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RESEARCH PAPER

Effects of replacing dietary black soldier fly larvae (BSFL) meal with fishmeal (FM) in diets for rabbitfish (*Siganus sutor*) reared in brackish water intertidal earthen ponds

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Abstract

The effects of replacing dietary fishmeal (FM) with black soldier fly larvae (BSFL) meal in rabbitfish (*Siganus sutor*) diets were evaluated over 90 days in intertidal earthen ponds at Kibokoni, Kilifi Creek. A total of 240 fish were assigned to a completely randomized design with four dietary treatments; each replicated three times. Fish were reared in hapa cages (1 m × 1 m × 1 m) with 1 mm mesh. Four diet treatments were formulated: (T1- 100 % BSFL, T2 – 75 % fish meal and 25 % BSFL, T3 – 50 % fish meal and 50 % BSFL, T4 – control commercial feed. Fish were stocked at a density of 20 fish/m^{-3} and fed twice daily at 5% of their body weight. The experimental fish had an initial mean weight of 11.64 ± 0.97 g and a mean length of 9.12 ± 0.14 cm. Final mean weight gain of the fish ranged from 22.75 ± 3.89 g in T2 (lowest) to 32.3 ± 4.05 g in T1 (highest). Feed conversion ratio (FCR) did not differ significantly among the diets ($P > 0.05$), while survival rate (SR) was highest in T2 (93.3 ± 4.73%) and lowest in T1 (71.1 ± 9.18%). The study provides information that will guide farming of rabbitfish in intertidal earthen ponds, and a possible replacement of fish meal with black soldier fly larvae. Further research on optimal stocking densities is strongly recommended.

Introduction

The long-term sustainability of aquaculture is closely linked to the formulation of cost-efficient feeds, which account for a significant share of production costs, primarily due to the high expense of protein sources. Escalating global prices of conventional feed ingredients, such as fishmeal, together with constraints on critical resources like land and water, highlight the need for innovative feeding strategies. The adoption of climate-smart practices and the integration of sustainable alternative protein sources—including insect meals, algae, and plant-based proteins—are crucial for reducing production costs, enhancing growth performance, and mitigating pressure on wild fish populations (Davis and Hardy, 2022).

A major challenge in the aquaculture industry is replacing fishmeal with alternative protein sources, as fishmeal is valued for its high protein content, balanced amino acid profile, superior digestibility, and palatability (Abdel-Tawwab et al., 2020; Xiao et al., 2018). While plant- and insect-based protein sources are generally more affordable and accessible than fishmeal, they often present limitations such as anti-nutritional factors (ANFs), reduced palatability, and imbalanced amino acid profiles, which can adversely affect fish growth and health. Addressing these limitations through advanced processing methods or the inclusion of functional additives is essential for the development of sustainable aquaculture feeds (Xiao et al., 2018).

The rising demand and competition for fishmeal, driven by human consumption as well as its use in livestock and aquaculture feeds, have resulted in increasing supply shortages. These deficits stem primarily from declining wild fish stocks (Pauly et al., 2002), worsened by overfishing and unsustainable harvesting practices. Furthermore, anthropogenic pressures such as pollution, coastal development, and habitat degradation further limit the availability of marine resources (Lewis and Maslin, 2015). Environmental challenges, including the spread of aquatic diseases and the effects of climate change—such as ocean warming, acidification, and ecosystem disruption—also reduce fish populations and reproductive capacity (Naylor et al., 2021). Together, these factors threaten the stability of fishmeal supply and underscore the urgent need for alternative, sustainable protein sources in aquaculture to decrease reliance on wild fisheries and support long-term feed security.

Approximately 55.86% wild catch and 44.14% aquaculture fish catch is diverted from human consumption to make fishmeal, a source of protein for animal feeds (Pauly et al., 2002). The competition between humans and animals has led to over exploitation of these fish species, and consequently, the cost of feed has become expensive and unaffordable to most farmers (Merino et al., 2012; Thurstan and Roberts, 2014; FAO, 2016a). This has dictated the search for alternative feedstocks for aquaculture which in turn can allow more fish for human consumption (Karapanagiotidis et al., 2011). There is therefore a need for an alternative cheap and easily available source of protein, which is not suitable for direct human consumption and meets the protein requirements (Kassahun et al., 2012). Moreover, global plea has been on the importance of sourcing locally raw materials (ingredients) to reduce over-dependence on imports (Folke et al., 2021; Sara et al., 2022).

Fish and soya bean meals are widely used as primary protein sources in animal feed due to the high protein requirement of fish (Hussain, et al., 2024). Fish-meal for many decades has been the major protein source in aquaculture, but due to unavailability and not cost-effective insects are gaining acceptance and attention due to their nutritional value (Makkar et al., 2014; Henry et al., 2015; Nogales Merida et al., 2018), low environmental footprint (Van Huis et al., 2017) and immune system benefits offered (Mousavi et al., 2020). However, in recent years, the prices of these protein sources, along with other commodities used in animal feed, have increased globally due to the rising demand for protein sources to support the growing human population (Tilman et al. 201;Kearney, 2010, Keyzer et al., 2005, Tilman et al., 2011). To address these challenges, several research have been conducted to find novelty ingredients that are replaced with fishmeal such as processed raw materials and fish proteins hydrolysates (Sidik et al., 2020), microbial biomass (Oliva-Teles and Goncalves, 2001; San Martin et al., 2020), poultry and pork by-products (Karapanagiotidis et al., 2019; Wu et al., 2018), but they lack essential elements such as balanced amino acids. These protein sources are used in feeds for livestock, companion animals (cats and dogs), and farmed fish to meet the increasing global demand (Hardy, 2010) hence leading to scarcity.

Black soldier fly larvae (BSFL) have been widely reported for their significant role in organic waste management (Czekała et al., 2020; da Silva and Hesselberg, 2020). The larval biomass produced through the bioconversion of organic wastes has been identified as a high-quality source of fats and oils (Kim et al., 2021) as well as proteins (Kinasih et al., 2020). Consequently, BSFL are increasingly utilized across various industries, including animal feed, biodiesel production, biopolymers such as chitin, and soil composting (Purkayastha and Sarkar, 2021). Notably, whole or processed BSF larvae or pupae can be incorporated into the diets of poultry, fish, pets, and pigs, serving as promising alternatives to conventional feed ingredients like soybean- and fish-based meals (Surendra et al., 2020). Black Soldier Fly larvae (BSFL) have a crude protein content of roughly 40–50% on a dry weight basis, providing a high-quality source of essential amino acids comparable to fishmeal. The protein levels and overall nutritional composition, however, can vary depending on factors such as larval age, the type of rearing substrate, and processing methods, including defatting (Czekała et al., 2020).

