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Cover image: The mudskipper *Periophthalmus argentilineatus* on the mangrove of Ulo Beach, Cabo Delgado, North Mozambique. © José Paula, 2006

Efficacy of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* on growth improvement of hybrid Nile and Rufiji tilapia populations

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Abstract

An investigation of the role of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* on growth performance of hybrids from *Oreochromis niloticus* and *Oreochromis urolepis urolepis* was carried out in plastic tanks at 10 fish/m^3 density and a salinity of 25 units. The probiotic treatments were 2, 4 and 6 g/kg feed while the control feed did not include any probiotics. Growth parameters were measured fortnightly and water quality parameters monitored every day. Survival rate and water quality factors revealed non-significant variations ($p > 0.05$). Final weight differed significantly among the treatments ($p < 0.05$). Furthermore, Food Conversion Ratio (FCR), Specific Growth Rates (SGR), and weight gain did not differ significantly ($p > 0.05$) between the control and 2 g/kg treatments. However, the 4 and 6 g/kg feed treatments showed significant differences ($p < 0.05$) from the control diet and 2 g/kg for both *L. plantarum* and *S. cerevisiae*. The condition factor did not show any significant difference ($p > 0.05$) among the treatments. Results of this study indicate that increasing the quantity of commercial probiotics in feeds improved growth rates. Condition factor observed in the treatments could be due to the role of the tested probiotics in improving the water quality of tanks. Therefore, probiotics could potentially be used to enhance coastal aquaculture development.

Keywords: probiotics, growth performance, salinity, hybrids, *Oreochromis* species

Introduction

Global fish production from aquaculture requires different farming techniques to achieve the goal of high productivity. One of the important aspects recently considered to improve aquaculture production is the application of food additives, particularly probiotics. The contribution of probiotics to global aquaculture production was noticed in the culture of Mozambique tilapia and Nile tilapia (Gobi *et al*., 2018; Al-Deriny *et al*., 2020). These authors reported improvement of growth performance and immunity when tilapia were fed probiotics. Involvement of probiotics in aquaculture production in Africa is based on experiences from Egypt. Ibrahem (2015) reported on the status of probiotic use in Egypt's aquaculture. An investigation on the effect of probiotics on growth performance, feed efficiency and immune response in Nile tilapia culture was also conducted in Egypt (Dawood *et al*., 2020). Little work has been carried out to improve the understanding and use of probiotics to enhance aquaculture production in East Africa, including in Tanzania.

Probiotic additives are live microbes that are fed to fish and thought to improve intestinal microbial balance and fish growth (Wang *et al*., 2005). When these live microorganisms are properly provided in recommended volumes they may result in a healthier host

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(FAO and WHO, 2002). Probiotics have the ability to inhibit pathogenic bacteria, and improve both water quality and immune response of host species (Verschuere *et al.*, 2000; Carnevali *et al.*, 2006), although a study by Khatun and Saha (2017) indicated that probiotics have no significant influence on water quality. Furthermore, probiotics produce digestive enzymes which improve fish nutrition (Carnevali *et al.*, 2006), provide disease resistance and anti-carcinogenic activity (Wang, 2007). The influence of probiotics on growth and disease resistance in *Oreochromis niloticus* has been discussed by several authors (e.g. Verschuere *et al.*, 2000; Carnevali *et al.*, 2006; Kim *et al.*, 2010). Probiotics differ from pre-biotics in that they add new microbes to the gut whereas the latter act as fertilizer to the important bacteria in the gut. *L. plantarum* and *S. cerevisiae* showed improved growth, enhanced enzymatic activity, increased stress resistance and feed utilization in *O. niloticus* (Maurilio *et al.*, 2002; Essa *et al.* 2010). It has been documented that probiotics can be important for pathogenic control in aquaculture systems (Cruz *et al.*, 2012). Therefore, probiotics are non-pathogenic and non-toxic (Kherraz *et al.*, 2012).

