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EFFECT OF STOCKING DENSITY AND DIET ON THE GROWTH AND SURVIVAL OF *Labeo victorianus* FISH

Running title: *Labeo victorianus*' response to stocking density and diets

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ABSTRACT

This study evaluated the effect of stocking density and diet on the growth and survival of *Labeo victorianus* fish. Stocking densities, diet and survival rates were studied under 27 hapas, 1.5m^2 wide and1m deep at the National Aquaculture Research and Development training center, Sagana in Kenya. Juveniles averaging 15g were stocked at 8/m^2, 16/m^2 and 24/m^2 then reared for 5 months. The juveniles were fed on three diets; natural feed and formulated diet with refined 30% and 25% crude protein ad libitum daily. The mortalities, daily weight and length gains, and water quality were monitored throughout the research period. The results showed that 8/m^2 stocking densities had the best survival (100%) and the highest weight (11.2–20.0 g) and length (3.5–4.4 cm) gains by the fish. There was a significant effect of dietary protein on growth performance of the reared fish (180% weight gain compared to the control). However, weight gains at 25% and 30% protein levels were not significantly different (P>0.05). The results suggested that stocking density and diets are critical on growth performance and survival of juvenile *L. victorianus*. This study has particular significance with regards to fish husbandry in terms of survival and production efficiency.

Keywords: Stocking density, *Labeo victorianus*, Fish, Tank, Feed evaluation, Protein

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1. INTRODUCTION

Currently, 800 million people suffer from chronic malnourishment and the global population is expected to hit 9.6 billion people by 2050, resulting to a huge challenge of feeding the population (Floros et al., 2010). The food insecurity has been aggravated in the developing countries because of declining food production efficiency coupled with increasing demands on food resources attributed to increasing human populations (Sasson, 2012). In particular, the over-reliance of sub-Saharan Africa on subsistence agriculture and the erratic climatic conditions have aggravated the food insecurity situation. Consequently, the region has been severely hit by chronic malnutrition (FAO, IFAD, and WFP, 2013). To mitigate the negative effects of the impending food shortage, strenuous efforts are being employed to increase both animal and plant protein production.

Fisheries and aquaculture have played and will continue playing a significant role in eliminating hunger, promoting health, and alleviating poverty. Fish is exceptionally nutritious and is a vital source of protein and essential nutrients, especially for many people in developing countries where fish is the main protein supply (FAO, 2014). However, in the recent past, the unsustainable capture of wild fish has depleted stocks while the demand for fish and fish products is rising especially as large populations in developing countries gain wealth and adopt middle-class diets higher in protein (Moorhead and Zeng, 2010; Chambel et al., 2013; Tânia et al., 2014). In line with this view, aquaculture has emerged as a key component to bridge the current food shortage gaps and ensure protein security (Beveridge et al., 2013; FAO, 2004). Moreover, this approach reduces the overreliance on natural resources and is more sustainable than the wild-caught fish.

Aquaculture status in Africa and specifically in sub-Saharan Africa is comparatively low despite its potential of lifting masses out of poverty and contributing to food and nutrition security (FAO, 2014). For instance in 2012, sub-Saharan Africa contributed a paltry 0.68 % of the world's aquaculture production despite every African country launching donor-supported fish farming projects in 1988 (FAO, 2014). Aquaculture development in Kenya dates back to the 1920s but had stagnated at subsistence level until a few years ago (FAO, 1966; Munguti, Kim, and Ogello, 2014). Serious attempts to expand aquaculture in Kenya were made in the early 1960s with the main objective of increasing animal protein consumption for the rural populations (FAO, 2004; Munguti, Kim, and Ogello, 2014). Since then, aquaculture has made little progress with some farmers abandoning fish ponds. This was mainly due to losses attributed to high costs of production (Munguti, Kim, and Ogello, 2014). However, with the intervention of the Kenyan government through the Fish Farming Enterprise Productivity Program (FFEPP) in 2009, there has been a tremendous increase in the number of fish farmers and aquaculture productivity across the country (Musa et al., 2012; Munguti, Kim, and Ogello, 2014)

The primary aquaculture products in Kenya are few and include the Nile tilapia (*Oreochromis* spp.), African catfish (*Clarias gariepinus*) and Common carp (*Cyprinus carpio*) (Munguti, Kim, and Ogello, 2014; FAO, 2004). Trout is another product but it is temperature-restricted; it is only produced at climatic regions with low temperatures, mainly in the Mt. Kenya region (Coche, Haight, and Vinc, 1994). There has been little focus in Kenya placed on domesticating new wild fish species, which may have significant potential for providing sustainable food supplies and improve local economies (Munguti, Kim, and Ogello, 2014). Therefore, there is need to reengineer aquaculture to develop new fish products that vastly expand the diversity, sustainability, and quality of aquaculture products to meet growing food security and nutrition needs.