In recent years, insects have become an important source of alternative protein in aquaculture (Stamer, 2015). There have been studies on the use of *H. illucens* meal as a replacer to fishmeal in aquaculture industry in farmed fish species such as, rainbow trout (Stadtlander et al., 2017), Atlantic salmon (Li et al., 2020), European sea bass (Magalhaes et al., 2017), Gilthead seabream (Karapanagiotidis et al., 2023), African catfish (Fawole et al.,2020), Nile tilapia (Tippayadara et al.,2021), yellow catfish (Xiao et al., 2018) and crustaceans such as pacific white shrimp (Cummins et al., 2O17), Red crayfish (Wang et al., 2022), with no studies on marine fishes such as Rabbitfish (*Siganus sutor*) despite its availability.

Studies by Ojha et al., (2020), indicates that, black soldier fly (*Hermetia illucens*) is the most farmed insect species for mass production due to its short life cycle, better feed conversion ratio (FCR), and efficient bioconversion (50-60%) and recovery of nutrients from various organic materials (Okemwa et al., 2022) thus gaining a vast popularity across the globe. From previous studies no harmful effects of *H.illucens* meal on digestibility nor growth performance of farmed fish species have been recorded (Renna et al., 2017; Xiao et., 2018). Insect meals are the current promising animal protein source, being the largest group of organisms in the ecosystem, with no current indications of scarcity in availability (Van Huis, 2013). Black soldier fly larvae (BSFL) (*Hermetia illucens*) larvae are largely considered as an important candidate species to be used for animal and aquatic feeds, due to its high protein content (37-63%) and balanced profile of essential amino acids profile (Maiolo et al.,2020 and Borroso et al., 2014), environmentally friendly and sustainable to be cultured (Cammack and Tomberlin, 2017; Van Huis, 2013). Some studies suggest partial replacement of fishmeal with insect meals, with recent reports showing a successful 100% replacement of fish meal without compromising the growth performance of fish (Hua et al.,2021 and Okemwa et al., 2022). Nevertheless, for the case of carnivorous farmed fish, a total replacement of fish meal products in the diets remains to be feasible and more studies are essential on herbivorous fish.

Alternative and cheaper fish ingredients have great potential for reducing feed costs. However, compared to traditional feed ingredients such as soybean and fish meals, they have difference in terms of quality, nutritional composition, digestibility and availability of minerals (Glencross, 2016; Hua et al., 2021)., and this might affect growth, nutrient utilization and whole-body composition of fish (Bonaldo et al., 2015; Aragao et al., 2020) more so, of herbivorous fish (Palma et al., 2020). Therefore, BSFL has great potential to meet global demand for fish products and replace limited and expensive fish meals to improve production and sustainable aquaculture industry.

Rabbitfish (*Siganus sutor*) are a small family of marine herbivorous fish that is widely distributed in the Indo west Pacific region (Woodland 1983; Jaikumar (2012). The species is economically important and relatively easy to rear and thus considered suitable for aquaculture (Jaikumar (2012). The fish has high tolerance to disturbance in environmental conditions, handling stress and crowding potential (Saoud et al., 2008). It is easy to breed and able to adapt to different water quality parameters. In their natural environment, rabbitfish feed on low energy and protein algae, which leads to varying and sub-optimal growth. Nevertheless, in captivity, rabbitfish can be reared to accept formulated diets thus making them suitable for commercial aquaculture (Jaikumar (2012). This is due to its herbivore to omnivorous feeding habit and feeding on both filamentous green algae and formulated feeds. Understanding the ideal feeding levels is crucial for maximizing growth and feed efficiency, as well as avoiding water quality degradation caused by overfeeding and improving the economic feasibility of aquaculture processing (Naylor et al., 2021).

Research on *S. sutor* in the Western Indian Ocean region has centered on its biology, capture and spawning aggregation, and little effort has been made in farming species (Mbaru et al., 2010; Akinyi et al., 2018), Therefore, the culture of *S. Sutor* in intertidal earthen pond cages is a new aspect in addition to feeding the species with BSFL formulated feed. The species is herbivorous (feeds on benthic algae, seaweed, and seagrass) and can easily be weaned to formulated feeds in captivity (Li et al., 2018). Rabbitfish are warm water fish, but their protein requirements are not well studied. To support cultivation activity, study to fish feed nutrition was conducted to evaluate the effects of replacing dietary fish meal and black soldier fly larvae meal on rabbitfish reared in brackish water.

Methodology

Study site

The study was carried out at Kilifi County, Umoja Self-help group inter-tidal earthen pond farm (Figure 1). The farm is located at Kibokoni village, along Kilifi creek about 10 km West of Kilifi Township at Longitude 039 500 3200E and Latitude 03 360 1200S. Fishponds are constructed in the intertidal mangrove flats classified as the brackish water zone. The area is inhabited by *Rhizophora mucronata* and *Avicennia marina* mangrove species on either side of the sea and land, respectively (Mirera, 2009). Ponds are designed to enable free exchange of ocean water during spring high tides with minimal or no exchange of water during neap tides.