Tilapia production faces challenges in a mixed culture condition of male and female fish. All-male fish populations are believed to have better growth performance than mixed sex culture (Banerjee and Chakraborty, 2010). Also, they control unnecessary reproduction (Mensah *et al.*, 2013), prevent stunted growth and produce fish with reliably uniform market size. Manual sexing, genetic manipulation, temperature influence in rearing facilities, hormonal reversal (Silva, 2013) and hybridization have been used in monosex tilapia production. Testosterone hormone is widely used for sex reversal of tilapia fry. Yet, both hormonal reversal and use of antibiotics have raised public worries. Dethlefsen and Relman (2011) argued that antibiotics may kill beneficial microbes in the gastrointestinal tract and have hence received injunctions in many countries. Alternatively, application of probiotics and natural all-male hybrids are encouraged in

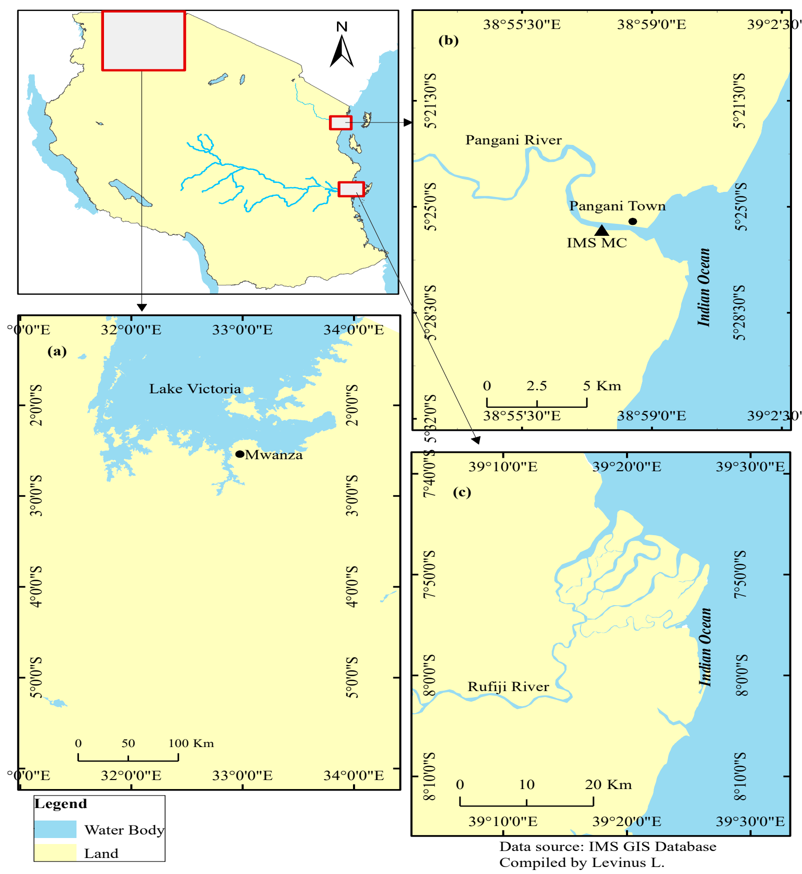

Figure 1. Map of Tanzania showing (a) Lake Victoria, (b) Pangani, and (c) Rufiji River. The study site was at Pangani, while broodstock were collected from Lake Victoria and the Rufiji River.

fish farming. However, little is known on the influence of commercial *L. plantarum* and *S. cerevisiae* to growth performance in tilapia hybrids. Therefore, this study was conducted to determine the influence of commercial probiotic feed supplements on the growth of all-male tilapia hybrids in brackish waters. The probiotics used were *L. plantarum* (bacteria) and *S. cerevisiae* (yeast). The hybrids were progenies of the hybridization between female Nile and male Rufiji tilapia populations. The hybridization between Nile and Rufiji tilapia populations produces 100 % male fish (Mapenzi and Mmochi, 2016).

Materials and methods

This study was conducted at the Institute of Marine Sciences Mariculture Center (IMS-MC) of the University of Dar es Salaam, located at Pangani, Tanga Region in Tanzania. Two experiments with two probiotics and three levels of diet treatments were conducted at IMS-MC. A total of 210 fish hybrids were stocked into 1000 L white plastic tanks at densities of 10 fish/m^3. In the first and second experiments, the symbols SC_{1-3} and LP_{1-3} were used to denote *S. cerevisiae* and *L. plantarum* at three treatments 2, 4 and 6 g/kg feed, respectively. The hybrids were raised at a salinity of 25 units which is considered optimum for hybrid growth (Mapenzi and Mmochi, 2016b). They were acclimated to the salinity for 13 days with an increase of 2 units per day and 1 unit on the last day. Each treatment was replicated three times making a total of 21 tanks including controls. *L. plantarum* and *S. cerevisiae* were added to basal feeds at 2, 4 and 6 g/kg feed respectively. The 40 % crude protein feed consisted of cassava flour, maize flour, shrimp and fish meals, sunflower seed cake and premix. Feed formulation was carried out by weighing each component followed by thorough mixing. Feed pellets were prepared using a meat mincer machine. Hybrids were fed at 5 % body weight twice a day. Feeding adjustment was effected depending on fish biomass increment. Control groups were not fed diets with probiotic additives.