One of the wild fish products with potential in aquaculture is the *Labeo victorianus*, a Labeine restricted to the Lake Victoria basin, locally known as *ningu*. Other Labeines are widely distributed, with at least 80 species, on the African continent and contributing 16.4% of the African cyprinid fauna (Skelton et al., 1991). *L. victorianus*, just like other labeines is potamodrometic and moves into affluent rivers to spawn in vegetated flooded pools (Fryer and Whitehead, 1959; Reid, 1985; Skelton *et al*., 1991; Weyl and Booth, 1999). However, due to it being a valued delicacy and its various uses by the riparian community in addition to its predictable migratory habits, considerable fishing pressure has been exerted on *L. victorianus* and is ecologically endangered (Rutaisire and Booth, 2005). Therefore, efforts to bring it into captivity can increase its production and aquaculture products diversity. However, *L. victorianus* has not been known to spawn by itself in captivity thus; it is induced to breed in hatcheries and with good induction techniques; the fish spawn in large numbers, in the culture units (Orina et al., 2014). Past trials of *L. victorianus* culture have reported various challenges including spawning, egg hatchability, slow growth and mortalities perhaps stemming from the environmental conditions (Rutaisire and Booth, 2005).

Therefore, to bring *L. victorianus* into captivity, understanding various parameters to optimize its production in order to compete with less expensive specimens collected from the wild is crucial. Stocking density and diet are the two key elements in aquaculture that affect growth, health, and welfare of fish (Chambel et al., 2015). Feeds make up a major proportion of fish production costs and optimization of growth rates is dependent on the quality and composition of the diet (Chambel et al., 2015). On the other hand, the stocking density can affect survival, behavior, survival, feeding, and water quality. Increase in stocking density increases the stress condition while reduced stocking densities may be sub-optimal in production (Sharm and Chakrabarti,

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1998). Identification of the optimum stocking density and diets for a species are thus; important factors that contribute to efficient management and productivity of fish under captivity (Rowland et al., 2006).

The aim of this study was to evaluate the effect of stocking density and diet on the growth performance and survival of juvenile *L. victorianus*.

2. MATERIALS AND METHODS

2.1 Breeding Site Identification and Brood Stock Collection

A preliminary survey for breeding grounds of *L. victorianus* based on gained information from other scientists engaged in *L. victorianus* research (Abwao et al., 2014; Orina et al., 2014) was carried out prior to the long rains at Mara and Migori rivers located in South Rift and part of South Nyanza, Kenya. Electro-fishing method was used for sampling. On the onset of long rains, sampling was carried out at the Mara River using the electro-fisher. Populations of gravid *L. victorianus* at the ratio of 2 male: 1 female were collected and acclimatized for one day by the river in a cage before transportation to the study site. Gravid brooders were identified by gently squeezing the belly from the head towards the anal area. Gravid male release milt while gravid female release eggs (golden green) (Abwao et al., 2014). The brooders were transported to the National Aquaculture Research and Development training center, Sagana in Kenya by packing them in water holding oxygenated polythene bags. On arrival, the brooders were acclimatized and held in well aerated tanks.

2.2 *L. victorianus* Induction and Spawning Procedure

Prior to inducement of selected female brooders, length and weight measurements were recorded against the donor males. A 2 ml solution comprising of 0.5 ml ovaprim and 1.5 ml saline solution was intramuscularly injected to the female brooders. The induced brooders were paired in 60 L aquaria with ready males at a ratio of 2:1 (male: female). Aquaria temperature was maintained at 26±1ºC with a flow rate of 10 L per hour. The aquaria were covered with black polythene and the fish left to engage in the breeding process. Spent brooders were transferred to other holding facilities and then introduced to ponds where they were subjected to a special diet to aid in quick recovery. Fries attained from the propagation process were reared in nursery ponds using artemia for the first two weeks and wean meal introduced in the third week. The fries were grown to fingering size of average weight of 15g.

