

Full Length Research Paper

Effect of low crude protein diets on the growth performance, survival and feed conversion ratio of the African Catfish, *Clarias gariepinus* (Burchell, 1822) larvae

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Improved culture of the African catfish (*Clarias gariepinus*) is important to addressing the increasing demand for fish especially in the developing countries. Hence, the present study evaluated the growth performance, survival and feed conversion ratio (FCR) of *C. gariepinus* larvae fed with locally formulated diet of low crude protein. The treatments consisted of crude protein (CP) contents of 35%, 30%, 25% and control (28 %). The fish larvae (average weight, 0.03±0.01 g and length, 20.0±0.01 mm) were stocked in glass aquaria at 7 fish/L, and reared at room temperature for 6 weeks. The fish larvae were sampled weekly for length-weight measurements. Prior to sampling, Water analysis was done for temperature, ammonia (NH₃) and pH. Fish larvae fed on the test diets exhibited significantly higher length and weight, specific growth rate (SGR), and survival than those fed with control diet (p < 0.005). Specifically, *C. gariepinus* larvae fed 35% CP diet exhibited better growth performance and feed utilization, and relatively higher survival rate than those fed 30% CP or 25% CP. These findings demonstrate that African catfish larvae can be reared with low CP diets and hence cheaper for small holder fish farmers. The present findings contribute towards the success and realization of the improved production of *C. gariepinus* larvae, which is essential for successful aquaculture development in developing countries.

Keywords: *Clarias gariepinus*, Catfish, Crude protein, Survival rate, Fish nutrition, Aquaculture

INTRODUCTION

Background

With the progressive decline of the wild capture fisheries and continued growing population all over the

world, aquaculture is expected to play a great role in ensuring sufficient fish in the market. In the recent past,

aquaculture has captured the attention of world food production agencies and governments, and has experienced significant growth. In 2013 and 2015, the relative share of aquaculture contribution to the global fish production and consumption was 44% and 50%, which is projected to increase to 52% and 57% by 2025 respectively (FAO, 2016).

As a rich source of easily digestible proteins with all essential amino acids, fish provides essential fats (e.g. long chain omega-3 fatty acids), vitamins (D, A, and B) and minerals (including calcium, iodine, zinc, iron, and selenium) particularly if eaten whole (FAO, 2016). The main utilization for non-food uses will continue to reduce into fish meal and fish oil. Other uses will be for ornamental, aquaculture purposes for instance fingerlings and fry, bait, pharmaceutical purposes, and as direct feed for aquaculture, livestock and other animals (FAO, 2016). Success in farming any fish species depends on good nutrition, favorable environmental conditions and application of best management practices (Ozbay et al., 2014). *C. gariepinus* is available in most inland water bodies in Africa, and due to their piscivory feed habit they have been used to control over-breeding in mixed sex tilapia culture in earthen ponds (Mac'Were et al., 2006). *C. gariepinus* reproduce in response to environmental stimuli such as rising water levels and inundation of low-lying areas. These events do not occur in captivity and hormone treatment is employed to ensure large scale production of fingerlings (Poumogne, 2008). In Lake Victoria, *C. gariepinus* is also utilized as bait in Nile perch fishery.

Several studies have reported on the effects of different feeds on growth performance of different life stages of fish species from the Lake Victoria basin (Rasowo et al., 2008; Munguti et al., 2009; Chepkirui-Boit et al., 2011; Musa et al., 2012; Mugo-Bundi et al., 2015; Ogello et al., 2017; Matanda et al., 2017; Kubiriza et al., 2018). However, the major constraints in aquaculture are good quality fish seed and feed (Aloo et al., 2017; Onura et al., 2018). Fish feed most often represents the greatest percentage of the total cost in aquaculture (Ali and Jauncey, 2004; De Silva and New, 2007). The highest cost in feed production is the ingredients, and therefore the key consideration in improving FCR should be maximization of feed ingredient utilization by the fish (Hasan and New, 2013). This ensures reduction in the output of solid phosphorus and nitrogen waste (Wanabe, 2002).