Fish sampling using a locally made scoop net was carried out by taking any five individuals from each tank after every two weeks. The fish were sedated using a 2 ml clove oil dose in 20 L of water prior to measurements, and were returned into tanks soon after measurements that took a maximum of 10 minutes. Total weight and length of each hybrid were measured using a BOECO BEB 61 digital balance and a wooden measuring board respectively, for determination of the length-weight relationship. The hybrids were kept unfed for 24 hours prior to sampling to reduce metabolic activities that would increase stress on the fish. Weekly replacement of 20 % of the water and siphoning of water sediments twice a week were practiced to control water quality in tanks. The pH and temperature were measured using a HI8424 pH meter, salinity with a DMT-10 Digital Hand-Held Marine Tester, and Dissolved Oxygen (DO) with a PDO-520 Dissolved Oxygen Meter each morning and evening. This study took 63 days for completion.

Analytical design

Growth performance of hybrids

The following fish growth parameters were established to determine the growth performance of the hybrids:

Specific Growth Rate (% day^{-1}) = $\left[\frac{\ln Wf - \ln Wi}{T}\right]$ x 100.......1

Food Conversion Ratio (FCR) =
$\frac{Total\ feed\ intake\ by\ fish\ (grammes)}{Total\ weight\ gain\ by\ fish\ (grammes)}$ 2

Survival Rate (%) = $\left[\frac{Nf}{Ni}\right]$x 100..3

Weight Gain (g) = $W_f - W_i$..4

Where T is the number of days of the experiment, W_i and W_f are the initial and final mean body weights, and N_f, N_i are the numbers of harvested and stocked fish, respectively.

The coefficient of condition $(K) = \frac{W}{L^3}$ x 100 (Pauly, 1983 5

Where W = Weight of individual fish (g), L = Total length of individual fish, K = condition factor

Length-weight relationship was calculated as W = a L^b (Ricker, 1978..6

which was transformed into common logarithm as log W = log a + b.log L 7

Where W = Weight of fish in gram (g), L = Total length of fish in centimeters (cm), a = proportionality constant, b = the value obtained from the length-weight equation/coefficient of regression.

Statistical analysis

Data on fish growth were pooled for each probiotic treatment. The homogeneity of variance and distribution of data were tested using Levene's and Kolmogorov–Smirnov tests respectively. The data were normally

Table 1. Average water quality parameters (SC_{1-3}) denotes *S. cerevisiae* supplemented feeds at 2, 4 and 6 g/kg levels respectively).

Probiotic Levels g/kg Feed				
Parameters	**Control**	**SC1**	**SC2**	**SC3**
Temp (°C)	27.28±0.12	27.23±0.12	27.21±0.12	27.25±0.12
DO (mg/L)	6.33±0.05	6.29±0.05	6.2±0.05	6.27±0.05
pH	7.29±0.05	7.2±0.03	7.18±0.03	7.2±0.04

Table 2. Average water quality parameters (LP_{1-3}) represents *L. plantarum* supplemented feeds at 2, 4 and 6 g/kg levels respectively).

Probiotic Levels g/kg Feed				
Parameters	**Control**	**LP1**	**LP2**	**LP3**
Temp (°C)	27.28±0.12	27.17±0.12	27.24±0.12	27.31±0.12
DO (mg/L)	6.33±0.05	6.45 ±0.05	6.39±0.03	6.26 ±0.06
pH	7.29±0.05	7.27±0.03	7.31±0.03	7.2±0.04

distributed and showed similarity of variance. Therefore, one-way ANOVA was conducted to analyze the data in Origin 9 software. The probiotic experiments were conducted separately hence the need for one-way ANOVA analysis. The Tukey test ($p < 0.05$) was used to identify the groups that showed significant differences. A t-test was conducted to determine the conformation of hybrids to the cube law. The length-weight relationship and condition factor (K) were calculated, and feed conversion efficiency determined.

Results

Water quality parameters

There was no significant difference in temperature observed among all treatments ($p > 0.05$, Tables 1 and 2). Furthermore, DO and pH did not differ significantly among treatments ($p > 0.05$, Tables 1 and 2) despite a slightly higher DO at LP_1. Water quality in the controls did not show any significant difference. In that regard, all water quality parameters were in acceptable ranges for fish farming.