2.3 Stocking Densities and Feeding Trials

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The fingerlings weighing averagely 15g were obtained from the nursery pond and stocked in 1m^2 hapas at triplicate stocking densities of 8, 16 and 24/m^2. Stocked fingerlings were acclimatized for three days before feeding. Treatment (T1) was subjected to 30% CP on-farm formulated diet; Treatment (T2) was subjected to natural pond productivity and also acted as control, while Treatment (T3) fed on 25% CP on-farm formulated diet. Treatments depending on formulated diet (T1 and T3) were all fed *ad libitum*.

2.4 Proximate Composition of the Experimental Formulated Diets

The samples of the ingredients of experimental diets were analyzed for crude protein and other components using the standard procedure (AOAC, 1984). The proximate composition of protein of each ingredient (Tables 1 and 2) were used in the simple Pearson square method as described by New (1987) to standardize the protein levels and complement with the basal feeds to supply energy. Each treatment except for control diet was mixed with 0.1% vitamin-mineral mix as recommended by New (1987). The formulated diets were then proximate-analyzed for crude protein, fiber, lipids, ash, carbohydrates and moisture contents.

Table 1: Proximate composition of ingredients of diet 1 (30% CP).

Ingredient	Protein (%)	Fiber (%)	Lipids (%)	Ash (%)	Carbohydrate (%)	Moisture (%)
Caridina nilotica	15.95	1.51	0.522	0.9	-	9
Cotton seed cake	8.4	7.66	1.992	3.2	2.976	11
Wheat Bran	5.52	1.61	1.656	1.2	32.61	9
Vitamin premix/mineral	0.13	-	-	-	-	-

Table 2: Proximate composition of the ingredients of diet 3 (25% CP).

Ingredient	Protein (%)	Fiber (%)	Lipids (%)	Ash (%)	Carbohydrate (%)	Moisture (%)
Caridina nilotica	15.95	1.51	0.522	0.9	-	9
Cotton seed cake	5.6	7.66	1.992	3.2	2.976	11
Wheat Bran	3.32	1.61	1.656	1.2	32.61	9
Vitamin premix/mineral	0.13	-	-	-	-	-

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2.5 Experimental Design

A 3 x 3 factorial design of 3 stocking densities and 3 feeding regimes was replicated for the study. A total of 27 hapas measuring $1m^2$ were mounted in an earthen pond measuring 800 m^2 and stocked with *L. victorianus* fingerlings. Prior to stocking, the ponds were drained, repaired and treated with lime at a rate of $200g/m^2$ to eradicate any aquatic fauna prior to the start of the experiment. They were then filled with screened river water and fertilized using Di-Ammonium Phosphate (DAP) and urea at the rates of 2 g/m^2 and $3g/m^2$, respectively. The ponds were then stocked with the fingerlings at $8/m^2$, $16/m^2$, and $24/m^2$.

2.6 Data Collection, Sampling and Recording

All fish in each hapa were weighed and length measurements taken and recorded at each sampling process. The wet weights were measured by use of an analytical balance (readability 0.01mg) (Sartorius, Germany) and total length by use of a mounted ruler to the nearest 0.01mm. Sampling was carried out twice a month at two weeks intervals to obtain data on water quality, number of fish, and length-weight measurement as a way of assessing the survival and growth performance of the stocked population. At the end of the experiment the ponds were drained and all fish harvested and weighed to the nearest 0.1g. The experiment was run for four (4) months thus seven (7) sampling sessions excluding the initial measurements were made. Water quality parameters monitored include:-Total ammonia nitrogen (TAN), dissolved oxygen (DO), pH, temperature, water turbidity, and conductivity. These were measured twice daily at the depth of 30 inches. Growth performance of the experimental fish was calculated as described by Abwao et al. (2014) as follows:

Weight Gain (WG) = final weight (g) - initial weight (g).