Larval nutrition studies are expanding to address the demand for fingerlings which are mostly sold to farmers as single pieces of fish. It was recommended more larval nutritional studies with standardized

experimental conditions and use of reference diets (Verret et al., 1987). For instance, proximate composition of protein, fat, lipid, moisture and ash content of fish feeds is important because of the dietary and medical importance of these nutrients in human health (Maithya et al., 2017). The same nutrients also play a crucial role in accelerating growth, reproduction and survival of fish. In particular, protein guarantees growth and health of fish (Kwikiriza et al., 2016). *C. gariepinus* fingerlings require diet with low CP of at least 35% [21]. Thus, diet with excess protein tend to be uneconomical by increasing feeding cost (Mustapha et al., 2014) and compromises water quality by increasing the total ammonium nitrate concentration (Mosha et al., 2016).

Therefore, in the present study, the growth performance, FCR and survival rates of *C. gariepinus* larvae fed on diets composed of different CP levels were evaluated.

METHODOLOGY

Experimental design

Four diets were fed to the larvae and evaluated in experiments conducted in hatcheries as previously described (Ajiboye et al., 2015) with some modifications. The fish larvae were stocked in replicates (initial mean weight of 0.03 g) in glass aquariums at 7 fish/L between 30th November 2017 to 12th January 2018. During the experiment, two-thirds of the culture water from each aquarium was replaced with clean water at every alternate day before feeding. Aeration was done using Resun Air pump model No. AC-9906 (Resun, China). Faeces in the containers were removed by siphoning. Any mortality was noted and dead larvae removed in the morning and evening before feeding. Each day, the fish larvae were fed three times i.e. 8.00, 14.00 and 17.00 hrs. Feeding criteria was at 20%, 15% and 10% body weight between 0-7 days, 7-21 days and 21-42 days respectively. The proximate compositions of the feed materials were also determined according to AOAC (1995) standard procedures.

Data collection

During initial stocking, a sub-sample of 20 individual fish larvae for each treatment were weighed (± 0.001 g) using an analytical balance (Shimadzu Analytical Balance AUW320 series) and total lengths measured (± 0.1 mm) to determine the initial size (t_0) and

weight (W_0) at stocking. Total length was measured by placing a fry on a transparent petri dish placed on a 1 × 1 mm graph paper. At different days of the week, a similar number of 20 fish were sampled and the individual weight (W_i) and total lengths (TL_i) measured to determine the size at that time (t_i) and restocked. The water quality in the culture was determined by measurement of water temperature ($^{\circ}\text{C}$), pH, dissolved oxygen (DO mgL^{-1}), conductivity (μScm^{-1}), ammonia ($\text{NH}_3 \mu\text{gL}^{-1}$), ammonium ($\text{NH}_4 \mu\text{gL}^{-1}$) and ORP (mV) using standard methods. The pH, ORP electrical conductivity and DO were determined using Hanna HI 9828pH/ORP/EC/DO meter and nitrate determined using a VIS-UV spectrophotometer. Nutrients levels were determined according to standard methods in both the reservoir tank and culture aquaria.

The following formulae were used to calculate the changes in weight and total length gain of individual fish larvae; FCR, SGR and survival rates.

Length gain of larvae (mm) = Average final length – Average initial length

Percentage gain in length (%) = [(Average final length – Average initial length) / (Average initial length)] × 100

Weight gain of larvae (g) = Average final weight – Average initial weight

Percentage gain in weight (%) = [(Average final weight – Average initial weight) / (Average initial weight)] × 100

SGR % / day = [Ln (final weight, W_{t_2}) - Ln (initial weight, W_{t_1})] / [$T_{t_2} - T_{t_1}$] × 100, where W_{t_2} = final live body weight (g) at time T_{t_2} , W_{t_1} = Initial live body weight (g) at time T_{t_1}

Percentage survival rate (%) = [(Total no. larvae that survived)] / [(Total number stocked)] × 100

FCR = [Feed given (g)] / [fish larvae body weight gain (g)]

Statistical analysis

Statistical analysis and graphs were performed using GraphPad prism software version 5.03. Data normality was verified using D'Agostino omnibus K12 test. Data are presented as mean ± standard error of the mean (SEM). Statistical differences between treatment groups were determined using one way analysis of variance (ANOVA) with Tukey's post-test. Statistical difference between two groups was determined by two-tailed Unpaired-t test. The correlation between variables was assessed using Pearson correlation tests. A difference with a p-value less than 0.05 was considered significant.

