Mi, M. Ren, A study of the potential effect of yellow mealworm (Tenebrio molitor) substitution for fish meal on growth, immune and antioxidant capacity in juvenile largemouth bass (Micropterus salmoides), Fish and Shellfish Immunology 120 (2022) 214–221.
[48] S. Jentoft, A.H. Aastveit, P.A. Torjesen, Ø. Anderser, Effects of stress on growth, cortisol and glucose levels in non-domesticated Eurasian perch (Perca fluviatilis) and domesticated rainbow trout (Oncorhynchus mykiss), Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 141 (2005) 353–358.
[49] J.O. Ogunji, W. Kloas, M. Wirth, N. Neumann, C. Pietsch, Effect of housefly maggot meal (magmeal) diets on the performance, concentration of plasma glucose, cortisol and blood characteristics of Oreochromis niloticus fingerlings, J. Anim. Physiol. Anim. Nutr. (Berl.) 92 (2007) 511–518.
[50] M.J. Madibana, M. Mwanza, B.R. Lewis, R. Toefy, C.H. Fouche, V. Mlambo, Black soldier fly larvae meal as a fishmeal substitute in juvenile dusky bob Diets: Effect on feed utilization, growth performance, and blood parameters, Sustainability 12 (2020) 9460.
[51] Y. Hu, Y. Huang, T. Tang, L. Zhong, W. Chu, Z. Dai, K. Chen, Y. Hu, Effect of partial black soldier fly (Hermetia illucens L.) larvae meal replacement of fish meal in practical diets on the growth, digestive enzyme and related gene expression for rice field eel (Monopterus albus), Aquac. Rep. 17 (2020). Article ID 100345.