Survival (%) was calculated based on the number of fish remaining in hapas in the earthen ponds as a percentage of the initial stocks. DO, pH, temperature, conductivity and TDS were determined *in situ* using Hanna multiple range test meter. Samples for biochemical oxygen demand (BOD) were collected in dark glass bottles and incubated at 20ºC for 5 days before determining the remaining DO.

2.7 Data analysis

The data obtained for the weight and length variables at various stocking densities and diets were entered into the computer using Microsoft Office excel 2007 before analysis was done. Analysis of Variance (one-way ANOVA) using SPSS Version 17 was carried out to test the effect of stocking density and diet on growth performance while the effect on survival was calculated as

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percentage of the difference between initial and final stock divided by the initial stock for each variable. If ANOVA indicated significant treatment effects, the Tukey's honestly significant difference (HSD) test was used to determine differences among individual treatment means (Snedecor, Cochran, and Snecdecor, 1989). Differences were considered significant at $P<0.05$.

3. RESULTS

3.1 Water Quality Parameters

The water quality parameters monitored in the experimental ponds per treatment were within the recommended range for fish culture (Boyd, 1990; Ayinla, 1994) (Table 3). Water temperature ranged from 23.7°C in the mornings to 28.9°C in the afternoons, dissolved oxygen from 2.1 mgl^{-1} in the mornings to 8.3 mgl^{-1} in the afternoons, and pH from 7.3 to 8.2 (Table 3).

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Table 3 Water quality parameters (Temperature, DO, pH and TAN) during the growing of *L. victorianus*.

Parameter	Experimental treatments								
	T11	**T12**	**T13**	**T21**	**T22**	**T23**	**T31**	**T32**	**T33**
DO morning (mgL^{-1})	2.6±0.1^{a}	2.6±0.2^{a}	2.6± 0.1^{a}	2.5±0.2^{a}	2.6±0.2^{a}	2.6± 0.2^{a}	2.7± 0.3^{a}	2.1± 0.1^{b}	2.6±0.2^{a}
DO afternoon (mgL^{-1})	8.2±0.9^{a}	8.3±0.7^{a}	8.2± 0.9^{a}	8.2± 0.2^{a}	8.2± 0.8^{a}	8.2± 0.7^{a}	8.2± 0.9^{a}	8.3± 0.9^{b}	8.2±0.2^{a}
Temperature morning (°C)	23.7± 0.7^{a}	24.0± 0.3^{a}	23.8± 0.6^{a}	23.8± 0.6^{a}	23.9± 0.3^{a}	23.7± 0.6^{a}	23.8± 0.7^{a}	23.8± 0.7^{a}	23.8± 0.6^{a}
Temperature afternoon (°C)	27.8± 2.7^{a}	28.8± 1.5^{a}	28.9± 1.4^{a}	28.7± 1.5^{a}	28.9± 1.6^{a}	28.9± 1.4^{a}	28.9± 1.6^{a}	28.9± 1.6^{a}	28.5± 1.5^{a}
pH morning	7.3± 0.2^{a}	7.3± 0.2^{a}	7.3± 0.2^{a}	7.3±0.2^{a}	7.3± 0.2^{a}	7.3± 0.2^{a}	7.3± 0.2^{a}	7.3±0.2^{a}	7.3±0.2^{a}
pH afternoon	7.9±0.3^{a}	8.1±0.1^{a}	7.9± 0.0^{a}	8.1±0.1^{a}	8.0± 0.0^{a}	8.1± 0.0^{a}	7.9± 0.1^{a}	8.2± 0.1^{a}	8.1±0.1^{a}
TAN (mgL^{-1})	1.1±0.0^{a}	1.1±0.0^{a}	1.1± 0.0^{a}	1.1±0.0^{a}	1.1±0.0^{a}	1.1± 0.0^{a}	1.1±0.0^{a}	1.1±0.0^{a}	1.1±0.0^{a}

The data are means±standard deviations of triplicate measurements. Superscripts with different letters indicate differences of the means.

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3.2 Growth Performance

Weight and length gains for the *L. victorianus* juveniles after the feeding trial are presented in figures 1 & 2, respectively. For all the treatments, weight doubled after 14 days (Figure 1) and there was significant increase in length during the same period of growth (Figure 2), then followed by a slowed growth rate and increase in length for all the treatments (Figures 1 & 2). The treatments with supplemented diets (T1 and T3) at all stocking densities independently experienced significant weight gains after 14 days, however the difference between 84th and 98th day of growth was insignificant. On the contrary, for the control (T2), growth stagnated after 14 days (Figure 1) and there was no significant length increase over the same period (Figure 2), indicating that they had attained full growth and length under the experimental conditions.

