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Performance of African catfish *Clarias gariepinus* larvae fed on formulated diets containing *Spirulina platensis* and *Eisenia fetida*

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Abstract

Profitable aquaculture depends on quality seed and fish feed used. The need to use significant amount of aquatic resource proteins in aquafeed presents economic and environmental challenges. Therefore, it is necessary to develop sustainable, cheaper, renewable and ecofriendly protein alternatives to replace aquatic resource proteins, hoping for their eventual elimination from larviculture. The current study evaluated Spirulina platensis and Eisenia fetida as dietary protein sources for Clarias gariepinus larvae (0.002g±0.04). Hundred percent Caridina nilotica in the control diet was partially replaced by either Spirulina platensis or Eisenia fetida at 25%, 50% and 75% to formulate six approximately isonitrogenous and isocaloric Clarias gariepinus larval diets (T1, 25%S. platensis +75%C. nilotica; T2, 50%S. platensis + 50%C. nilotica; T3,75%S. platensis +25%C. nilotica; T4,25%E. fetida + 75%C. nilotica; T5,50%E. fetida +50%C. nilotica; T6 ,75%E. fetida +25%C. nilotica). Diets were randomly assigned to 21 glass aquaria in triplicate. Twenty-five larvae per liter were randomly distributed into each aquarium, 48 hours after hatching. The larvae were fed at 20% body weight decreasing to 10% by fourth week of the experimental period, five times a day for eight weeks. Growth performance, nutrient utilization and survival response were evaluated in controlled culture conditions. Each diet formulation cost was also estimated. A combination of 50% Eisenia fetida and 50% Caridina nilotica performed significantly ($p<0.05$) better in growth, nutrient utilization and survival at a relatively low formulation cost. Possibly because of higher levels of methionine, lysine, isoleucine, leucine, valine, arginine, glutamic and phenylamine which are responsible for enhanced growth and survival. However, these parameters reduced in larvae fed on 50% Spirulina platensis and 50% Caridina nilotica due to higher crude fiber. Caridina nilotica can be replaced with either Eisenia fetida up to 75% or 25% of Spirulina platensis without negative effect on growth, nutrient utilization and survival.

Keywords: *Spirulina platensis, Eisenia fetida, Caridina nilotica, Clarias gariepinus larvae, Fish larval nutrition, Protein alternatives*

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Introduction

Aquaculture production of African catfish (*Clarias gariepinus,* Burchell 1822) was projected to increase by 65% globally and 54% in Sub-Saharan Africa by 2030 (Troell *et al.,* 2014; Troell *et al*., 2019). The growth has been attributed to the species tolerance to adverse conditions, faster growth and higher feed conversion efficiency (Chepkurui-Boit *et al*., 2011). However, the realization of its production potential is dependent on availability of quality seed and affordable quality larval feed (Obiero *et al*., 2019). *Clarias gariepinus* larval diet is unique because of the difference of the larval stage from all other life stages in feeding physiology, ontogeny of digestive capacity and nutritional requirements (Verreth *et al* 1992; Hamre *et al*., 2013). *Clarias gariepinus* larvae experiences drastic ontogenic shifts, has a small mouth gape and selective food preference that is skewed to small particle size with high protein levels (Verreth *et al*., 1992; Hamre *et al* 2013; Rathore *et al*., 2016). Further, the larvae are very sensitive to diets fed within the first weeks of exogenous feeding (Onura *et al*., 2018). This problem has been attributed to slow gut maturation and an extended larval period that demands unique nutritional and feeding care (Verreth *et al*., 1992; Ngugi *et al*., 2007).

Quality fish larvae with optimal growth and survival depends on quality of culture water, broodstock, feed quantity and, dietary protein composition and quality (Kpogue *et al.,* 2013). Protein requirements varies between and within species, age and culture conditions. According to Jobling (2012), there is limited information on the exact nutritional specifications for larval feeds. However, earlier studies reported requirement levels for *C. gariepinus* larvae of 50 - 59% crude protein (Uys and Hecht 1985; Vital *et al*., 2016), 9-11% lipids (Uys and Hecht 1985; Stickney and Hardy 1989), 20-21% carbohydrate (Uys and Hecht, 1985) and digestible energy of 3042kCal/kg – 3824kCal/kg (Machiels and Henken, 1985; Uys, 1989; Yilmaz *et al*., 2006). Information on amino acids and mineral requirements for *C. gariepinus* larvae remain scanty except for methionine at 2.5% dietary protein dry matter (Uys and Hecht 1985**)**. Despite limited information on fish larval nutrition, *C. gariepinus* larvae have been weaned on dry formulated diets with varying outcomes (Uys and Hecht 1985; Verreth *et al*., 1992; Chepkurui-Boit *et al*., 2011; Kpogue *et al*., 2013; El-sebaie *et al.,* 2014). This has resulted to variable larval quality and sustained shortage of quality and quantity *C. gariepinus* seed for stocking to match aquaculture intensification (Obiero *et al*., 2019). Therefore, sustained efforts to improve formulated diets in fish larval nutrition for sustainable aquaculture are of importance.

Caridina nilotica, (Roux 1833), has to some extent, replaced the nutritionally balanced fishmeal in aquaculture feed industry (Radhakrishnan *et al.,* 2014; Cashion *et al*., 2017; Han *et al*., 2018). However, exploitation of both *C. nilotica* and fishmeal for aquafeed is a threat to the viability and health of wild fisheries through disruption of food chains supported by these species (Rasowo *et al.,* 2008, Chepkirui Boit *et al*., 2011, Cashion *et al.,* 2017). It is also in competition with human food and livestock feed. Thus, negatively impacting the United Nation's sustainable development goal on ensuring food and nutritional security for all. Consequently, a continued search for high quality, sustainable, economical, renewable and readily available protein alternatives to ensure nutritional quality and aquatic resource integrity in aquafeed is a priority (Coffey *et al*., 2016; Han *et al*., 2018).

Unfortunately, plant protein alternatives to fishmeal have been limited due to imbalanced amino acid profile, presence of anti-nutritional factors that reduce diet digestibility, non-soluble carbohydrates and fiber which also increases aquatic pollution (Ytrestøyl *et al.,* 2015; Montoya-Camacho *et al*., 2019). On the other hand, animal protein alternatives to fishmeal presents challenges of increased microbial contamination, zoonotic infections, fatty acid rancidity and prohibitive cultural beliefs in most developing countries (Kobayashi *et al.,* 2015). Hence, variable success of fishmeal alternatives to enhance feed quality for optimal larval growth while ensuring ecological sustainability. This has necessitated the need for further research on feasible proteins alternatives. As such, non-conventional protein sources of spirulina (*Spirulina platensis,* Geitler, 1925*)* and earthworm (*Eisenia Fetida,* Savigny,1826), have gained interest in the recent past through innovation, science and technology

to change ways of conducting economic initiatives in the face of climate change and for responsible aquaculture.

