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Partial replacement of fish meal with soldier termite in juvenile Mozambique tilapia: Effects on growth performance, blood serum chemistry and histomorphology

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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*
Dry matter, %	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;

$$\text{thermal-unit growth coefficient (TGC)} = 1000 \times \frac{\text{final weight (g)}^{\frac{1}{3}} - \text{initial weight (g)}^{\frac{1}{3}}}{\text{temperature (°C)} \times \text{days}};$$

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 $(cm)^3 \times 100$;

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 $P < 0.05$

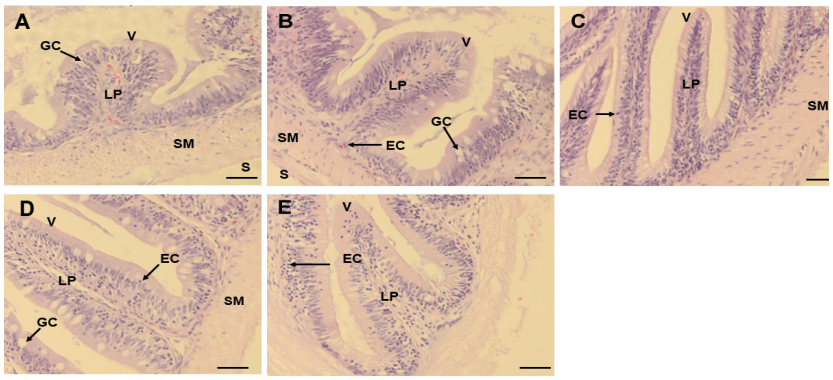

Figure 1. Micrograph of *Oreochromis mossambicus* fed diets with soldier termite meal as a fish meal replacement (A) diet D1 (0% of soldier termite), (B) diet D2 (10%), (C) diet D3 (30%), (D) diet D4 (50%), (E) diet D5 (70%)
V – villi, LP – lamina propria, GC – goblet cell, SM – submucosa, S – serosa, 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.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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