RESULTS

Proximate nutrient composition and physico-chemical parameters

Proximate composition analysis was done on the four diets: 35% CP (T1 Diet), 30% CP (T2 Diet), and 25% CP (T3 Diet) and 28% CP (T4 Diet) and the results are given (Table 1). The treatments were classified based on the diet fed. There was no significant difference in the physico-chemical parameters of the culture conditions among the four treatment groups (Table 2) at the beginning and end of the experiments ($p > 0.05$). Of note, the parameters were within the acceptable standard limits. Taken together, the data presented show that all the fish larvae culture conditions were similar and that only CP level was the independent variable.

Growth performance

The length gain of the fish larvae grown under different diets were significantly different ($p < 0.05$). Tukey's post test revealed that larvae fed on 35% CP had the highest length gain ($p < 0.05$; Figure 1). The mean length gain per day by fish larvae fed on the four diets followed a similar pattern (Table 3). Conversely, the weight gain of the fish larvae fed on the four diets was not significantly different ($p > 0.05$; Figure 2). However, it was observed that larvae fed on 35% CP had significantly highest weight gain per day compared to the rest ($p < 0.05$; Table 3).

In the present study, no significant difference in SGR was observed among the fish larvae fed on the four diets ($p > 0.05$; Figure 3). Further, throughout the study, the highest SGR was observed in larvae fed 35% CP (Figure 4). Considered together, the findings implied that locally formulated feeds particularly 35% CP, contributes better to *C. gariepinus* larval growth than the commercial feeds.

Feed utilization and survival rate

In the present study, feed utilization by the fish larvae was quantified in terms of the FCR. There was no significant difference in the FCR of larvae fed on the four diets ($p > 0.05$; Table 3). However, the FCR increased gradually over the six-week period for the fish larvae fed on the four diets with 35% CP showing a considerably higher FCR (Figure 5). Further, a positive correlation was observed between FCR and weights of larvae fed

Table 1: Proximate contents of locally formulated diets

Parameter (%)	T1 (35% CP)	T2 (30% CP)	T3 (25% CP)	T4 (Control; 28% CP)
CP	35.06 ± 0.258	30.78 ± 0.342	25.51 ± 0.333	28.02 ± 0.253
Crude fibre	6.44 ± 0.174	8.29 ± 0.136	9.59 ± 0.30	5.53 ± 0.10
Crude lipid	10.54 ± 0.036	10.11 ± 0.038	13.48 ± 0.027	12.56 ± 0.047
Ash	8.77 ± 0.059	7.47 ± 0.251	7.00 ± 0.030	12.08 ± 0.277
Moisture	11.72 ± 0.144	11.29 ± 0.178	11.86 ± 0.232	10.48 ± 0.146
NFE	27.46 ± 0.232	32.07 ± 0.273	32.57 ± 0.704	31.33 ± 0.234

Note: Values represent % mean ± SEM; NFE = Nitrogen Free Extract

Table 2: The physico-chemical parameters of the treatment groups.

Parameters	T1	T2	T3	T4
Temp(°C)	18.9±2.6	18.8±2.7	18.8±2.9	19.2±2.6
DO(mgL ⁻¹)	6.2±0.9	5.8±0.8	6.3±0.8	5.8±0.7
Cond(µgcm ⁻¹)	360.2±36.7	316.7±7.2	306.5±7.5	308.4±3.2
TDS(mgcm ⁻¹)	55.1±8.7	56.3±11.0	61.1±5.2	55.7±0.5
NH ₄ (µg ⁻¹)	308.6±91.3	325.8±131.9	302.5±86.9	305.5±92.8
NH ₃ (µg ⁻¹)	45.8±4.4	42.2±6.7	41.3±6.9	38.1±4.4
Nitrates(µg ⁻¹)	178.7±8.6	173.4±26.8	143.5±12.1	160.6±11.2
Nitrites(µg ⁻¹)	87.2±2.2	89.5±3.5	92.1±6.3	92.6±4.5
TP(µg ⁻¹)	483.2±63.7	421.1±24.2	420.9±38.5	464.1±56.6
Silicates(mg ⁻¹)	5.3±1.4	4.2±0.4	4.3±0.3	3.9±0.2

Note: The values represent the mean ± SD taken during the experiment period.