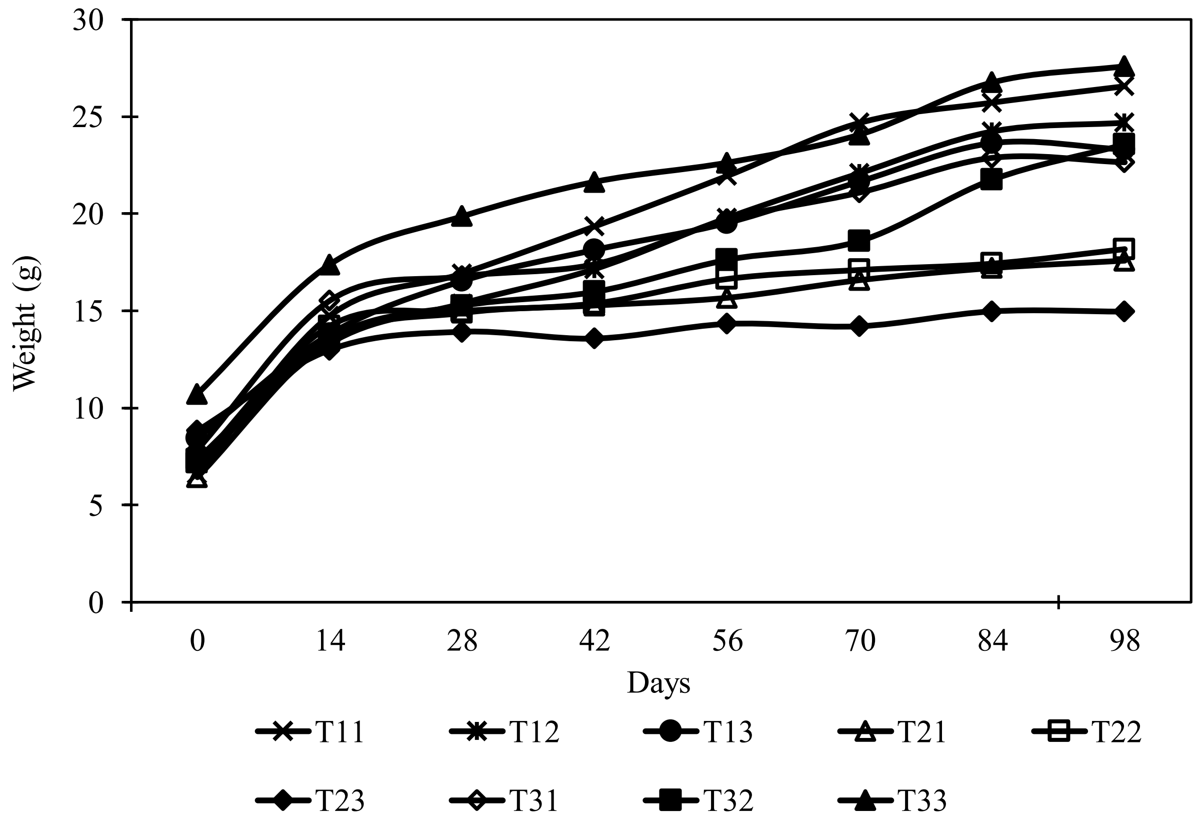

Figure 1: Growth trends of *L. victorianus* in hapas at different stocking densities and diets over 98 days period.