Spirulina platensis is a photosynthetic cyanobacterium which has high nutritional composition comparable to yeast and milk powder for larval nutrition (Radhakrisnan *et al.,* 2014; Abd- El Alim *et al*., 2018). The species has digestible sugars on its cell wall, lacks cellulose, variable protein content (43- 70%) depending on methods of culture and analysis, reasonably balanced amino acids profile, 4-10% lipids with a total fatty acid content of 81.2mgg^{-1} dry weight, 15-25% carbohydrate and is an excellent source of vitamins, minerals, carotenoids and presence of antioxidant pigment making it a better protein compared to other vegetable protein sources (Radhakrisnan *et al.,* 2014; Guedes *et al*., 2015; Liestianty *et al.,* 2019). According to Salmeán *et al*., (2015), the species has a digestibility of 85% compared to casein and high digestive coefficient demonstrating its nutrient availability to organisms. Presenting *S. platensis* as a complete nutritional source in aquaculture that may be fed to larvae through live feed enrichment or as a formulated diet (Velasquez *et al.,* 2016). *Spirulina platensis* supplemented in diets has also been found to increase immunity and physiological responses in fish (Abd-El Alim *et al*., 2018). Further, *S. platensis* enhanced growth, feed utilization, disease resistance and stress tolerance when it replaced fishmeal in the diets of *Pangasianodon gigas* (Tongsiri *et al.,* 2010), *C. gariepinus* (Promya and Chitmanat, 2011) and *O. niloticus* (Velasquez *et al*., 2016). Although data on *S. platensis* replacing fishmeal in fish larval diets maybe available, it is important to re-examine its performance in relation to other non-conventional protein source alternatives like *E. fetida* in *C. gariepinus* larvae.

On the other hand, use of digestible earthworm in aquafeed leaves minimal ecological footprint in the environment compared to fishmeal and plants proteins (Stanković *et al*., 2011; Troell, *et al*., 2019). For instance, *E. fetida* is a vermiculture worm with a nutritional composition closer to fishmeal and chicken egg though with higher levels of limiting amino acids (Antonova *et al*., 2021). The species has sufficient quantities of crude protein (50- 70%) with balanced amino acid profile, crude lipids (5- 10%) dry weight (NRC, 1993), gross energy of 4000kCal/kg (Castro-Bedriñana, *et al*., 2020), 5-21% carbohydrate, 6.6 - 10.5mg/g protein total fatty acids with sufficient levels of polyunsaturated fatty acids (Gunya and Masika, 2021). *Eisenia fetida* also has high levels of vitamins and minerals compared to fishmeal and soya bean in addition to high solubility in digestive enzymes (Gunya and Masika, 2021). Therefore, *E. fetida* is an efficient feed ingredient that is important in fish larval rearing and tropical fish nutrition (Dedeke *et al*., 2013; Mohanta *et al*., 2016). This species has been used successfully in poultry and pig feed nutrition (Castro-Bedriñana, *et al*., 2020), however, its use in aquaculture has variable results (Musyoka *et al*., 2019). Dedeke *et al.,* (2013), reported reduced growth for *C. gariepinus* larvae fed on frozen *E. fetida* meal and 100% worm meal (Ng *et al*., 2001; Dedeke *et al.,* 2013), *Oncorhynchus mykiss* fry (Hilton, 1983.), *Herobranchus longifilis* fingerling (Mohanta *et al.,* 2016). However, increased growth was observed in *Labeo rohita* fry fed on *E. fetida* (Mohanta *et al.,* 2016). Research on earthworm inclusion in fish feed compared to other non-conventional protein sources remain scanty (Mohanta *et al*., 2016: Saravanan *et al*., 2015). Thus, more studies on *E. fetida* use in aquaculture for improved larval quality and enhanced growth while, prioritizing aquaculture profitability and sustainability are a necessity.

Both *S. platensis* and *E. fetida* are natural food ingredients for fish in the natural environment and occupy low positions in the food chain with high protein levels comparable to fishmeal. The fact that both can be cultured under controlled environmental conditions allows for their nutritional manipulation to fit different species and life stages of fish. In addition, *S. platensis* and *E. fetida*, do not compete for arable land for their production and only uses small space giving high land use efficiency by lessening the burden on natural resources. These protein alternatives, are produced commercially on nutritional medium and compost which can be enriched to improve their nutritional value as maybe demanded (Kumlu *et al.,* 2021). *Spirulina. platensis* and *E. fetida* are eco-friendly protein alternatives which are sustainably produced and have high biomass to ensure quantity and quality in aquafeed. Also, information on proximate composition of *S.*

platensis and *E. fetida* are available. However, the use of *S. platensis* or *E fetida* to replace *C. nilotica* either partially or completely in formulated feed in fish larval nutrition remain scanty.

This study evaluated the effects of *S. platensis* or *E. fetida* protein in the rearing of *C. gariepinus* larvae. To achieve this, dry diets were formulated by partially replacing *C. nilotica* in the control at three levels with either *S. platensis* or *E. fetida*. Effects of formulated diets on nutrient utilization, growth and survival of *C. gariepinus* larvae was determined. Also, the cost of formulating each of the test diets was estimated. The results of the study will be a guide in the formulation of high-quality *C. gariepinus* larval feed for enhanced aquaculture production to ensure food security and improved livelihood.

Materials and Methods

Larvae production

A mature female (1.19kg) and two males (864 ± 5.4g) of *C. gariepinus* were sourced from the pre-evaluated Makindi fish farm, Kiambu County, Kenya. The brooders were selected according to Viveen *et al.*, (1985), transported to the Department of Biology in the Faculty of Science and Technology, University of Nairobi (UoN), where they were conditioned for two weeks in half filled 100 L plastic tanks at 27˚C. The broodstock were fed on Skretting commercial diet twice a day (10am and 4pm) at three percent of the wet body weight (WBW). Gravid female was induced, stripped, fertilized and extruded eggs incubated on a Kakhaban mat in a 50 L plastic basin filled to 45 L at 29˚C according to Graaf and Jansen (1996). The incubator was continuously aerated with a single air stone in a flow through system at a flow rate of 2.5L/minute to ensure a renewal rate of three times per hour. Hatching occurred 24 hours after incubation.

Table 1. Chemical composition (% dry matter) of ingredients used in formulating diets for feeding C. gariepinus larvae

Ingredient	Moisture	Ash	CP	CF	EE	Ca^{2+}	P^{3+}	NFE
Wheat Pollard	5.00	3.97	16.1	7.30	5.93	0.26	1.44	66.7
DCP	1.00	-	-	-	-	20.1	18.1	-
Corn	6.10	1.33	7.14	2.93	5.48	0.04	0.33	82.8
C. nilotica	7.30	12.0	63.9	1.46	3.93	3.49	1.46	18.7
E. fetida	83.89	6.71	64.3	1.25	11.0	0.75	0.78	16.7
S.platensis	9.90	13.8	61.8	1.43	2.67	1.87	0.81	20.3

DM = dry matter, CP =crude protein, CF= crude fiber, EE= Ether extract (lipid), Ca^{2+} =calcium, P^{3+}=Phosphorous, NFE= nitrogen free extract DCP=Dicalcium Phosphate

Feed ingredients

Spirulina platensis and *E. fetida* were protein alternatives used to partially replace *C. nilotica* in *C. gariepinus* larval diet. *Spirulina platensis* powder was sourced from Nasio Trust in Kakamega County, Kenya, packed in air tight zip lock plastic bags and stored in closed carton boxes to prevent exposure to light. Live *E. fetida*, cultured according to Sharma and Garg (2018), were sourced from a reputable integrated fish farmer in Nyandarua County, Kenya. The negatively phototactic *E. fetida* were handpicked into plastic buckets, transported to UoN, Department of Biology. The *E. fetida* were left in the buckets for 12 hours, allowing for recovery from handling stress and evacuate their guts so as to reduce the foul smell and toxic coelomic fluid. Subsequently, *E. fetida* were thoroughly cleaned and rinsed as described by Pucher *et al.*, (2014). Afterward, the *E. fetida* were batch weighed and oven dried at 60˚C to a constant dry weight to minimize loss of the dry matter content, to reduce the unpleasant odour and off flavors that could decrease the attractiveness of

formulated diets before storage at 4˚C until use. The other ingredients sourced from reputable distributors were corn, wheat pollard, Dicalcium phosphate, Ascorbic acid, DL- Methionine, HCL-Lysine and vitamin-mineral premix. The commercial feed (Skretting Gemma micro-150) of 100-200 µm particle size was sourced from an Aqua-shop.