Table 3: The daily growth performance of fish larvae fed on different diets

Parameter	T1 (35% CP)	T2 (30% CP)	T3 (25% CP)	T4 (28% CP)
Daily length gain (mm/day)	0.23 ± 0.01 ^b	0.17 ± 0.01 ^a	0.15 ± 0.01 ^a	0.16 ± 0.00 ^a
daily weight gain (g/day)	0.006 ± 0.00 ^b	0.006 ± 0.00 ^a	0.005 ± 0.00 ^a	0.004 ± 0.00 ^a
Survival rate (%)	70 ± 1.60 ^a	80 ± 1.60 ^b	75 ± 1.40 ^c	78 ± 1.70 ^d
FCR	0.706 ± 0.05 ^a	0.830 ± 0.05 ^a	0.823 ± 0.05 ^a	0.905 ± 0.09 ^a

Note: Different letters per row represent statistical significance between the different diets as determined by one-way ANOVA with Tukey's post-test ($p < 0.05$).

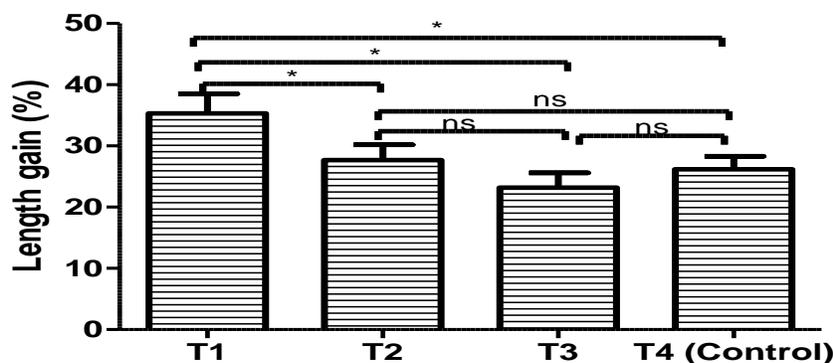


Figure 1: Length gain by *C. gariepinus* larvae fed on different CP levels. The length gain by *C. gariepinus* larvae fed on diets containing 35% CP (T1), 30% CP (T2), 25% CP (T3) and 28% CP (T4) over a six-week period. Bars represent mean ± SEM. The measurements were taken in triplicate every week. Statistical analysis was performed using one way-ANOVA with Tukey's post-test (*, $p < 0.05$; ns, $p > 0.05$).

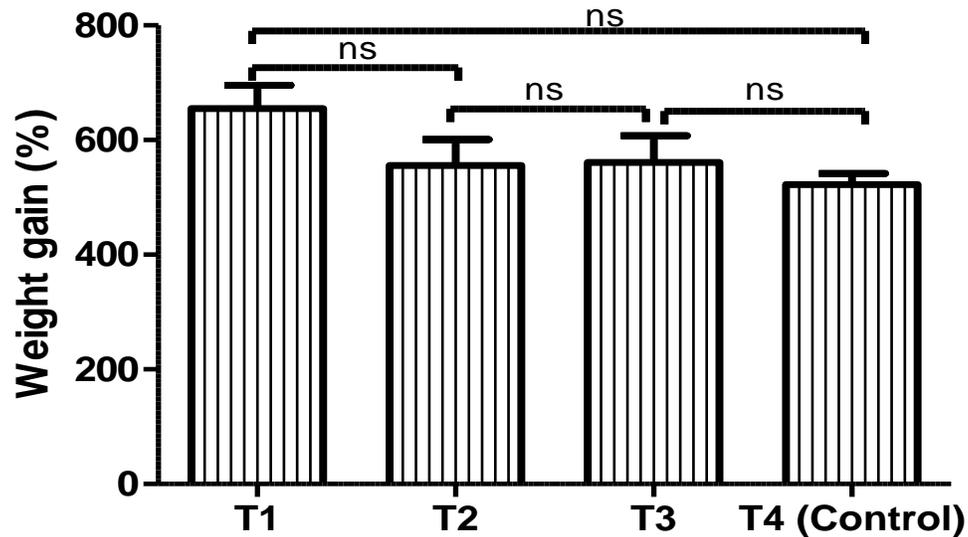


Figure 2: Weight gain by *C. gariepinus* larvae fed on different CP levels. The weight gain by *C. gariepinus* larvae fed on diets containing 35% CP (T1), 30% CP (T2), 25% CP (T3) and 28% CP (T4) over a six-week period. Bars represent mean \pm SEM. The measurements were taken in triplicate every week. Statistical analysis was performed using one way-ANOVA with Tukey's post-test (*, $p < 0.05$; ns, $p > 0.05$).