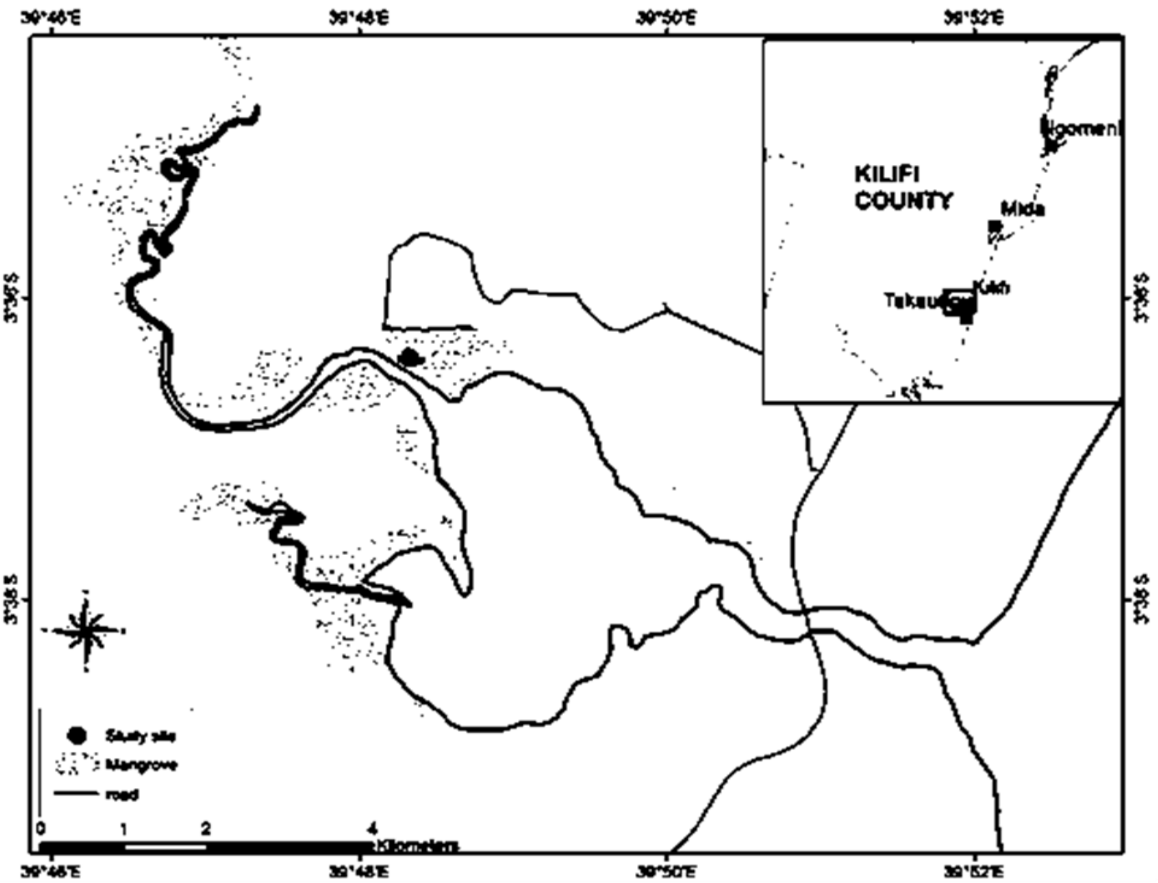

Figure 1. Map showing study site for mariculture intertidal ponds

Experimental design

A 90-day feeding trial was conducted to evaluate the effect of graded inclusion levels of black soldier fly larvae (BSFL) as a replacement for fish meal in the diet of *Siganus sutor* (rabbitfish). Four dietary treatments were tested: T1: 100% BSFL (0% fish meal), T2: 75% BSFL (25% fish meal replacement), T3: 50% BSFL (50% fish meal replacement) and T4: Commercial feed (control). Each treatment was conducted in triplicate, totaling 12 experimental units. The feed treatments were randomly assigned to hapa net cages installed in earthen ponds to minimize location bias. The hapas were constructed using 1 mm mesh size netting, with dimensions of 1.5 m × 1.0 m × 1.2 m (L×W×H). Juvenile *S. sutor* were stocked at a density of $20m^3$ (fish per cubic meter), with initial average weights ranging from 33.76 ± 0.68 g to 36.06 ± 0.99 g, and lengths from 8.62 ± 0.16 cm to 8.89 ± 0.13 cm. Fish were fed twice daily at 09:00 and 16:00 hours, at a rate of 5% of body weight. To protect the experimental fish from predators, each hapa was enclosed within a larger outer net cage made of 5 mm mesh. Daily net cleaning was performed during routine monitoring to prevent clogging and maintain optimal water circulation within the hapas.

Hapa nets cages and earthen ponds

The experiment was conducted in an existing intertidal earthen fishpond measuring 20 m × 40 m (800 m^2), located along the coastal zone. Prior to the start of the experiment, the pond underwent renovation, which included the installation of inlet and outlet pipes to ensure proper water exchange. To ensure biosecurity and reduce pathogen load, the pond was drained, limed, and sun-dried for one week. This process aims to eliminate parasites, residual organic matter, and other undesirable organisms. The pond was subsequently refilled with natural seawater during spring high tides using inlet pipes fitted with fine mesh screens (1mm mesh size) to prevent the entry of predators and other unwanted aquatic organisms. Hapa net cages were constructed from black nylon mesh with a 1 mm mesh size, each measuring 1.5 m × 1.0 m × 1.2 m (length × width × height). The cages were suspended within the pond using corner stake nets to maintain structural integrity and ensure that each hapa retained enough water volume for accurate stocking density. These hapas served as individual experimental units for the different dietary treatments.

Experimental fish

A total of 240 juvenile fish were used across all experimental replicates. The fish were caught from the wild along the Kilifi Creek by artisanal fishers using seine nets. Immediately after capture, fish were sorted and transferred into clean seawater containers equipped with portable aerators to maintain optimal oxygen levels during handling and preparation for transport. To minimize stress and mortality during transportation, ice blocks were added to the containers to gradually lower the water temperature, thereby reducing fish metabolic activity. Upon arrival at the experimental site, the fish were held in earthen ponds for a period of 14 days to allow for acclimatization to captive conditions. Species identification was conducted prior to stocking to ensure that only *Siganus sutor* were used in the experiments. A trained fish taxonomist, assisted by a standard taxonomic key, was engaged to distinguish *S. sutor* from other species using morphometric characteristics as described by (FAO, 2012).

Feed preparation

Black soldier fly larvae (BSFL) were sourced from experimental culture at Nyamone Fish Farm, located on the south coast of Kenya. The larvae were initially dried, followed by oven-drying to ensure complete moisture removal. Once dried, the BSFL were ground into a fine powder for use in feed formulation. Other feed ingredients including fishmeal, wheat bran, maize bran, and cassava flour (used as a binder) were procured from Majengo market Mombasa County. These ingredients were individually sun-dried, ground, and sieved through a size 40 mesh to achieve a uniform particle size.

Each ingredient was weighed using a digital analytical balance, and the formulation of experimental diets was performed using the Pearson's Square Method (Millaminaet et al., 2002), based on the desired protein levels for each treatment. The ingredients were thoroughly mixed, after which a vitamin-mineral premix was incorporated and blended into the dry mixture.

To bind the feed into pellets, cassava flour was mixed with water in a 1:4 ratio (i.e., 50 g cassava powder in 200 ml water per 1 kg of feed) to produce a stiff dough. The feed mixture was then pelleted using a 1.5 mm die to produce uniform pellets. The pellets were shade-dried to a final moisture content of approximately 10%, then stored at –20°C until use.

A proximate composition analysis of the primary feed ingredients was carried out at the natural product laboratory, KEMFRI headquarters prior to diet formulation to determine their nutritional content. The composition and proximate analysis of the formulated diets are presented in Table 1.