Diet formulation

Six diets, approximately isonitrogenous (55% crude protein) and isocaloric (3800 kCal/kg digestible energy) and a control were formulated and prepared based on a reference diet described in Uys, (1989). Allowing for loses during formulation, digestible energy in the formulations was calculated based on the ingredient digestible energy (DE) book values in NRC (1993) and least cost inclusions were considered. Control diet (T_7) contained 100% *C. nilotica* providing 55% crude protein (CP). *Caridina nilotica* was replaced by either *S. platensis* or *E. fetida* to give three levels of 25%, 50% and 75% test diets (T_1 = *25%S. platensis +75%C. nilotica,* T_2 = *50%S. platensis +50%C. nilotica,* T_3 = *75%S. platensis + 25%C. nilotica,* T_4=*25%E. fetida +75%C. nilotica,* T_5 = *50%E. fetida +50%C. nilotica* and T_6 = *75%E. fetida +25%C. nilotica*) as shown in Table 2. Varying levels of corn oil were added to raise crude lipid levels closer to recommended range of 9-11% in *C. gariepinus* larvae (Uys, 1985; Stickney and Hardy 1989), with equal proportions of vitamin-mineral premix, sodium chloride, ascorbic acid, Dicalcium phosphate, DL- methionine and HCL-lysine as indicated in Table 2.

Dry ingredients for diet formulation were individually ground using a KD -318 kitchen blender (Ningbo Ambel international Co. ltd, Guangdong, China) and sieved through 125µm mesh to ease handling, thoroughly mixed using egg beater hand whisk to homogeneous mixture unto which hot water was added to gelatinize carbohydrates before kneading it to a dough with uniform consistence. Thereafter, the dough was pelletized using a manual pelletizer to make 200µm, 250µm and 500µm pellet size for different larval sizes, assuming an increase in the mouth gape with age. Feed pellets were dried in the oven (Gallenhamp Catnoin INC 700110M, Gallenkamp and company, Chester, United Kingdom) at 60°C to a constant dry weight before storage in zip lock bags in air tight plastic containers at room temperature. Formulation was done once for consistency and diets were fed within the eight weeks of study period.

Table 2: *Ingredient proportions (%) in formulated diets for feeding C. gariepinus larvae*

	Diets						
Ingredients	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Corn	3.62	2.21	0.12	9.38	9.94	10.1	4.54
Wheat pollard	7.03	8.17	9.13	2.49	1.5	1.06	5.12
Corn oil	3.29	3.56	4.69	2.07	2.5	2.78	4.28
C. nilotica	63	42	21	63	42	21	84
E. fetida	0	0	0	21	42	63	0
S. platensis	21	42	63	0	0	0	0
DCP	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vit- mineral premix	1	1	1	1	1	1	1
Salt (NaCl)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Ascorbic acid	0.01	0.01	0.01	0.01	0.01	0.01	0.01
DE (kCal/kg)	3804	3802	3801	3804	3800	3802	3802
Cost ($/kg)	13.8	26	38.3	3.27	5.02	6.78	1.52

,DCP= dicalcium phosphate, DE=calculated Digestible energy, T_1 = 25% S. platensis +75% C. nilotica, T_2 = 50% S. platensis +50% C. nilotica, T_3 = 75% S. platensis + 25% C. nilotica, T_4=25% E. fetida +75% C. nilotica, T_5 = 50% E. fetida +50% C. nilotica, T_6 = 75% E. fetida +25% C. nilotica, T_7 = 100% C. nilotica and. Vit- mineral*

premix provided the following per kg feed vitamin A, 5000 IU; vitamin D3, 1000 IU; vitamin E, 150 IU; vitamin K3, 3 mg; Vitamin B1, 10mg; vitamin B2, 15 mg; vitamin B6, 7.5mg; vitamin B12, 0.025 mg; Niacin 100 mg; Pantothenic acid 27.5mg; Biotin, 0.5mg; Folic acid, 3mg; Choline 500 mg; vitamin C, 300 mg; manganese 75 mg; iron, 20 mg; zinc, 22.5 mg; Copper, 2.5 mg; Cobalt, 0.1 mg; iodine 0.7 mg; selenium 0.06 mg

Chemical analyses of feed ingredients and formulated diets

chemical composition of dietary ingredients and formulated diet samples were analysed using the Association of Agricultural Chemists, (1998), standard procedures and summarised in Tables 1 and 3. Nitrogen (N) content was determined in a Micro-Kjeldahl (Gerhaelt Bonn 001460, Ultratronic GmbH, Gilching Bayern, German) after acid digestion. Crude protein was estimated using the Jones Factor (N x 6.25). The Ether extract (EE) or lipid content was analysed using the Soxhlet extraction method with ether as an extraction solvent. Percentage (%) nitrogen free extracts (NFE) were determined by difference method. Ash content was determined by incineration for eight hours at 550 ˚C in a Muffle furnace (Wc Heraeus, hanau 170, 220v, Milan, Italy) to ensure minimal breakdown of calcium carbonate.

Amino acid compositions of formulated diets were analysed at Evonik Nutrition GMbH, Wolfganag, Germany and summarized in Table 4. Performic acid oxidation was done before hydrolysis to oxidize Methionine and Cysteine to Methionine sulphate and Cysteic acid respectively, according to AOAC (1998). Performic acid was neutralized as described by Greenfield and Southgate (2003). Protein was hydrolyzed using 6M HCl to release amino acids before neutralizing the hydrolyzed samples with sodium citrate at a pH of 2. Individual amino acids were quantified using high performance liquid chromatography (HPLC). Since Tryptophan was completely degraded during hydrolysis and Tyrosine was destroyed by oxidation, they were not quantified. Calcium was estimated using an Atomic Absorption Spectrophotometer (model 210, Malvern Panalytical, Westborough, USA) at 420 nm and Phosphorus determined using atomic absorption spectrophotometer at 220 nm (SpetraAA, 220FS FL, Varian, polo California, USA) at the Faculty of Agriculture, Department of Soil Science, UoN.

Diet formulation cost

Cost of formulating each diet was estimated based on the proportions of each ingredient included in the diet, market price of the ingredients at the time of formulating in Kenya and transportation cost. Price and transport were the only criterion considered assuming that all other formulation factors remained constant.

Experimental design

Feeding trials were carried out for eight weeks in 21, 50 L glass aquaria, filled to 40 L with tap water, with each of the seven treatments in triplicate. Forty-eight-hours post hatching and allowing 10% mortality, 25 *C. gariepinus* larvae (individual mean weight of 0.002g) per liter were randomly distributed into the glass aquaria. The larvae were not fed in the first 10 hours after stocking to allow recovery from handling stress. The first feeding of the larvae consisted of a combination of 75% commercial diet (Gemma micro150) with 25% each of the respective formulated (T_1-T_6 and control) diets in random triplicate aquaria. Consequently, commercial diet decreased by 25% while respective formulated diet increased by 25%. By the 4th day, larvae were fed on 100% each of formulated diet until the end of eight weeks. Diet particle size was increased from 200µm to 250µm after the first week and 500µm after the 4th week of experiment. The *C. gariepinus* larvae were hand fed five times a day at 20% wet body weight for the first 2 weeks (Verreth *et al.,* 1992) and at 10% wet body weight until the end of the experiment. The feeding ration was adjusted weekly based on wet body weight calculated from weekly samples. Water temperature was thermostatically maintained at 28˚C and each aquarium was gently aerated with single air stone except when larvae were feeding. Every morning, before feeding excess feed were gently siphoned out together with approximately 50% of bottom aquarium water which was replaced by an equal volume of fresh tap water so as to ensure high water quality. A natural photoperiod regime of 12 hours light and 12

hours dark was maintained throughout the experimental period.