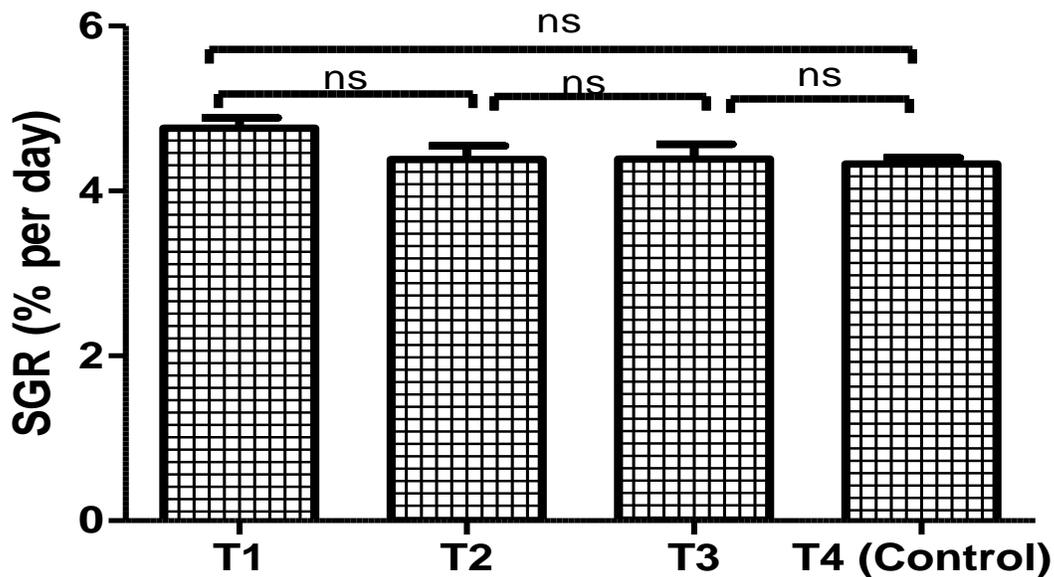


Figure 3. The SGR of *C. gariepinus* larvae fed on different CP levels. The percent SGR (per day) of larvae fed on diets containing 35% CP (T1), 30% CP (T2), 25% CP (T3) and 28% CP (T4) over a six-week period. Bars represent mean \pm SEM. The measurements were taken in triplicate every week. Statistical analysis was performed using one way-ANOVA with Tukey's post-test (*, $p < 0.05$; ns, $p > 0.05$).