Table 1. Results of the proximate analysis of the experimental diets

Ingredients (g/kg feed)	Dietary treatments FM: BSFL			
	T1 (100 % BSFL)	T2 (75 % BSFL)	T3 (50 % BSFL)	T4 (0 % BSFL)
Fish meal	0	425	325	525
Maize bran	195	195	195	195
Wheat bran	260	260	260	260
BSFL	525	100	200	0
Vitamin premix	5	5	5	5
Mineral premix	5	5	5	5
fish oil	10	10	10	10
Total	1 kg	1 kg	1 kg	1 kg
Proximate composition				
Moisture (%)	8.6	9.9	10.2	9.5
Crude lipid (%)	5.45	4.32	4.11	4.95
Crude protein (%)	27.4	24.6	25.3	30.9
Ash (%)	6.45	8.10	8.64	8.21
Crude fiber (%)	3.43	3.77	3.27	4.13
NFE	48.65	49.31	48.48	42.31

NFE = 100% - (% crude protein + % crude fat + % crude fiber + % ash + % moisture)
[a] Vitamin Premix (per 100 g premix): thiamin hydrochloride, 0.15 g; retinol acetate, 0.043 g; Ca pantothenate, 0.3 g; riboflavin, 0.0625 g; niacin, 0.3 g; ascorbic acid, 0.5 g; biotin, 0.005 g; pyridoxine hydrochloride, 0.225 g; para-aminobenzoic acid, 0.1 g; folic acid, 0.025 g; α-tocopherol acetate, 0.5 g; cholecalciferol, 0.0075 g; menadione, 0.05 g; inositol, 1 g. [b] Mineral Premix x (per 100 g premix): NaH_2PO_4, 10.0 g; $CaCO_3$, 10.5 g; KH_2PO_4, 21.5 g; $Ca(H_2PO_4)_2$, 26.5 g; KCl, 2.8 g; $AlCl_3 \cdot 6H_2O$, 0.024 g; $MgSO_4 \cdot 7H_2O$, 10.0 g; $MnSO_4$ 6 H_2O, 0.143 g; KI, 0.023 g; $ZnSO_4$ $7H_2O$,0.476 g; $CuCl_2$ 2 H_2O, 0.015 g; $CoCl_2 \cdot 6H_2O$, 0.14 g; Calcium lactate, 16.50 g; Fe-citrate, 1g

Proximate analysis

Experimental diets were analyzed for proximate analyses to determine the nutritional composition at KMFRI laboratory (Table 1). Analysis was done on a dry matter basis using standard methods of the Association of Official Analytical Chemists (AOAC, 2003). The dry matter was done by drying pre-weighed samples in an oven at 105^0C for 16 hours to reach a constant weight. Nitrogen was analyzed using the Kjeldahl method, Crude protein, lipids, and fibers were determined using the procedures outlined by (AOAC, 2003) Ash content was determined by burning the samples in a muffle furnace at 550^0C for 4 hours.

Water quality parameters (Table 2) were monitored bi-weekly. Physic-chemical parameters such as temperature, dissolved oxygen, pH, total ammonium nitrogen (NH_4^+–N), and nitrite nitrogen (NO2-N) were measured biweekly. Temperature (°C), dissolved oxygen (mg L-1) and pH were measured in situ using a multiparameter water quality meter model number H19828 (Hanna Instruments Ltd., Chicago, USA). Water samples for Chlorophyll-(chl-a), total ammonium nitrogen (NH_4^+–N), (mg L-1) and nitrites-nitrogen (NO2-N) (mg L-1) were collected monthly using standard methods by (APHA, 2017), at each treatment hapa in a plastic cylinder, mixed and then sub-sampled to obtain a representative sample for laboratory analysis. The samples were stored in a cooler box on ice and transported to Kenya Marine and Fisheries Research Institute (KMFRI) nutrient laboratory where analysis was undertaken.

Table 2. Mean (±SD) of water quality parameters observed in intertidal earthen ponds

Parameters	Mean and StDev	Min	Max
Temperature(^{0}C)	29.45 ± 0.58	28.8	30.20
DO (mg/L)	5.1 ± 0.03	5.18	5.32
Transparency (cm)	31.5 ± 0.12	30.23	44.2
PH	7.78 ± 0.08	7.57	7.88
Salinity(mg/L)	41.56 ± 1.23	39.27	44.67
PO_4(mg/L)	0.44 ± 0.01	0.453	0.45
NO_2(mg/L)	0.2 ± 0.03	0.2	0.24
NO_3(mg/L)	1.5 ± 0.01	0.93	1.5
NH_3(mg/L)	0.02 ± 0.05	0.021	0.023

The results shown are the Mean (±SD) of water quality parameters observed in intertidal earthen ponds

Fish sampling

Monthly sampling of fish was undertaken and involved measurement of individual weight (for daily feed amount adjusted) and total length. To reduce stress, fish were handled under mangrove shade in a plastic basin halfway filled with clean pond sea water. During sampling, water was aerated (6,815 liters per hour) using 12 Volt aquarium aerators to ensure adequate dissolved oxygen concentrations. Random samples of 20 fish were collected from each replicate for weight and length measurements. Weighing was done using a digital balance (0.01g) (model KERN 572-33, Germany) and total length was measured using a measuring board (0.10 cm) as described by (Froese, 2006; FAO, 2022). Fish growth performance under different treatments was evaluated in terms of wet weight (g), daily weight gain (DWG, g day-1), specific growth rate (SGR, % day-1), percentage survival (%) and feed conversion ratio (FCR) (Table 3). Both at the beginning and end of the experiment, fish from each treatment were kept for further proximate analysis. Calculation was done using the following formulas:

SGR (%) = (Ln final weight–ln initial weight)/rearing day*100

Weight gain = Mean final weight–mean initial weight

DWG = final weight–mean initial weight/culture days

DGR = Mean final weight–mean initial weight/culture days

FCR = Feed (g) (dry weight)/live weight gain (g)

Weight gain (g) = Final weight -initial weight

Survival (%) = Final number harvested/initial number stocked*100

Weigh gain (%) = final weight-initial weight/ Initial weight*100

Table 3. Mean (±SE) growth performance of *S. sutor* under different dietary treatments in cages installed in intertidal earthen ponds

Parameters	T1	T 2	T3	T4
Initial BW(g)	36.06 ± 0.99	34.53 ± 0.78	35.33 ± 0.78	33.76 ± 0.68
Final BW (g)	68.36 ± 0.42	57.28 ± 0.33	61.67 ± 0.42	62.67 ± 0.41
Initial length (cm)	8.89 ± 0.133	8.62 ± 0.16	8.72 ± 0.073	8.82 ± 0.21
Final Length (cm)	10.51± 0.15	9.92 ± 0,24	9.78 ± 0.19	10.24 ± 0.89
BWG (g)	32.30 ± 4.05	22.75 ± 3.89	26.34 ± 1.81	28.9 ± 0.14
ADLG	0.36 ± 0.05	0.253 ± 0.03	0.29 ± 0.02	0.321 ± 0.04
ADWG	3.80 ± 0.02	2.30 ± 0.00	3.60 ± 0.048	1.60 ± 0.05
SGR	3.68 ± 0.09	3.88 ± 0.08	3.74 ± 0.09	3.65 ± 0.02
SR (%)	71.10 ± 9.18	93.30 ± 4.73	86.70 ± 1.41	84.40 ± 7.03
FCR	2.11 ± 0.20	2.51 ± 0.20	2.34 ± 0.3	2.16 ± 0.31

* Significance was tested at ($p<0.05$) for growth performance of *S. sutor* under different dietary treatments in cages installed in intertidal earthen ponds

Carcass collection and analysis

At the beginning of the experiment (Day 1), three fish from each treatment were randomly sampled and immediately stored at –20°C for subsequent analysis of initial body composition. Upon completion of the 90-day feeding trial, an additional three fish per treatment were randomly selected from each dietary treatment, euthanized, and dissected to obtain whole-body carcass samples and kept frozen at -20ºC for proximate analysis as shown in table 4. The chemical analysis of fish carcass was conducted following Association of Official Analytical Chemists (AOAC, 2003).