Data collection

Every morning before feeding, dissolved oxygen, temperature and pH were measured in each aquarium using a multiparameter water quality measuring meter (Multi WTW 3630 IDS, xylem analytics, Brandenburg, Germany). Plastic bottles (100ml) were filled with sample water from each glass aquaria, stored at -18˚C for later analysis of total NH_3-N using a Hanna Ammonia Medium Range meter (ISM HI96715C, Hanna instruments, Waitakere, New Zealand) before converting it to ammonia according to the manufacture's conversion table. After the initial determination of body weight and length at stocking, body weight was followed by scoop netting of 30 *C. gariepinus* larvae from each aquarium (with replacement) and individually weighed to the nearest 0.001g using an ASB-220-C2 analytical weighing balance (Shambhavi impex, Mumbai, India). Total body length was measured on a wet measuring board to the nearest 0.1 cm. This was done weekly for the first month and every fortnight thereafter. For Specific growth rate (SGR), 30 larvae were removed at stocking and the end of experimental period (without replacement), individually weighed and oven dried (model TS 8000S, Termaks, Bergen, Norway) at 104˚C for four hours to a constant weight with a precision of 0.001g. The information obtained was used to determine larval growth, daily growth rate, SGR, nutrient utilization and survival as;

Weight gain (g) = final weight – initial weight

Length gain (cm) = final length – initial length

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

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

Where Ln = Natural Logarithm

Feed Conversion Ratio (FCR) was estimated as feed consumption (g) per wet weight gain (g) while Protein Efficiency Ratio was estimated as wet weight gain (g) per protein consumption (g). Total counts of larvae in each aquarium were recorded at the end of the 1st, 4th and 8th week of the experimental period and live individuals presented as survival compared to stocking densities. Larvae removed for SGR determination were added to the surviving larvae numbers.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics Version 20 (International Business Machines Corporation, Armonk, New York, USA). One-way ANOVA was performed to test the effects of dietary treatments on water quality, growth, nutrient utilization and survival. When significant differences were established, treatment means were compared using John Wilder Tukey's Honest Significant Difference post hoc test (Tukey, 1977) and considered different at $p < 0.05$. Prior to data analysis, all data sets were subjected to normality and homogeneity of variance tests using Kolmogorov Smirnov's (Zar, 1999), and Levine's tests (Levene, 1961) respectively. All estimated values are expressed as mean ± standard error. (SE).

Results

Nutrient utilization

There was differential nutrient utilization based on protein source and inclusion level as shown in Figure 1 and Table 5. Feed Conversion Ratios (FCRs) in Figure 1 and Protein Efficiency Ratios (PERs) in Table 5 were fairly similar at the end of week one. However, *S. platensis* containing diets had the best FCRs (Figure 1) and PERs (Table 5) in week two of the study with a similar trend observed in week three for *E. fetida* fed larvae. The best FCR throughout the experimental period was observed in larvae fed on a combination of 50% *E. fetida* with 50% *C. nilotica* (1.3-1.74) and worst FCR in the range of 1.5-3.0 was posted by larvae fed on 50% *S. platensis* and

50% *C. nilotica* combination. Nutrient utilization by larvae fed on control diet (100% *C. nilotica*) was not different from those feds on 25% inclusion level of either *E. fetida* or *S. platensis* proteins after the third week of experimental period. Larvae fed on a combination of 50% *E. fetida* with 50% *C. nilotica* converted nutrients better to attain a higher PER of 1.30 at the end of the study compared to all test diets. *Spirulina platensis*-based diets posted poor FCRs in range of 2-3 at the end of experimental period with corresponding lower PERs.

Growth performance

Growth parameters were affected by level of *E. fetida* or *S. platensis* in the diet as shown in Table 6. After eight weeks, larvae fed on a combination of 50% *E. fetida* and 50% *C. nilotica* (T_5) had higher ($p< 0.05$) mean weight gain (0.43g), daily growth rate (0.82% g/day), total body length (4.27cm) and SGR (9.37%/ day) compared to all other formulated diets. In contrast, diet T_2 (50% *S. platensis* + 50% *C. nilotica*) performed poorer ($p <0.05$) in larval body weight gain (0.20g), daily growth rate (0.38%g/day), total body length (2.97cm) and SGR (7.31 %/day) compared to the other formulated diets. This growth performance for larvae fed on T_2 was not different ($p>0.05$) from diet T_3 (75% *S. platensis* + 25% *C. nilotica*) fed larvae. Evaluated growth parameters of larvae fed on control diet was similar to those fed on diet T_1 throughout the experiment. ***S****pirulina platensis* inclusion beyond 25% in a diet resulted to a reduction in growth parameters compared to similar *E. fetida* inclusion levels where growth only reduced beyond 50% *C. nilotica* replacement**.** Also, all diets containing *E. fetida* protein had higher growth values than *C. nilotica* as a single protein source.

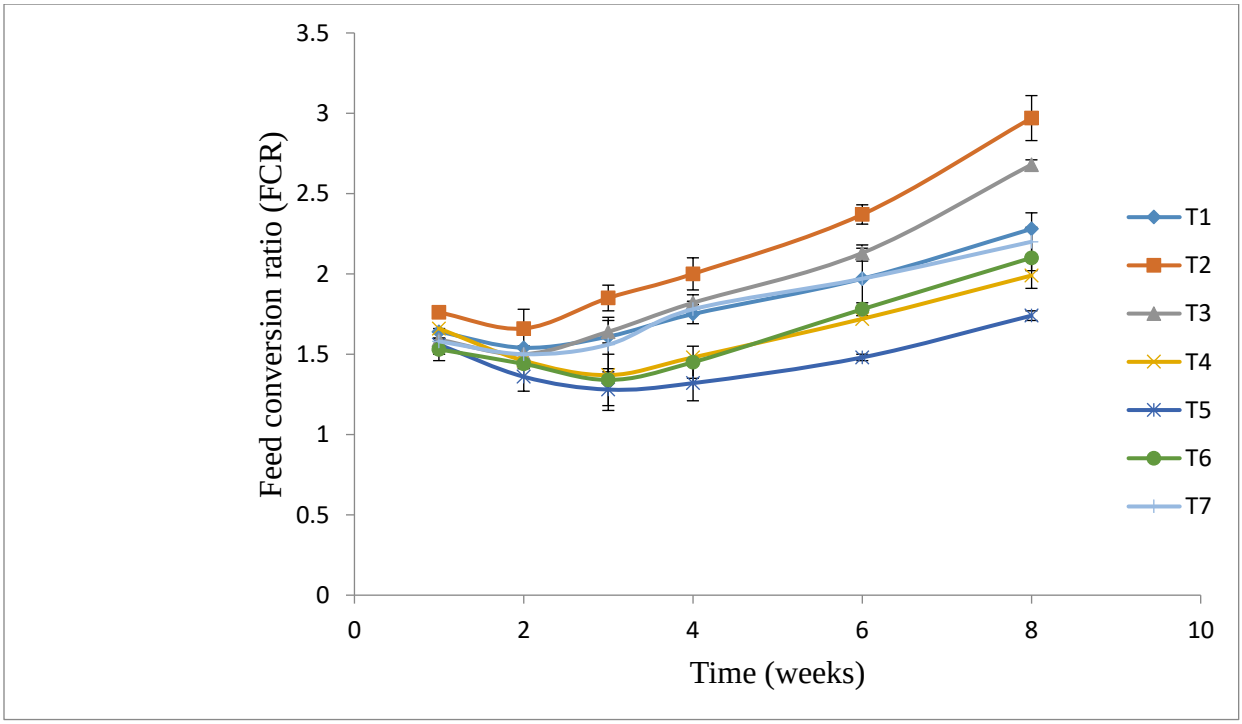

Figure 1. Feed Conversion Ratio (FCR) for C. gariepinus larvae fed on the different formulated diets