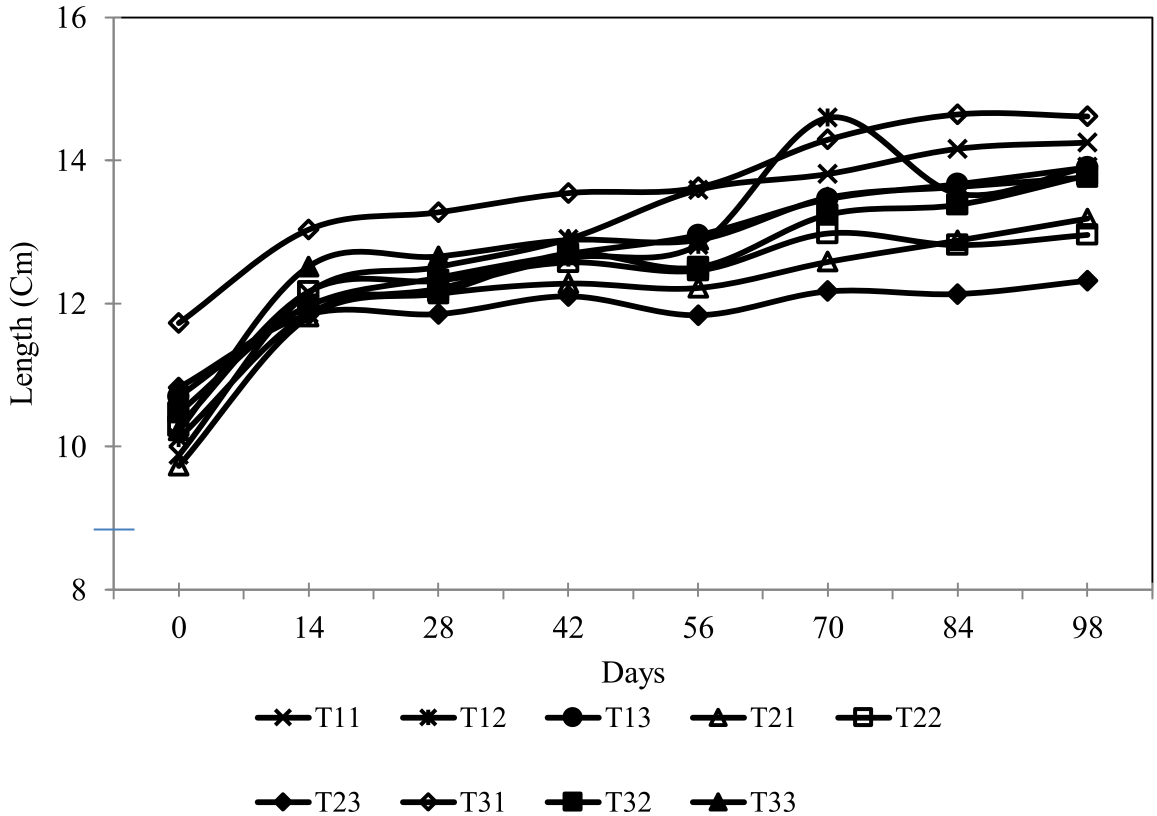

Figure 2: ***L. victorianus*** **length gain in the hapas at different stocking densities and diets over a period of 98 days.**

The weight and length gains at different feeding regimes and stocking densities after 7 weeks are reported in Tables 4 and 5, respectively. The weight and length gains seemed different hence; the growth performance was analyzed for the effects of diet and stocking densities using one-way ANOVA. For the effect of diets on weight gain, the P-value corresponding to the F-statistic of one-way ANOVA was lower than 0.05, suggesting that the effect of one or more diet treatments were significantly different. Thus; Tukey's HSD test was performed, which indicated that growth performance increased significantly ($P<0.05$) with application of protein diets to the feeds of fish (Table 4), but there was no significant difference in weight gain with the diet of 30% protein and that of 25% protein ($P>0.05$), suggesting that feeds with lower protein content could be economical in raising fish.

Table 4: *L. victorianus* weight gain based on diet and stocking during 7 weeks growth period in hapas

Diet	Stocking density	Initial Weight (g)	Final Weight (g)	Weight gained (g)	Means of weight gained (g)
Diet 1 (30% CP)	1	6.6± 1.5	26.6 ±6.2	20.0±4.7[d]	
	2	7.2± 1.8	24.7± 7.3	17.5±5.5[d]	17.4±5.1[a]
	3	8.5± 3.6	23.3± 8.4	14.8±4.8[d]	
Diet 2 (Control)	1	6.4± 1.4	17.6± 4.7	11.2±3.3[e]	
	2	7.2±2.2	18.2± 4.6	11.0±2.4[e]	9.5±2.3[b]
	3	8.8± 3.3	15.0± 4.5	6.2±1.2[f]	
Diet 3 (25% CP)	1	7.9± 1.9	22.7± 6.1	14.8±4.2[g]	
	2	7.4± 2.8	23.6 ±7.9	16.2±5.1[g]	16.0±4.1[a]
	3	10.7± 3.0	27.6±6.9	16.9±3.9[g]	

The data are means±standard deviations of triplicate measurements. Different superscript letters on the weight gained indicates that the means are significantly different within each diet. Different superscript letters on the means of weight gained indicates that the means are significantly different between the diets. Stocking densities: **1**, 8/m^2; **2**, 16/m^2; and **3**, 24/m^2.