Data analysis

All statistics were performed using the SPSS package (Version 23.0). Data was analyzed using One-Way Analysis of Variance (ANOVA), to compare the variation between treatments, followed by a comparison of means (Tukey's HSD test) to test the effects of growth performance on fish ($P < 0.05$). Growth performance curves were generated using excel. To evaluate whether the dataset followed a normal distribution, quantile–quantile (Q–Q) plots were generated and visually inspected.

Results

Water quality parameters in intertidal earthen ponds stocked with Siganus sutor

The mean water temperature was 29.45 ± 0.58°C, ranging from 28.8°C to 30.2°C, which is optimal for tropical marine fish. Dissolved oxygen levels were stable, with a mean of 5.10 ± 0.03 mg/L, supporting good respiration and metabolic functions in fish. Water transparency showed moderate variation, with a mean of 31.5 ± 0.12 cm, and a maximum of 44.2 cm, indicating balanced water clarity conditions. The pH was slightly alkaline (7.78 ± 0.08), remaining within a healthy range (7.5–8.0). Salinity averaged 41.56 ± 1.23 mg/L, consistent with marine environments and suitable for *S. sutor* physiology. Nutrient concentrations for Phosphate (PO_4) 0.44 ± 0.01 mg/L, Nitrite (NO_2–N): 0.20 ± 0.03 mg/L, Nitrite (NO_2–N): 1.50 ± 0.01 mg/L and Ammonia (NH_3): 0.02 ± 0.05 mg/L (Table 2).

Proximate composition of formulated fish feed

The experimental diets (T1–T4) involved varying substitution levels of fishmeal with BSFL meal: T1 (100% BSFL) full replacement, T2 (75% BSFL), T3 (50% BSFL) and T4 (0% BSFL) 100% fishmeal (control). As BSFL inclusion increased fishmeal decreased from 525 g/kg (T4) to 0 g/kg (T1), BSFL increased from 0 g/kg (T4) to 525 g/kg (T1) where other ingredients remained constant, ensuring that changes in nutrient composition are attributable to the FM–BSFL substitution. The results indicate moisture content (%) ranged from 8.6% (T1) to 10.2% (T3), protein content was high in T4 (30.9%), lowest in T2 (24.6%) while T1 (100% BSFL) had 27.4%. Nitrogen-Free Extract (NFE) was high in T2 (49.31%), T3 48.48 and T1 (48.65%) respectively while T4 (42.31%) had the lowest NFE. Ash content was high in fishmeal-based diets (T2–T4), while T1 (6.45%) had the lowest. Crude Fiber (%) was slightly lower in T3 (3.27%), highest in T4 (4.13%) (Table 2).

Growth performance

The growth performance of fish was under different treatments (T1–T4) ($p < 0.05$) as summarized in (Table 3). Initial body weights were statistically similar across treatments ($p > 0.05$), indicating homogenous starting conditions. However, significant differences ($p < 0.05$) were observed in final body weights, body weight gain (BWG), average daily weight gain (ADWG), and feed conversion ratio (FCR). T1 recorded the highest final body weight (68.36 ± 0.42 g), followed by T4 (62.67 ± 0.41 g), T3 (61.67 ± 0.42 g), and T2 (57.28 ± 0.33 g). A similar trend was observed in BWG and ADWG, where T1 significantly outperformed all other treatments. The lowest growth performance was observed in T2, which had the least BWG (22.75 ± 3.89 g) and ADWG (2.3 ± 0.002 g/day). T2 recorded the highest specific growth rate (SGR, 3.88 ± 0.08%) ($p > 0.05$).

In terms of feed utilization, statistically significant differences ($p < 0.05$) were observed in FCR among treatments, with T1 exhibiting the most efficient FCR (2.11 ± 0.20), while T2 had the highest FCR (2.51 ± 0.20). Survival rate (SR) varied significantly ($p < 0.05$), with T2 achieving the highest SR (93.3 ± 4.73%) and T1 the lowest (71.1 ± 9.18%) (Table 3).

All treatments (T1–T4) showed a progressive increase in mean weight gain over time (Figure 2).

Among the four treatments, T1 resulted in the highest weight gain over the culture period, followed by T4 and T3, while T2 showed the lowest performance. There was no overlap between T1 vs T2 at day ~90 (P>0.05) (Figure 2).