Table 5: *Protein efficiency ratio (mean±SE, n=30) for C. gariepinus larvae fed on different formulated diets*

Time (weeks)	Diets								
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	F	P
1	1.12±0.08 ^{a}	1.04±0.01 ^{a}	1.14±0.03 ^{a}	1.10±0.03^{a}	1.18±0.03^{a}	1.19±0.05^{a}	1.16±0.06^{a}	2.15	0.17
2	2.62±0.02 ^{ab}	2.37±0.02 0^{a}	2.48±0.03 ^{a}	2.97±0.56^{ab}	3.80±0.29^{ab}	3.83±0.10^{ab}	2.60±0.36^{ab}	4.16	0.01
3	1.23±0.14 ^{b}	0.94±0.22 ^{a}	1.11±0.05 ^{a}	1.33±0.04^{b}	1.46±0.17^{d}	1.40±0.15^{c}	1.20±0.17^{b}	5.48	0
4	1.07±0.01 ^{abc}	0.87±0.04 ^{a}	0.95±0.01 ^{a}	1.23±0.01^{bc}	1.40±0.13^{cd}	1.24±0.08^{bc}	1.03±0.05^{ab}	12.29	0
6	0.99±0.03 ^{ab}	0.84±0.02 ^{a}	0.90±0.02 ^{ab}	1.05±0.02^{bc}	1.33±0.02^{cd}	1.13±0.02^{bc}	0.96±0.08^{ab}	21.02	0.01
8	1.00±0.08 ^{b}	0.68±0.10 ^{a}	0.88±0.0.11^{a}	1.03±0.05^{b}	1.30±0.03^{c}	1.08±0.00^{b}	1.01±0.07^{b}	27.49	0

^{abc} *means in the same row without common letters are different p<0.05,* T_1 *= 25% S. platensis +75% C. nilotica,* T_2 *= 50% S. platensis +50% C. nilotica, T3 = 75% S. platensis + 25% C. nilotica,* T_4 *= 25% E. fetida +75% C. nilotica,* T_5 *= 50% E. fetida +50% C. nilotica,* T_6 *= 75% E. fetida +25% C. nilotica,* T_7*= 100% C. nilotica, SE = standard error*

Clarias gariepinus larval survival

Effects of all dietary treatments tested on *C. gariepinus* larval survival was within a range of 60- 78% as shown in Figure.2. Highest mortalities within a range of 12- 30.34% were recorded in week one of the experimental period compared to subsequent weeks of the study. *Clarias gariepinus* larvae fed on a combination of 50% *E. fetida* and 50% *C. nilotica* had a significantly higher percentage survival of 78% compared to all test diets. The lowest survival during the study was observed in larvae fed on 25%*S. platensis* + 75%*C. nilotica* (T_1). *Eisenia fetida* containing diets had higher % survival compared to corresponding *S. platensis* inclusion level in the diet. Percentage survival of larvae fed on control diet (T_7) was similar to those fed on diet T_4

Water quality

Water quality parameter (temperature, pH, Dissolved Oxygen and NH_3) values remained relatively constant throughout the experimental period (Table 7). As expected, NH_3 was slightly higher in diets containing *E. fetida* protein (range 0.04- 0.05mg/l) compared to all other diets evaluated though it was not significantly (p>0.05) different.

Table 7. Mean water quality parameters (mean ±SE, n=3) for C. gariepinus larvae raised in glass aquaria for 8 weeks

Water parameter	**Diets**								
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**F**	**P**
Temp(°C)	26.74±0.22	26.94±0.27	26.84±0.29	26.95±0.24	26.98±0.29	26.84±0.26	26.94±0.29	0.95	0.55
pH	7.29±0.04	7.31±0.02	7.30±0.03	7.32±0.01	7.33±0.03	7.33±0.04	7.30±0.01	1.32	0.33
DO (mg/l)	5.40±0.15	5.42±0.13	5.26±0.18	5.46±0.08	5.42±0.13	5.36±0.19	5.41±0.14	1.20	0.12
NH_3(mg/l)	0.03±0.00	0.04±0.01	0.03±0.00	0.04±0.00	0.04±0.01	0.05±0.01	0.04±0.01	0.80	0.59

T_1 = 25% S. platensis +75% C. nilotica, T_2 = 50% S. platensis +50% C. nilotica, T_3 = 75% S. platensis + 25% C. nilotica, T_4=25% E. fetida +75% C. nilotica, T_5 = 50% E. fetida +50% C. nilotica, T_6 = 75% E. fetida +25% C. nilotica, T_7= 100% C. nilotica, SE = standard error, DO = Dissolved Oxygen, Temp=Temperature. All values without a superscript means they are not significantly different

Diet formulation cost

Formulating a diet in this study had economic effects as shown Table 2. Diet formulation cost increased with increase of *S. platensis* or *E. fetida* proportions in the diet. A combination of 25% *C. nilotica* and 75% *S. platensis* was the most expensive diet to formulate at $38.3 compared to all other combinations except the control with least formulation cost of $1.52. Diet T_5 was moderately cheap to formulate at $5.02.

Discussion

Nutrient utilization

A reduction of FCR (Figure. 1) in all diets from week one to their respective low values before increasing until the end of experiment suggested, differential nutrient utilization based on protein source and was comparable to earlier reviews on larval nutrition by Hamre *et al.,* (2013) and Rønnestad, *et al*., (2013). Higher FCRs in week one could have meant feed wastage, probably because of undifferentiated feed acquisition processes, digestion constraints, slow and drastic intestinal ontogeny and absence and/or reduced peptic activity in fish larvae at start of feeding (Verreth *et al*., 1992; Hamre *et al*., 2013). Additionally, feeding *C. gariepinus* larvae at 20% body weight as recommended by Verreth *et al.,* (1992), possibly provided low amount of nutrients for optimal utilization at the start of feeding. An observation comparable with Uys, (1989), underfeeding reports for *C. gariepinus* larvae at 25% body weight. Possibly because the larvae demand huge amounts of nutrients for effective utilization to match its faster growth rate of up to 100% body weight hence, poor FCR in week one (Hamre *et al.,* 2013).

Lower FCR and higher PER in week two for *S. platensis* containing diets and control compared to week three in *E. fetida* diets suggested variable abilities of formulated diets in stimulating digestive functionality in *C. gariepinus* larvae. *Spirulina platensis* containing diets could have stimulated maturation of digestive morphology and physiology earlier for effective utilization compared to *E. fetida* due to variable diet influence on gut functionality (Zambonino-Infante *et al.,* 2008). However, *S. platensis* and 100% *C. nilotica* diets posted the poorest FCRs at the end of study that suggested low nutrient utilization and probable environmental pollution due to decomposition of unconsumed feed. An observation that could probably be attributed to self-renewal ability of the intestine in adapting to feeding changes due to variable particle sizes used during the current study period (Rønnestad *et al*., 2013).