For the effect of diets on length gain, the P-value corresponding to the F-statistic of one-way ANOVA was lower than 0.05, suggesting that the effect of one or more diet treatments were significantly different. Subsequent Tukey's HSD test indicated that length of fish increased significantly ($P<0.05$) with application of protein diets (30%) to the feeds of fish (Table 5), while the diet with 25% protein had no significant effect on length gain compared to the control ($P>0.05$). However, the effect of both diets with protein supplementation on length gain of fish was not significantly ($p>0.05$) different, suggesting that feeds with higher protein content could be preferred for fish length gain, when compared with the control.

Table 5 ***L. victorianus*** **length gain based on diet and stocking during 7 weeks growth period in hapas**

Diet	Stocking Density	Initial Length (cm)	Final Length (cm)	Length gained (cm)	Means of length gained (cm)
Diet 1 (30% CP)	1	9.9± 0.6	14.3±0.9	4.4±0.4[d]	
	2	10.1± 0.8	13.9± 1.4	3.8±0.6[de]	
	3	10.7± 1.2	13.9±1.5	3.2±0.4[e]	3.8±0.5[a]
Diet 2 (Control)	1	9.7± 0.6	13.2± 1.0	3.5±0.7[f]	
	2	10.3±0.9	13.0± 1.2	2.7±0.6[f]	
	3	10.8± 1.3	12.3± 1.2	1.5±0.7[g]	2.6±0.7[b]
Diet 3 (25% CP)	1	10.2± 0.7	13.8± 1.2	3.6±0.4[h]	
	2	10.5±0.9	13.8 ±1.5	3.3±0.4[hi]	
	3	11.7± 1.1	14.6± 1.3	2.9±0.2[i]	3.3±0.3[ab]

The data are means±standard deviations of triplicate measurements. Different superscript letters on the length gained indicates that the means are significantly different within each diet. Different superscript letters on the means of length gained indicates that the means are significantly different between the diets. Stocking densities: **1**, $8/m^2$; **2**, $16/m^2$; and **3**, $24/m^2$.

For the effect of stocking density on weight and length gain, one-way ANOVA indicated significant differences ($P<0.05$) between the different stocking densities within the same diets, with those with low stocking densities performing better than their counterparts with high stocking densities (Tukey's HSD test) (Tables 4 and 5). There was a positive correlation between weight gain and length increase ($R^2=0.7067$), which suggests that the more the weight gain, the longer the fish. This suggested that low stocking densities and high protein supplementation are better in achieving higher weight and length gains of the fish, which could be favorable for high fish productivity.

3.3 Survival Rate

The mean survival rates of the fingerlings are shown in Figure 3. The mean survival rates ranged between 46 and 100% in all the treatments. The highest survival rates were observed in stocking density 1 ($8/m^2$) with 100% survival, followed by stocking density 2 ($16/m^2$) and then stocking 3 ($24/m^2$) in all the treatments. The results showed that there was no significant difference in survival rates between the different dietary treatments at stocking density 1 and 3. All these results indicate that there was an inverse relationship between survival rates and stocking density. This means that for high returns from fish, low stocking density could be preferable.