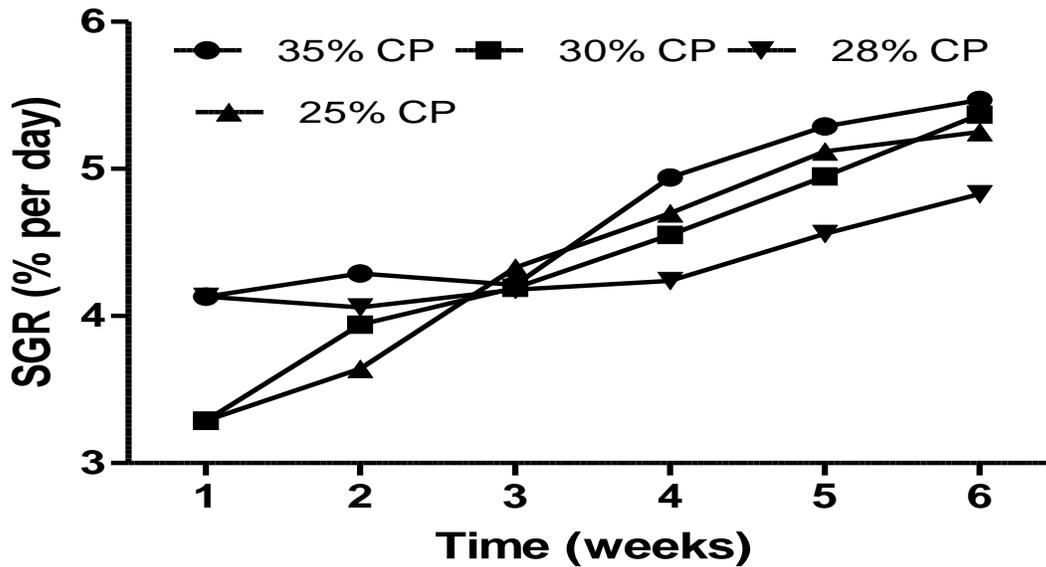


Figure 4: SGR (%) per day of *C. gariepinus* larvae fed on different levels of CP. The percent SGR of *C. gariepinus* larvae fed on 35%, 30%, 28% or 25% CP over a six-week period were calculated. The experiments were conducted in triplicate. Each plot represents the mean \pm SEM.

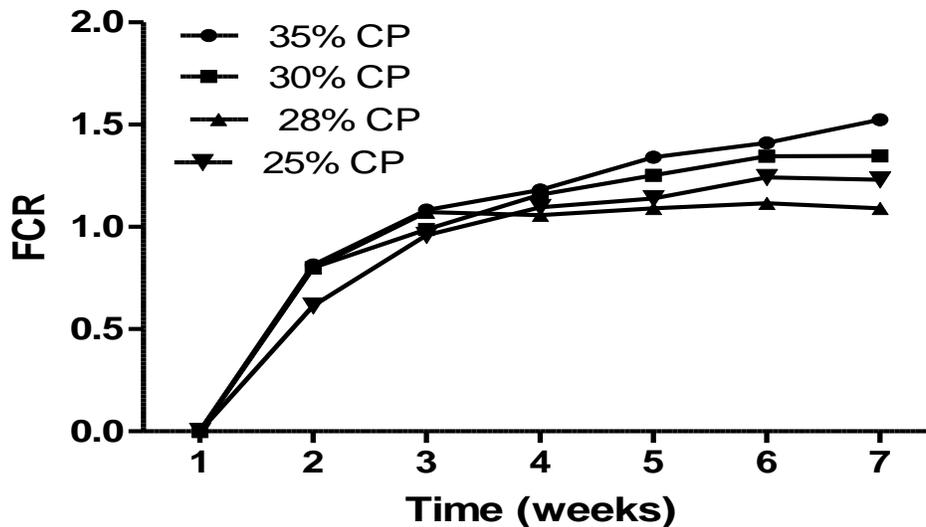


Figure 5: The FCR of *C. gariepinus* larvae fed on four diets. The FCR of *C. gariepinus* larvae fed on 35%, 30%, 28% or 25% CP over a six-week period were calculated. The FCR were calculated in triplicates weekly over a six-week period. The experiments were conducted in triplicate. Each plot represents the mean \pm SEM.

35% CP diet between larvae weight gain and FCR ($r = 0.738$; $p < 0.01$, $n = 72$).

The survival rate of the fish larvae grown under different diets were significantly different ($p < 0.05$). A post hoc analysis revealed that larvae fed on 30% CP had the highest length gain ($p < 0.05$), followed by 28% CP, 25%

CP and 35% CP in that order ($p < 0.05$; Table 3). However, the mean survival rate per week was considerably highest for 35% CP (Figure 6). These findings implied that the survival rate is not dependent on the level of CP.