Of the formulated diets, diet T_5 fed larvae converted the diet significantly ($p<0.05$) better to attain lower FCR of 1.74±0.03 at the end of the study period. An indication of *C. gariepinus* larval ability to digest and absorb combined nutrients from 50% *E. fetida* and 50% *C. nilotica* compared to all other combinations and control. The FCR value for diet T_5 was comparable to those reported for *Parachanna obsura* (Vital *et al*., 2016), and *Labeo rohita* fry (Mohanta *et al*., 2016). These similarities could be attributed to *E. fetida* meal processing and high dietary lipid used in both studies that could have resulted to increased acceptability and positive utilization. The current study results were also comparable to other non-convention protein sources of fermented fish offal for *Labeo rohita* fry (Mondal *et al*., 2011), and garden snail fed to *C. gariepinus* larvae (Sogbesan *et al*., 2006), but were not in agreement with the FCR of 5.58 reported for *C. gariepinus* larvae fed on the Eudrilid earthworm, *Libyodrilus violaceus* by Dedeke *et al*., (2013), despite use of 54-55% crude protein diets. Variations were possibly because of differences in worm species which affects worm meal digestibility, feeding protocol, personnel performance and chitin levels in the diets depending on worm age and culture substrate (Patil and Biradar, 2017). The current study *E. fetida* were harvested within two weeks of hatching from soft precomposed substrate compared to river sourced *L. violaceus* with unedified age in Dedeke *et al.,* (2013), hence, variable diet digestibility and utilization based on chitin content differences in the two studies (Musyoka *et al*., 2019).

Diet T_2 fed larvae posted poorer nutrient utilization values despite having high crude fiber of 1.77% DM that could have slowed gut evacuation, enhanced digestion, absorption and nutrient utilization due to longer contact time with digestion surfaces and enzymes (Rønnestad, *et al*., 2013). However, increased gut evacuation

rates associated with high feeding frequency, five times a day, in the current study could have compromised digestion and utilization of diet T_2 because of the minimal time for protease buildup since diets influences digestive enzyme secretion and activity differently (Hamre *et al.,* 2013). An indication of variable solubilities of formulated diets in digestive enzymes for effective nutrient utilization (Ahmad *et al*., 2021). Despite variations in age, feeding rates and frequencies, FCR with 50%*S. platensis* and 50%*C. nilotica* (T_2) were comparable to those reported for *O. niloticus* juveniles (Badwy *et al.,* 2008; Velasquez *et al*., 2016) and *O. mossambicus* fry (Olvera-Novoa *et al.,* 1998). These researchers reported increased FCR with higher levels of *S. platensis* inclusion in the diet and were in agreement with the findings of Sivakumar *et al.,* (2018).

Higher FCR ranges of 2-3 in *S. platensis* containing diet could be attributed to higher crude fiber above 1.6% DM recommended for catfish larvae by Uys (1989), low lipid content range of 7.57-8.82%, which decreased with increasing levels of *S. platensis* in the diet, diet water stability and use of diets with small particle size. Hamre *et al*., (2013), associated higher FCRs with low dietary lipid in larval nutrition while Dupree and Sneed, (1966) reported reduced digestibility of diets containing higher crude fiber. Poor FCR in *S. platensis* diets were probable indicators of nutrient loss which agrees with the findings of Dias *et al*., (1997) and, Radhakrishnan *et al*., (2016), who reported decreased diet water stability with increasing *S. platensis* levels. Furthermore, particle size of 200µm-500µm, was prone to leaching due large surface area, possibly resulting to limited nutrient availability for effective utilization hence, increased antioxidant enzymatic activities that enhanced larval physiological stress. Additionally, diets T_2 and T_3 had a rough texture and colored the aquarium water quickly during feeding as compared to all other diets, suggesting diet disintegration and loss of nutrients resulting to poor FCR.

Protein efficiency ratio (PER) value of 1.30 for *Clarias gariepinus* larvae fed on a combination of 50% *E fetida* and 50% *C. nilotica* (T_5) indicated better protein utilization compared to diet T_2 and T_3 with PER of 0.68 and 0.88 respectively. These PER values for diet T_5 fed larvae were comparable to those reported for *Labeo rohita* fry (Mohanta *et al.,* 2016). Low PER in diet T_2 fed larvae could have been due to low methionine and lysine levels in the diet compared to all other formulated diets and a control as shown in Table 4. An indication of low-quality diet in protein utilization as explained by Enyidi, (2017). Diet T_2 PER values were lower than those reported for *C. gariepinus* larvae (Raji *et al*., 2018), *O. niloticus* juvenile (Badwy *et al*., 2008; Velasquez *et al*., 2016). Observations attributed to differences in processing and diet digestibility which affects feed quality and amino acidity digestibility (Boney and Moritz, 2017).

Growth performance

Higher growth by larvae fed on diet T_5 (Table 6) was an indication of a diet that was efficiently assimilated into body systems for optimal growth. This could be attributed to higher levels of methionine, lysine, isoleucine, leucine, valine and phenylamine in diet T_5 (Table 4), which have been reported to be responsible for enhanced growth performance (Hien *et al.,* 2018). Although, there is limited knowledge on quantitative requirements of amino acid for *C. gariepinus* larvae, the observed levels of these essential amino acids in T_5 were possible evidence that could have driven diet metabolic use and positive growth response in larvae fed on this diet. Kolkovski *et al*., (2009), Andersen *et al.,* (2016), and Hien *et al.,* (2018), have reported these amino acids to be responsible for improved growth, enzyme activity, absorption, and general health in fish larvae. Improved growth with 50% *C. nilotica* + 50% *E. fetida* has also been reported for *C. gariepinus* fingerlings and fry (Dedeke *et al*., 2010, Olele, 2011) and, *Cirrhinus cirrhosus* fry (Beg *et al*., 2016). This could be explained by a synergetic advantage of nutritional content and energy blending from combining two animal proteins despite differences in culture system (Herawati *et al*., 2016). However, in the present study, the growth performance by T_5 fed larvae was better than those reported for *C. gariepinus* larvae (Dedeke *et al*., 2013), *Labeo rohita* fry (Mohanta *et al*., 2016) and *Paracanna obscura* fingerlings (Vital *et al*., 2016). Such disparities could have been due to quality differences in *E. fetida* meals which is influenced by worm culture period, substrate and meal processing (Musyoka *et al*., 2019). Possibly because Dedeke *et al*., (2013), had reported variable nitrogen content

and digestibility of worm meal depending on chitin levels that is influenced by age and substrate. Growth variability could also be as a result of changes in larval culture conditions, feeding rate and frequency, stocking density, genetics and nutritional status of brood stock (Vandecan *et al*., 2011). According to Chepkurui-Boit *et al*., (2011), a higher feeding rate, 20% decreasing to 10% wet body weight, as in the current study avails more feed to the larvae for ingestion and utilization for enhanced growth than the 5% used by Dedeke *et al*., (2013).