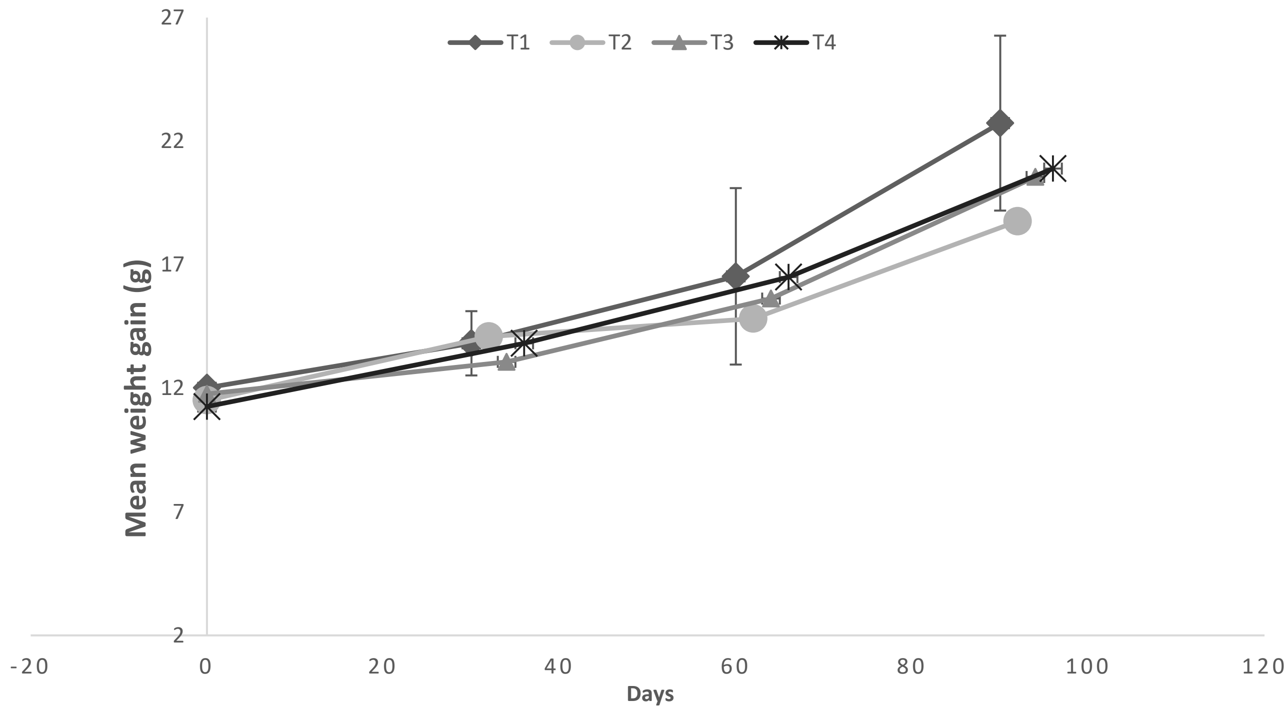

Figure 2. Growth trend (means ±Stdev) of rabbitfish (*S. sutor)* reared in brackish intertidal earthen ponds and fed with formulated diets

Table 4. Proximate Carcass Composition of *Siganus sutor* reared in Intertidal earthen Pond under different diets

Diet fed to *S.sutor*						
	Initial (Day 1)	**T1**	**T2**	**T3**	**T4**	**± SEM**
Dry matter	20.21	24.50	23.23	23.30	23.08	±0.71
Crude protein	50.23	60.98	56.71	57.98	58.22	**±1.79**.
Crude Lipid	7.67	17.45	14.76	9.21	20.34	±2.41
Ash	35.21	17.11	19.32	20.41	17.05	±3.41

Dry matter content ranged from 23.08% to 24.50% among the experimental diets (table 4), showing a modest increase from the initial value of 20.21%. The highest dry matter content was observed in T1 (24.50%), while T4 had the lowest (23.08%) among treatments.

Protein levels increased significantly in all diets compared to the initial baseline value (50.23%) where T1 had the highest crude protein content (60.98%), followed closely by T4 (58.22%) while T2 and T3 had slightly lower protein content, 56.71% and 57.98% respectively. Treatment1 recorded the lowest dry matter content, suggesting that the fish were well-fed and in good physiological condition, with increased protein deposition associated with active tissue development. Crude lipid content showed marked variation across treatments where the highest lipid content was in T4 (20.34%), more than double the initial diet (7.67%). T1 and T2 also had high lipid levels (17.45% and 14.76% respectively), whereas T3 was the lowest (9.21%). Ash content, is an indicative of total mineral matter, decreased in all experimental diets relative to the initial formulation (35.21%). T1 and T4 had the lowest ash content (17.11% and 17.05%) which is an indicative of mineral deficiency, while T3 had the highest (20.41%) among the treatments which would mean poor-quality feed ingredients,

Survival rate of Siganus sutor fed experimental diets in intertidal earthen pond cages

The survival rates (%) of *Siganus sutor* across the four diet treatments (T1–T4) showed notable variation ($p > 0.05$) (figure 3). T2 had the highest survival rate (93.3 ± 4.73%) and T1 recorded the lowest survival (71.1 ± 9.18%).

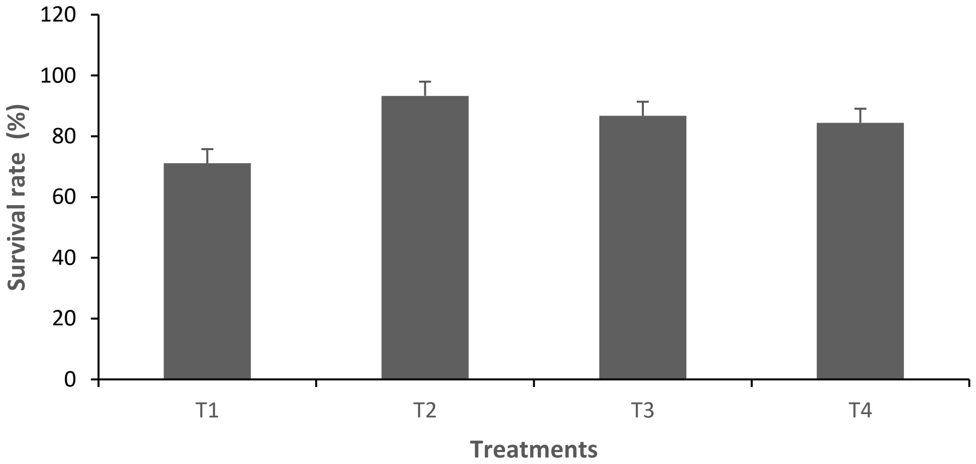

Figure 3. Percentage survival (mean ± SD) of *Siganus sutor* fed on different dietary treatments in cages installed in intertidal earthen ponds

Discussion

Growth performance

We observed that all experimental fish in cages were in good health throughout the experimental period. The study established that the wild collected *S. sutor* seed responded well to all experimental diets, in support by (Dewolu et al. 2010). According to Li et al. (2018), *S. sutor* readily accepts artificial feeds when introduced to diets, have lower protein demand than carnivorous fish Lupatsch (2007) thus, makes them attractive for both small- and large-scale aquaculture Li et al., (2018).

The current study, T1's significant growth trend (figure 2), compared to the other groups, may be attributed to enhanced feed palatability, which aligns with findings from El-Sayed (2006), who reported that protein-rich and well-balanced diets significantly enhance growth performance in aquaculture species. The sharp incline in weight gain for T1 in the final 30 days, indicates that the impact of the treatment intensified as the experiment progressed. Conversely, T2 recorded the lowest mean weight gain throughout the period, with its growth curve remaining consistently below those of the other treatments. This reduced performance could reflect inadequate nutrient composition, feed intake suppression, or metabolic inefficiencies, as suggested by De Silva & Anderson (1995), who found that even small deficiencies in energy can greatly impair fish growth. T3 and T4 demonstrated intermediate performance, notably, T4 showed improved weight gain in the latter phase, potentially indicating compensatory growth, a phenomenon where organisms experience accelerated growth after a period of nutrient limitation (Ali et al., 2003). The convergence of T3 and T4 curves in the mid-phase, followed by divergence, supports this hypothesis. All treatments (T1–T4) showed a consistent increase in mean body weight over time, indicating that the feeds or environmental conditions supported general growth. However, T1 demonstrated the highest mean weight gain, particularly evident in the final phase (Day 60 to Day 90), suggesting superior nutritional quality or better physiological response (Ali et al., 2020).