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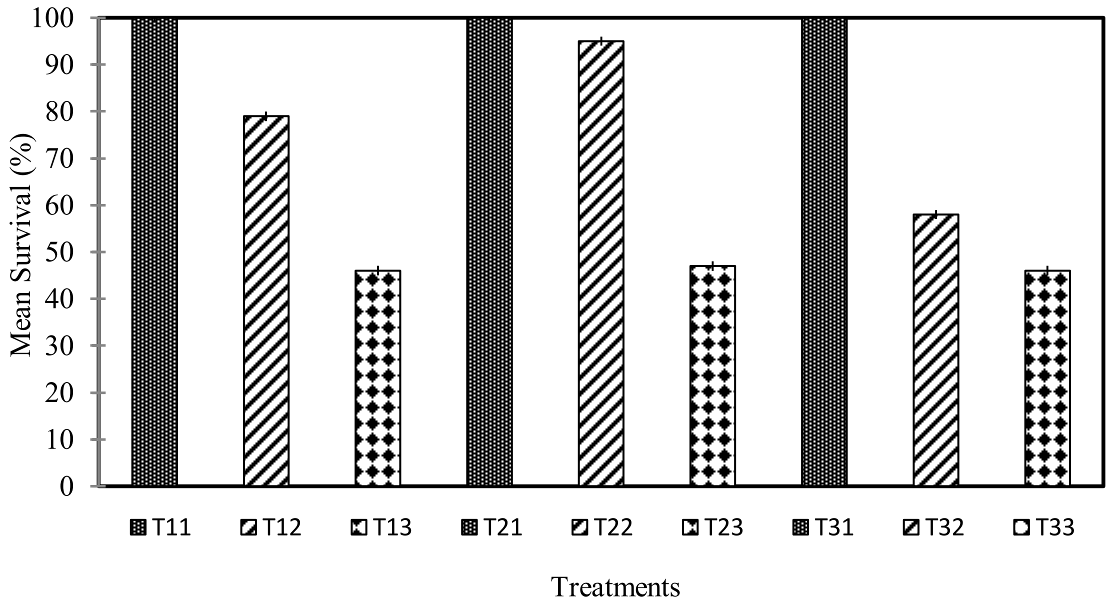

Figure 3: Survival rates of *L. victorianus* at different diets and stocking densities

4. DISCUSSION

Although there has been a global increase in the number of farmed fish species since 1950 (FAO, 2006), very few species indigenous to Africa have been cultured under captivity possibly because their survival under captivity has not been studied in details (Rutaisire et al., 2013). Growth data parameters, survival and mortality are great tools for evaluating the effect of feed and its value composition on fish species. This study sought to find the relationship between feeding and stocking density on the growth performance of *L. victorianus*; parameters that are useful in designing growing regimes in aquaculture for maximum productivity. The growth trend indicated a direct effect of stocking density, diet and a combination of the two factors on the growth of *L. victorianus*. Growth differentiation for aquaria treatments stocking density of 8 /m^3 for all diets gave the best final growth response and survival rates, indicating that density has an effect on the growth performance of the fish. The competition for food can limit the growth rate of fish and lead to poor weight gain (Stickney, 1994). That may have been the case in this study where fish stocked at higher stocking densities had poor survival rates. This is an indication that *L. victorianus* are not hardy and cannot survive poor conditions including high stocking densities and when reared under captivity, this factor should be considered.

Water quality plays a significant role in the biology and physiology of fish and may impact on the health and productivity of the culture system (Boyd, 1990). However, throughout this

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experiment, water quality across all the treatments was within the favorable range required for most fish (Boyd, 1990); the variation in fish growth and survival in this study may not therefore be strictly attributed to the characteristics of water quality parameters.

The natural food items in the pond might have satisfied the dietary requirements of *L. victorianus*. However, sustainable aquaculture can only be possible if quality feed or ingredients of feed are readily available to fish. The good growth performance of the fish fed with feed containing 25 and 30% proteins indicates that the feeds contained well balanced nutrients as seen in the proximate composition of the feeds to promote growth (weight and length). Owori-wadunde (2012) had demonstrated the pond-grown *L. victorianus* attained twice the average weight than those grown in the wild and of the same age group. Therefore, supplementing the diets of fish with quality feeds enhances the growth rate of *L. victorianus* compared to its counterparts in the wild.

In conclusion, *L. victorianus* can adapt to conditions under captivity due to their growth performance under different conditions demonstrated in this study. The diets containing 25 and 30% protein content had a similar effect on growth performance of the fish at the different stocking densities. Therefore, better performance of fish can be realized at low stocking density where there were high survival rates. Feeds with lower protein content could be economical for *L. victorianus* feeding. However, an optimum stocking density and feed need to be determined where survival rate is high for maximum results.

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