Table 6: Growth performance (mean ±SE, n=30) of *C. gariepinus* larvae fed on different formulated diets

	Diets								
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**F**	**p**
IW(g)	0.002±0.04	0.002±0.04	0.002±0.04	0.002±0.04	0.002±0.04	0.002±0.04	0.002±0.04	-	
FBW (g)	0.26±0.04^{bc}	0.20±0.05^{a}	0.23±0.08^{ab}	0.35±0.06^{d}	0.43±0.05^{e}	0.29±0.03^{c}	0.26±0.04^{bc}	317.59	0.00
DGR (%g/day)	0.50±0.02^{bc}	0.38±0.00^{a}	0.44±0.01^{ab}	0.67±0.04^{d}	0.82±0.01^{e}	0.56±0.01^{c}	0.50±0.02^{bc}	94.52	0.01
IL (cm)	0.60±0.01^{a}	0.60±0.01^{a}	0.60±0.04^{a}	0.6±0.01^{a}	0.6±0.01^{a}	0.6±0.01a	0.6±0.01^{a}	-	-
FL (cm)	3.41±0.02^{c}	2.97±0.20^{a}	3.33±0.16^{ab}	3.80±0.00^{d}	4.20±0.27^{e}	3.60±0.20^{d}	3.40±0.03^{c}	146.42	0.01
SGR(%g/day)	8.29±0.03^{b}	7.31±0.40^{a}	7.82±0.16^{a}	8.82±0.03^{c}	9.37±0.02^{d}	8.43±0.14^{b}	8.27±0.03^{b}	359.16	0.00

^{abc} means in the same row without common letters are not significantly *different p<0.05, ^{abc} means in the same row without common letters are different p<0.05, T_1 = 25% S. platensis +75% C. nilotica, T_2 = 50% S. platensis +50% C. nilotica, T_3 = 75% S. platensis + 25% C. nilotica, T_4 = 25% E. fetida +75% C. nilotica, T_5 = 50% E. fetida +50% C. nilotica, T_6 = 75% E. fetida +25% C. nilotica, T_7= 100% C. nilotica, SGR specific growth rate, FBW=final body weight, DGR=daily growth rate, IW=initial weight, IL= initial length, FL= final length, SE=standard error*

Larvae fed on a combination of *50% S. platensis* + 50% *C. nilotica* (T_2) reported significantly low growth performance at the end of the study compared to all other test diets. An observation made despite higher levels of alanine, glycine and arginine amino acids in diet T_2 (Table 4) that are responsible for enhanced feeding activity, digestive physiology and growth in fish larvae (Kolkovski *et al.,* 2009). An indication that these amino acids could be abundant in diet T_2 though with limited availability for *C. gariepinus* larval utilization. Nandeesha *et al*., (1998), reported reduced gut protease and lipase activities with increasing *S. platensis* levels in larval nutrition. Possibly resulting to compromised nutrient utilization for effective growth in T_2 and T_3 fed larvae hence reduce growth. The current growth performance by T_2 fed larvae was comparable to those of *Carassius auratus* larvae fed on 50% *S. platensis* replacing fishmeal (Gowsalya and Kumar, 2018), in similar culture conditions. Weight and SGR by diet T_2 fed larvae were lower than those reported by Badwy *et al*., (2008), where *S. platensis* was freeze dried compared to the sun drying in the current study.

Probably because freeze drying retain higher nutrients necessary for improved growth except for fatty acids (Musyoka *et al*., 2019).

Proximate analysis of formulated diets revealed relatively lower lipid content of 7.57- 8.82%, decreasing with increasing levels of *S. platensis* compared to *E. fetida* where lipid content increased with inclusion levels as shown in Table 3, an observation also made by Nandeesha *et al.,* (1998). The low lipid content could have reduced diet T_2 palatability, attractiveness, supply of fuel for cell functioning and, essential fatty acids necessary for catfish optimal larval growth and development in *S. platensis* containing diets (Radhakrishnan *et al*., 2016). However, this may be difficult to reconcile with the higher growth performance observed in Table 6 with 25% *S. platensis* fed larvae. Thus, the lower lipid content in *S. platensis* containing diets may not be enough reason to exclude them in larval nutrition since,

lipid content could be increased through manipulation at diet formulation and in culture medium.

Table 3. Chemical composition (% dry matter) of formulated diets used for feeding C. gareipinus larvae

	Diets						
Chemical proportions	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Moisture	11.40	8.20	5.40	5.70	5.80	5.00	6.90
Crude Protein	54.8	54.6	54.2	54.9	55	54.8	54.8
Ether Extract	8.82	8	7.57	8.97	8.98	10.4	8.79
Calcium	2.71	2.37	2.03	2.47	1.89	1.31	3.03
Phosphorus	1.29	1.17	1.04	1.24	1.09	0.92	1.42
Crude fiber	1.67	1.77	1.83	1.49	1.44	1.35	1.63
Ash content	13.8	14.6	15	14.2	14.3	12.9	14.2
NFE	21	21	21.4	20.4	20.3	20.5	20.6

DM = dry matter, NFE nitrogen free extract, T_1 = 25% S. platensis +75% C. nilotica, T_2 = 50% S. platensis +50% C. nilotica, T_3 = 75% S. platensis + 25% C. nilotica, T_4 = 25% E. fetida +75% C. nilotica, T_5 = 50% E. fetida +50% C. nilotica, T_6 = 75% E. fetida +25% C. nilotica, T_7= 100% C. nilotica

Clarias gariepinus larval survival

The highest survival by diet T_5 (78%) followed by T_6 (72%) could be attributed to higher levels of methionine, methionine + cystine, arginine, lysine and glutamic acid (Table 4) and higher lipid content in Table 3 compared to all other formulated diets and control. According to Wu *et al.,* (2017), and Mukhtar *et al*., (2017), these amino acids ensure higher feed intake and efficiency, increases digestive enzyme activity and fat proportions to provide energy necessary for survival. Dabrowski, (1984), and López (1995), reported increased fish larval survival in *Salmo salar* in the presence of higher levels of arginine, glutamic and methionine. Survival in the present study was much lower compared to 99% reported for *Parachanna obscura* fingerling (Vital *et al*., 2016), *C. gariepinus* fingerlings (Djissou *et al*., 2016), but was higher than those reported for *C. gariepinus* fry by Dedeke *et al*., (2013). A variation possibly attributed to differences in antinutritional coelomic fluid quantities in the *E. fetida* meal that affects its palatability and effective nutrient utilization for optimal survival (Gunya *et al*., 2016).

Clarias gariepinus larvae fed on a combination of 25%*S. platensis* + 75%*C. nilotica* (T_1) posted the lowest survival (60%) shown in Figure. 2, despite having a lipid level of 8.82% DM and relatively higher levels of methionine + cystine, arginine, lysine and glutamic acid (Table 4). Water quality parameters evaluated (Table 7) were within satisfactory ranges (Ngugi *et al.,* 2007), and were not significantly different despite relatively higher ammonia in *E. fetida* diets probably because of higher lipid levels. Therefore, lower survival in T_1 fed larvae could not be attributed to poor water quality. Nevertheless, 25%*S. platensis* + 75%*C. nilotica* (T_1) diet had a higher moisture content of 11.4% shown in Table 3 than the recommended less 10% (Tacon and Metian, 2015). Although moisture does not contribute to the nutritional value of the diet, it could reduce diet shelf-life by encouraging bacterial growth and production of aflatoxins that could have enhanced handling and physiological stress of the larvae (Jobling, 2016). Bacterial and aflatoxin activities could also result in nutrient modification and low availability thus, affecting optimal larval survival of larvae fed on T_1 (Gunya *et al.,* 2016). Reduced diet digestibility at high moisture content due to changes in diet specific dynamics necessary in breaking the feed during digestion has also been suggested by Oehme *et al.,* (2014). The lower survival with T_1 in the present study could not compare with 77% survival reported for *C. gariepinus* larvae by De