Treatment 1 (T1) demonstrated the highest final body weight (68.36 g) and body weight gain (32.3 g), suggesting a more effective nutrient profile or higher palatability of the feed. High weight gain often correlates with better protein quality and digestibility (NRC, 2011). These results align with findings by El-Sayed (2006), who noted that optimal protein and energy levels in feed significantly enhance growth rates in saline juvenile fish and improve feed utilization. In contrast, T2 showed the lowest growth performance, despite similar initial weights. This may suggest either nutrient deficiencies or lower feed intake, as previously reported by Fagbenro et al. (1998), who showed that sub-optimal feed formulations can limit growth even under favorable environmental conditions.

Additionally, combining two or three animal-based protein sources has been documented to promote a balanced amino acid profile and compensate for the high EE in the larvae (Phonekhampheng, 2008). However, this assumption was challenged in the present study, where some BSFL were combined with fish meal T2 (25% BSFL and 75% fish meal) and T3 (50% BSFL and 50% fish meal) and the results showed a single protein T1 (100% BSFL) performing well as compared to combined protein. Consequently, Kroeckel et al., (2012) whose study on reared juvenile turbot on diets containing different propositions of BSFL (33%), substitution levels of fish meal by BSFL led to a decrease in the growth performance parameters.

The observed body weight gain (BWG) in this study was relatively low, indicating a sluggish growth rate throughout the experimental period. This reduced performance is likely attributable to the high stocking density of 20 fish per m^3. Such density levels may have led to increased competition for space, feed, and dissolved oxygen, thereby negatively affecting growth performance. These findings are consistent with previous studies that have demonstrated a negative correlation between high stocking density and growth rate (Ellis et al., 2002; Montero et al., 1999; North et al., 2006). For instance, El-Sayed, (2002), reported a significant decline in growth performance of cultured fish at elevated stocking densities. High densities are known to induce chronic stress, which can suppress feeding behavior, impair metabolism, and reduce overall fish health, ultimately leading to suboptimal growth outcomes Baldwin, L. 2011. However, the effect of stocking density may be species-specific. For example, in seabass, studies have shown enhanced growth rates under higher stocking densities, likely due to the species' tolerance to crowding or improved social interaction under certain conditions (Martins et al., 2011; Diogenes et al., 2019). These contrasting findings highlight the need to consider species behavior and environmental context when determining optimal stocking densities. In the case of *Siganus sutor* (rabbitfish), the current results suggest that growth performance is sensitive to stocking density, with higher densities likely impairing individual growth potential. This observation is further supported by broader aquaculture literature, which reports that overstocking commonly results in reduced growth rates across various cultured fish species due to elevated stress levels and competition for limited resources (Holm et al., 1990, Irwin et al., 1999, Yousif, O. M. 2002.)

Survival rate

The highest survival rate (93.3%) was seen in T2, suggesting that although growth was poor, the fishexperienced less physiological or environmental stress. According to Stickney (2000), survival rate is not only a function of feed quality but also tank conditions, stocking density, and water quality. In contrast, T1’s low survival (71.1%), despite its excellent growth, suggests possible trade-offs between aggressive feeding behavior and stress-induced mortality, as discussed by Portz et al. (2006) in the context of stress physiology in aquaculture species. Similar findings were found by El-Sayed, A.-F. M., & El-Ghobashy, A. (2011) whose study on *Siganus rivulatus* found survival rates of between 75.0 - 80.3 % with a diet of CP 40 %. We found that treatments with low CP 24.6 attained higher survival and low growth rate while those with higher CP 27.4 had low survival and higher growth rate. The treatment with 100 % fish meal replacement with BSFL had the lowest survival which provides a gap for further investigation to establish any underlying issues to inform development of an insect-based diet for rabbit fish (Barroso et al., 2014)

Proximate composition

Protein is the most critical macro-nutrient in aquafeeds, essential for growth, tissue development, enzyme synthesis, and various metabolic functions in fish (NRC, 2011; Abdel-Tawwab et al., 2010; Wilson, 2002. In the present study, the diet formulated with black soldier fly larvae (BSFL) containing the highest crude protein (CP) level of 27.4% resulted in superior growth performance compared to the BSFL diet with 24.6% CP. The reduced growth observed in fish fed the lower protein diet (24.6% CP) suggests that insufficient dietary protein may have limited nutrient availability for tissue synthesis, metabolic functions, and overall somatic growth. The observed difference aligns with previous findings indicating that dietary protein levels below optimal thresholds can negatively impact growth performance in cultured fish species, particularly when alternative protein sources like insect meal are used (Henry et al., 2015; Makkar et al., 2014).

Despite these differences, carcass protein content remained relatively consistent across all treatments, suggesting that the dietary protein was adequately utilized and retained for somatic growth. This aligns with findings by Henry et al. (2015), who reported that partial replacement of fishmeal with black soldier fly larvae (BSFL) can maintain protein retention when diets are properly balanced for essential amino acids. The similar protein levels in fish carcasses across treatments also indicate that protein synthesis and deposition were not compromised by BSFL inclusion up to certain levels. However, diets with lower protein content may result in reduced growth rates over longer durations if not supplemented with limiting amino acids such as methionine or lysine, which are typically lower in insect meals compared to fishmeal (Makkar et al., 2014).

Moreover, protein content in carcasses is a good indicator of lean tissue development, and its consistency across treatments implies that all diets supported healthy physiological conditions, provided energy and other nutrients were balanced. The availability of an ingredient depends on its accessibility and nutrient content, as stated by Adéyèmi et al., 2020.

Studies where fishmeal was used as control diet, formulated with 30.9% CP, did not result in superior growth performance when compared to the black soldier fly larvae (BSFL) diet, which contained 27.4% CP NRC 2011. These findings suggest that *Siganus* species may exhibit efficient growth even on lower-protein diets, particularly when the protein source is highly digestible and well-balanced in essential amino acids. The better performance of the BSFL diet, despite its lower protein content, supports the hypothesis that herbivorous fish such as rabbitfish have relatively low protein requirements, likely due to their natural feeding habits and efficient utilization of alternative protein sources. This aligns with previous research indicating that dietary protein needs vary according to feeding guild, and that herbivorous species generally thrive on lower protein diets compared to carnivores.

Ash includes both macro and micronutrients essential for various physiological functions, including bone development, enzyme activation, and osmoregulation in fish. However, while minerals are vital, fish require them in relatively small quantities, and excessive ash content can negatively affect diet quality and digestibility (Dewanggani et al.,2021).

In the present study, ash content across all treatments ranged from 6.45% to 8.64%, falling well below the recommended maximum threshold of 12% for formulated aquafeeds Dewanggani et al.,2021. Notably, fish fed with diets containing lower ash content (6.45%) exhibited improved growth performance, likely due to better digestibility and nutrient bioavailability. In contrast, higher ash content diets were associated with reduced growth, supporting previous findings that excessive ash can impair nutrient absorption and lower feed utilization efficiency (Usman and Kamaruddin 2020).