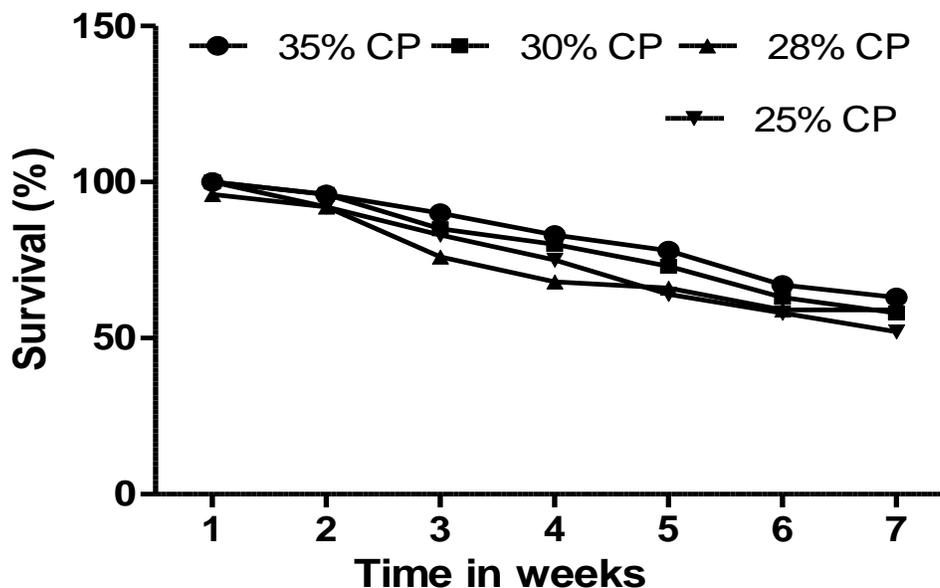


Figure 6: Survival rate of *C. gariepinus* larvae fed on different diets. The percent survival of fish fed on 35% CP, 30% CP, 28% CP or 25% CP was calculated weekly. The experiments were conducted in triplicate. Each plot represents the mean \pm SEM.

DISCUSSION

Water quality is of prime importance in fish culture systems for optimal growth, survival and reproduction. Thus, any evaluation of the growth performance, survival or reproduction of fish must control for water quality in the experimental set ups. In view of this, the present study monitored the culture conditions in respect of pH, dissolved oxygen, temperature, turbidity, total ammonia and nitrate throughout the six weeks. The water quality parameters were found to be similar and within the acceptable water quality conditions required for *C. gariepinus* growth (Ajiboye et al., 2015). Temperature is the most important variable affecting the growth of larvae and early juveniles (Ngugi et al., 2007). In the natural environment, the optimal temperature for growth appears to be 30°C; however, temperatures in the range of 26°C – 33 °C are known to yield acceptable growth performance (Britz and Hecht, 1987). At temperatures below this range, growth rates decrease but survival is still good. The current study recorded water pH within the range of 6.5 to 8.6 which was within the acceptable range of 6.5 to 9.0. However, fish are sensitive to unionized ammonia and

the optimum range is 0.02 mg/L to 0.05 mg/L in the pond water. The water DO and pH are important factors in water quality with influence over other nutrients. In indoor culture, high feeding rates may contribute to increase of uneaten feed and together with fish wastes can cause a significant deterioration in water quality. This could be the reason for high N and P elements measured. However, the frequent cleaning of the containers and the replacement of water ensured maintenance of good water quality. When concentrations of ammonia and nitrite exceed the recommended guidelines, toxicity effects can affect growth rates (Boyd, 1998). Lack of uptake of available soluble P may result in high concentration indoor setup, but often much high N and P in outdoor ponds results from application of fertilization and manure. Newly developed feeds should aim at being nutrient – dense in order to reduce the output of solid, phosphorus and nitrogen waste (Wanabe, 2002) as this also contributes to improved water quality in fish culture systems. In the present study the rate of pollution due to the burden of crude protein

was minimized by aeration and use of low crude protein diet.

In this study *C. gariepinus* larvae weight and total length significantly increased during the six weeks experimental period with the highest mean weight gain observed on fry feed on CP 35%. The high growth rate could be attributed to the high nutritional composition of the feed meal with 35% crude protein. However the experimental indoor temperature in the present study was not within the optimal temperature range of 26 – 33°C. In general, rearing of *C. gariepinus* larvae during 42 days in this experimental study setup, exhibited allometric growth with between 55.6% and 67.2% of the length and weight respectively. *C. gariepinus* larvae under the 35% CP diets exhibited positive allometric growth compared to the rest of the treatments. The larvae fed the experimental diets recorded better SGR than those fed the control diet. Larvae fed the highest 35% CP diet recorded the highest survival rate and considerably high FCR.