Chavez *et al.,* (2018) and *O. mosammbicus* fry by Olvera-Novea *et al.,* (1998). This could be attributed to differences in the biochemical composition of *S. platensis* (Guedes *et al.,* 2015). Higher survival (65-78%) by *E. fetida* containing diets in the current study could be attributed to higher lipids levels that provided necessary fatty acids, increased energy while enhancing feed intake. Nevertheless, a combination of 25% *C. nilotica* and 75% *E. fetida* posted lower growth compared to diet T_5 despite having higher lipid content of 10.4% dry matter. An observation that could be attributed to reduced digestibility due to increased chitin content with increased proportion of *E. fetida* in the diet (Musyoka *et al.,* 2019). The lower survival with *S. platensis* diets compared to *E. fetida* have also been reported in *Chitala chitala* fed on *S. platensis* and *Tubifex tubifex* meal (Sarkar *et al.,* 2006). An indication of compromised nutritional value in *S. platensis* containing diets compared to those having *E. fetida*. Possibly because of limited *S. platensis* protein digestibility as a result of higher crude fiber (Hua *et al.,* 2019). The low survival with *S. platensis* containing diets was in spite of high levels of glycine and alanine in Table 4, contradicting the observations of Dabrowski (1984), where increased survival was related to high levels of glycine and alanine in fish larvae. Histidine has been reported to increase survival as it provides energy necessary for survival Liaqat *et al.,* (2017). The current study could not confirm this observation because *S. platensis* fed larvae posted low survival despite having higher levels of histidine compared to *E. fetida* diets as shown in Table 4.

Diet formulation cost

Formulation costs increased with increasing proportions of *S. platensis* or *E. fetida* in the diet and this agreed with findings of Mugo-Bundi *et al.,* (2015). *Clarias gariepinus* larvae fed on *S. platensis* diets performed economically poorer by increasing formulation cost compared to all other diets. Possibly because of increased cost of ingredient due to monopolistic *S. platensis* market in the developing world and use of labor-intensive shallow ponds with complex culture media compared to solid waste technology recommended by Pelizer *et al.,* (2015). The control diet was the cheapest to formulate and this was attributed to its availability in the open-air markets. Diets containing *E. fetida* were relatively cheaper and had enhanced biological performance of all parameters evaluated compared to *S. platensis* diets. An observation attributed to ease of culture and its availability resulting to low ingredient cost.

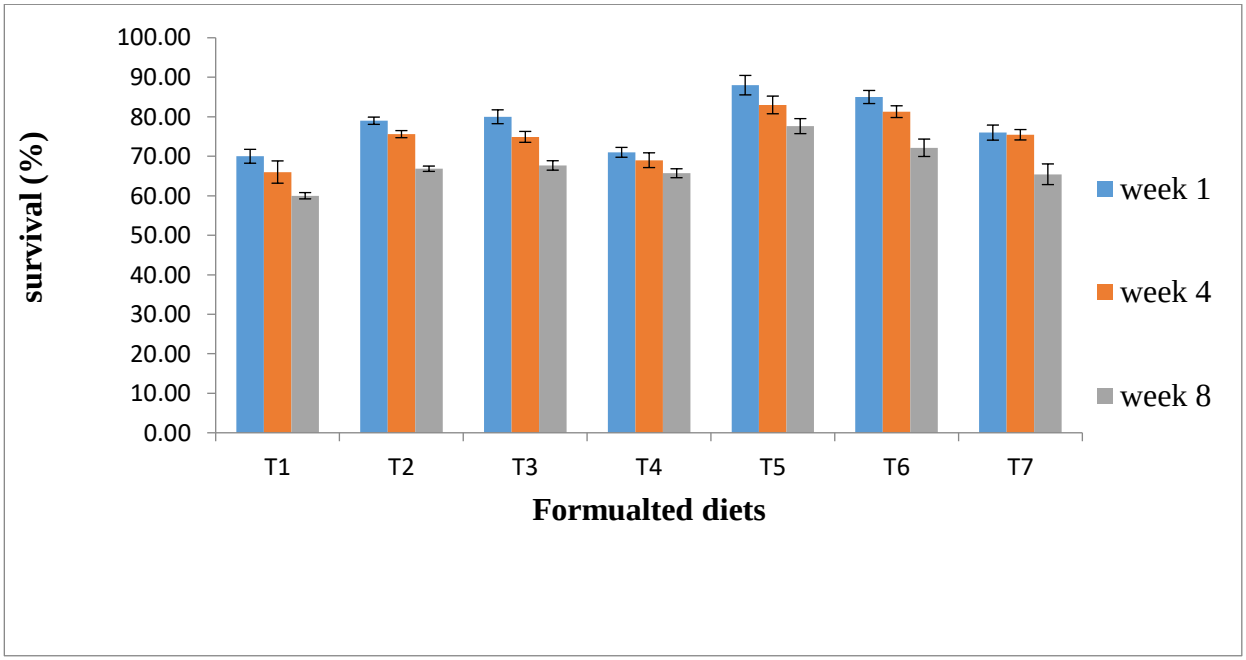

Figure 2: Survival of C. gariepinus larvae fed on the different formulated diets

Table 4. Amino acid (g/100g diet) composition (as is basis) of different formulated diets used for feeding C. gariepinus larvae

	Diets						
Amino acids	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Methionine	0.91	0.73	0.67	0.84	1.12	1.3	0.92
Cysteine	0.4	0.43	0.5	0.47	0.38	0.4	0.42
Methionine + cysteine	1.61	1.46	1.52	1.53	1.57	1.73	1.47
Lysine	2.42	2.49	2.61	2.14	2.88	2.97	2.25
Threonine	1.79	1.89	2.01	1.79	1.89	2.12	1.71
Arginine	2.35	2.37	2.22	2.04	2.46	1.17	2.23
Isoleucine	2.22	2.33	2.49	2.16	2.43	2.5	2.01
Leucine	3.8	3.9	4.15	3.55	3.93	4.1	3.39
Valine	3.12	3.33	3.43	2.82	2.69	2.46	2.67
Histidine	0.94	1.02	1.31	0.93	0.59	0.6	0.83
phenylalanine	2.43	2.57	2.67	2.2	1.91	1.89	2.12
Glycine	2.65	3.1	2.95	2.45	2.55	2.52	2.71
Serine	1.72	1.65	1.63	1.58	1.75	1.98	1.57
Proline	2.67	2.97	3.04	2.31	1.79	1.8	2.28
Alanine	3.2	3.23	3.46	3.22	3.01	3.1	3.17
Aspartic acid	3.9	3.82	3.94	3.71	4.43	4.51	3.69
Glutamic acid	6.2	6.07	6.05	5.64	6.4	6.31	5.83

T_1 = 25% S. platensis +75% C. nilotica, T_2 = 50% S. platensis +50% C. nilotica, T_3 = 75% S. platensis + 25% C. nilotica, T_4 = 25% E. fetida +75% C. nilotica, T_5 = 50% E. fetida +50% C. nilotica, T_6 = 75% E. fetida +25% C. nilotica, T_7= 100% C. nilotica

Conclusion

This study demonstrates possible *C. nilotica* partial replacement by either *E. fetida* up to 75% or 25% *S. platensis* in the diet without negative effects on nutrient utilization, growth and survival. A combination of 50%*C. nilotica* and 50%*E. fetida* had the highest positive effects in all parameters evaluated on *C. gariepinus* larvae at a relatively low formulation cost. Observation attribute to higher levels of methionine, lysine, isoleucine, leucine, valine, arginine, glutamic and phenylamine which are responsible for enhanced growth and survival Higher levels of *S. platensi*s gave diets with poor nutrient utilization and growth due to their high crude fiber.

Recommendations

This study recommends a combination of 50% *E. fetida* and 50% *C. nilotica* for *C. gariepinus* larval rearing. An evaluation of the non-protein proportions of *E. fetida* in larval diets, with respect to palatability, digestibility and coelomic fluid is also recommended.

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