Importantly, ash itself does not contribute metabolizable energy to the diet and is generally considered non-digestible Ringgita and Erwanto (2015). Diets with elevated ash levels have also been linked to toxicity risks and gastrointestinal irritation, particularly when certain minerals accumulate beyond tolerable levels Ringgita and Erwanto (2015).

Lipids are essential for the growth and survival of fish. Studies have shown that high levels of lipids in the diet indicate that there is enough energy available for growth and survival (Syahrizal et al., 2022). The results of this study indicate that fish fed with a high lipid diet showed better growth by 5.45 for T1(100% BSFL). The high lipid content in black soldier fly larvae (BSFL) increases the dietary energy of feeding. Animals tend to achieve early cravings with high-energy diets, which could have limited the intake of fish fed with whole BSFL. Additionally, fish growth occurs when there is an excess of free energy left after being used for body maintenance, basal metabolism, and fish activity. If the diet produces low energy, it will not be sufficient for the fish growth process (Herdiyanti et al., 2007). The poor growth of rabbitfish may be due to the recorded low lipids in all treatments, which could have resulted from a lack of sufficient energy.

According to Shelley and Lovatelli (2011), Feed Conversion Ratio (FCR), is the amount of feed needed to produce one kilogram of fish. FCR is a crucial factor in the success of feeding because it determines the amount of feed consumed, the increase in body weight, and the production cost (Boonyaratpalin, 1989). The best FCR was observed in T1 (2.11), indicating more efficient feed utilization. This is consistent with De Silva & Anderson (1995), who reported that lower FCR values indicate optimal protein-to-energy ratios in aquafeeds. T2, with the poorest FCR (2.51), may have experienced feed wastage, poor palatability, or inefficient digestion (Tacon & Cowey, 1985). Moreover, Chiu (1989) suggested that feed efficiency could be measured accurately if feeding is appropriately managed, and excess food is kept to a minimum. The feed conversion rate (FCR) was slightly higher for all treatments that included black soldier fly larvae (BSFL) and fishmeal (100%) with a value of 2.11 and 2.16, respectively. Introducing a formulated diet in a confined environment may have led to higher FCR in all treatments due to rabbitfish being herbivorous.

Water quality conditions

The range of mean values of temperature, DO, pH, and other parameters in all treatments in this study remained within the acceptable and tolerable levels for both growth and survival of rabbit fish. Studies have indicated that rabbitfish grow well at different ranges of salinity and can tolerate varied water quality parameters (Tidwell and Allan, 2001). In the current study, the averages of water temperature, pH, salinity, dissolved oxygen and nitrite (NO_2), nitrate (NO_3), and total ammonia (NH_3-N) values in all treatments were within the acceptable limits for rabbit fish (*Siganus sutor*) growth in brackish water (ANZECC, 2000; EPA, 2003; Saoud et al., 2007b; Saoud et al., 2008).The good water quality in the ponds can be associated with the free flushing of water during spring high tides (Mirera, 2011). Frequency of water renewal has a significant effect on the growth performance and nutrient utilization of fish (Absalom and Omenaihe, 2000; Okomoda et al., 2016).

Studies by Kohno et al., (1988) showed *S. guttatus* growth was improved by rearing at low temperatures, which agreed with our findings that had mean temperatures of 29.45±0.58. Rabbitfishes are tolerable to a wide range of salinities ranging from 14-37 ppt (Ben-Tuvia 1966).

The current study salinities ranged from 39.27-44.67, nevertheless, Boyd & Tucker, 2012, found out that rabbitfishes may not tolerate very low salinities. Dissolved oxygen (DO) is very vital for the growth and survival of fish, the values recorded in this study were acceptable for rabbitfish growth. Studies from Chapman (1996) reported that low values of DO (5 mg/L) result in lower functioning and survival of fish, this observation aligns with our findings where DO levels ranged narrowly between 5.18 and 5.32 mg/L, remaining just above the critical threshold. We also highlight the significance of managing water quality to acceptable levels in culture systems for a maximized growth performance of *S.sutor*. Extremes in water quality parameters may impact on the growth performance and survival of *S. sutor*. Salinities of more than 30ppt have been observed to cause growth depression with reduced digestibility and feed conversion efficiency in milkfish (Jana et al., 2006).

Recommendations

The findings of this study indicate that farming rabbitfish (*Siganus sutor*) in cages installed in intertidal earthen ponds is both feasible and promising. The use of Black Soldier Fly Larvae (BSFL) meal as a replacement for fishmeal in aquafeeds demonstrated strong potential. Diets incorporating BSFL resulted in superior growth performance compared to traditional fishmeal-based feeds. This suggests that BSFL can be a sustainable and cost-effective protein alternative, especially for herbivorous marine species such as rabbitfish. This outcome points to the need for further research on optimal stocking densities, feed competition, and environmental management to reduce stress and mortality in cage-farming systems.

The tested diets had protein levels ranging from 24.6% to 30.9%, and the fish still showed satisfactory growth and carcass protein retention. This implies that herbivorous fish such as rabbitfish may perform well on lower-protein diets, provided the protein source is digestible and nutritionally balanced. It is therefore recommended that future feed formulations maintain crude protein levels above 27% and ensure amino acid balance, particularly when insect-based ingredients such as BSFL are used. Furthermore, this study underscores the potential for BSFL to be reared on domestic and market waste, offering a climate-smart innovation in aquaculture. Such an approach can help reduce feed costs and organic waste while contributing to local economic development.

Conclusion

In conclusion, the successful incorporation of BSFL into rabbitfish diets represents a significant advancement toward sustainable aquaculture. The study highlights the potential of insect-based feed systems to address the rising costs and limited availability of fishmeal, while also supporting local feed production and environmental sustainability. To fully realize these benefits, further field trials in sea cages and commercial-scale systems are recommended. These will help validate the current findings and establish best practices for broader adoption of BSFL-based aquafeeds in marine aquaculture. The current findings strongly recommend incorporating BSF at 50%, alongside other protein sources, to enhance growth while reducing costs.

Author Contributions

D. Okemwa contributed to project conceptualization, methodology, writing original draft preparations: Prof. Ngugi Charles and Dr. Mirera David contributed to review and editing the original draft supervisory. All authors contributed to the manuscript; all authors read and approved the manuscript for publication. We certify that this is our original scientific research work, and it has not been submitted or published anywhere. The authors are responsible for all the content in the manuscript.

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Conflict of interest: The authors declare no conflict of interest, and the funders had no role in the design of the study, data collection, analyses, data and results interpretation, manuscript writing, and in decision making in publication of the findings.

Conflict of Interest

The authors declare that there are no conflicts of interest that might arise as a result of the publication of this manuscript and the information therein.

Ethics Statement

This study was conducted as part of a master's thesis and received ethical approval from the National Commission for Science, Technology and Innovation (NACOSTI)

Data Availability Statement

We certify that the data used in this article were collected from the study and can only be availed through the request and permission of the third-party authors.

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