Good quality fish feeds are necessary aquaculture inputs to address the challenges of lack of efficient and inexpensive farm made feeds for different stages of fish development. The most commonly used feed ingredients are *C. nilotica* and *Rastrineobola argentea*, with wheat or rice bran, sunflower or cotton seed cake and cassava as binders (Munguti et al., 2014). The major ingredients (sunflower seed cake, cotton seed cake, *C. nilotica*, wheat bran) CP content ranged from 14 % to 60 % (with a high dry matter of 91 % to 94 % content) (Munguti et al., 2014), as found in this study's proximate analysis. Evaluation of some of the most commonly used sources of protein for culture of *Clarias gariepinus* and *Nile tilapia*, shows that crude fiber (CF) is generally higher on feed ingredients of plant origin and range between 55 g/Kg - 368 g/Kg dry matter while nitrogen free extracts (NFE) and ash content are higher in the feedstuffs of plant origin with an exception of maize bran which have the lowest value (Munguti et al., 2012). Cotton (*Gossypium sp*) seed cake provided the best option as source of processed plant based protein in the present study.

Fish may adapt their metabolic functions to the dietary substrates, through a regulation in enzyme secretion, in order to improve the utilization of feed ingredients (Caruso et al., 2009). Although not provided, such specific information could provide more explanation on the feed conversion rate uptake for larvae growth. Fish meal has traditionally been used as the major protein source for formulated fish feeds due to the high protein content, balanced amino acid profile, high digestibility, palatability, and as a source of essential n-3 polyenoic fatty acids (Jackson, 2006). However, many

experimental studies have formulated relatively cheap mixed plant based dietary protein sources for the Nile tilapia, and there is a growing need for feeds to cater for the larval stage of *C. gariepinus*. Studies have suggested *Caridina nilotica* to replace fishmeal in the early feeding stage of *C. gariepinus* (Chepkirui-Boit et al., 2011).

In a study by Nyonje et al. shell free/decapsulated artemia was utilized, for the first feeding of fry before weaning to dry starter diets of 40 % to 50 %. The survival of the catfish fry and fingerlings ranged from 40 % to 60 % in all the hatcheries. In the present study much higher survival rates are reported for the larvae of *C. gariepinus*. The study results showed a progressive increase in the FCR, weight gain and total length of *C. gariepinus* larvae. Previously in a 2 weeks experiment, Verreth et al. (1987) also found that dry feeds enriched with an acetone extract of Artemia fed to *C. gariepinus* larvae (mean weight 2.2 ± 0.2 mg) resulted in low growth and extremely low survival rates (< 20%), compared to micro-encapsulated egg (34.4 % -50.9 % protein; high survival rates 64 % - 93 %, but low growth rates) and dried decapsulated Artemia cyst diets (53.2 % - 58.6 % protein; best growth and survival rates). Egg diets are high in fats and provide most of the essential nutrients and best growth was attained from addition of casein and vitamin/mineral mix to egg diets. The larvae hepatocyte ultrastructure indicated a nutritional deficiency (Verreth et al., 1987).

Hatcheries are protective environments used to produce fry and fingerlings for stocking fish ponds. The demand for fish seed led to investment in seed production by private farmers who own most of the hatchery production units in Kenya (Nyonje et al, 2018). The rearing the young fish (fry) until they are able to survive in an open pond environment is part of the activities in hatcheries. The study utilized catfish larvae of an initial 20 mm. A rearing size of 20 mm to 30 mm is suitable for stocking into production ponds or for use as baitfish. Therefore, this study results contribute towards the success and realization of the improved production of key candidate fish culture species, and improved hatchery management practices.

CONCLUSIONS

Feeding *C. gariepinus* larvae low crude protein in locally formulated feeds for six weeks, than optimally recommended produced comparatively good growth performance in terms of weight gain, SGR and FCR. Although the larvae fed the highest CP diet (35 % CP) had the highest growth performance, there was almost

similar growth performance between larvae fed on 25% CP and 28% CP (recommended commercial feed). The study supports the strategies towards ensuring maximum farmer profits by utilization of less expensive protein sources, in combination with improved feed nutritional quality. This will contribute to increased availability of specific life stage feed source, a common problem in larval rearing for sustainable aquaculture production.

Competing interests

The authors declare that they have no competing interests.

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