Table 3. Results of one-way ANOVA for growth parameters of hybrids treated with *S. cerevisiae.*
[2]Superscripts "*a*, *b* and *c*" in a row indicate significant variations among treatments with control inclusive ($p < 0.05$, Tukey test). [1]The SC_{1-3} levels refer to 2, 4 and 6 g/kg of *S. cerevisiae*.

Probiotic levels g/kg Feed					
Parameters	**Control**	**SC1**	**SC2**	**SC3**	**P**
Initial mean weight (g)	$16.85±0.15^a$	$15.61±0.16^a$	$15.37±0.15^a$	$17.17±0.18^a$	0.07
Final mean weight (g)	$58.03±2.9^a$	$58.5±2^a$	$68.05±2.5^b$	$72.85±1.9^c$	0.01
Weight Gain (g)	$42.11±4.36^a$	$42.26±4.45^a$	$51.58±4.79^b$	$53.86±4.18^b$	<0.001
Survival rate (%)	$90±0.2^a$	$91±0.8^a$	$93±0.8^a$	$94±1.7^a$	0.1
FCR	$1.2±0.08^a$	$1.2±0.08^a$	$0.73±0.06^b$	$0.68±0.04^b$	0.02
SGR (%/day)	$2.0±0.14^a$	$2.1±0.14^a$	$3.2± 0.13^b$	$3.5± 0.11^b$	0.02

Table 4. One-way ANOVA hybrid growth parameters results when treated with *L. plantarum*. [2]The superscripts "*a*, *b* and *c*" across rows specify significant differences among treatments ($p < 0.05$, Tukey test). [1]The levels $LP_{1\text{-}3}$ refer to of 2, 4 and 6 g/kg treatments.

	Probiotic levels g/kg Feed				
Parameters	**Control**	**LP_1**	**LP_2**	**LP_3**	**p**
Initial mean weight(g)	16.85±0.15^a	15.99±0.16^a	16.47±0.19^a	18.99±0.17^a	0.06
Final mean weight (g)	58.03±2.9^a	59.19±1.7^a	69.52±2.4^b	75.03±1.7^c	<0.001
Weight Gain(g)	42.11±4.36^a	41.59±3.82^a	54.15±4.7^b	57.86±4^b	0.03
Survival rate (%)	90±0.2^a	92±1.6^a	93±1.7^a	93±0.8^a	0.06
FCR	1.2±0.08^a	1.9±0.07^a	0.94±0.63^b	0.66±0.03^b	0.01
SGR (%/day)	2.0±0.14^a	2.08±0.12^a	3.44±0.13^b	4.2±0.1^b	0.02

Growth performance

Initial mean weight did not differ significantly among the tested feeds ($p > 0.05$, Tables 3 and 4). Final average weight showed a significant variation in both probiotics ($p < 0.05$, Table 3, 4). The *S. cerevisiae* 2 g/kg feed final weight and the control differed significantly from the 4 and 6 g/kg feed, respectively (Table 3). Similarly, for *L. plantarum* the 4 and 6 g/kg treatments showed significant deviation from the 2 g/kg and control (without probiotics) treatments in terms of average final weights (Table 4). The weight gains at 4 and 6 g/kg treatments were observed to differ significantly from 2 g/kg and the control in *S. cerevisiae,* respectively (Table 3). Despite slightly higher values in survival rates for the 4 and 6 g/kg treatments in *S. cerevisiae* and *L. plantarum*, survival was not significantly affected by treatment diets, including control (Table 3, 4). Hybrid weight increment after treatment with *S. cerevisiae* and *L. planatrum* are shown in Figures 2 and 3 respectively.

Length-weight relationship (LWR)

The hybrid length-weight relationship (LWR) showed negative allometric growth among all treatments (Figs. 4 and 5). Results of the present study indicate that hybrids fed on *S. cerevisiae* and *L. plantarum* probiotics showed allometric growth. Their "b" values were 2.4 for all *S. cerevisiae* treatments while the values ranged from 0.4-2.4 across *L. plantarum* treatments.

Condition factor (K)

The average "K" values were similar across all *S. cereviasae* treatments, ranging from 2.1-2.2. The values ranged from 2.2-2.5 for *L. plantarum* treatments. The control experiment showed clear similarity to *S. cereviasae* for average "K" values. None of the values

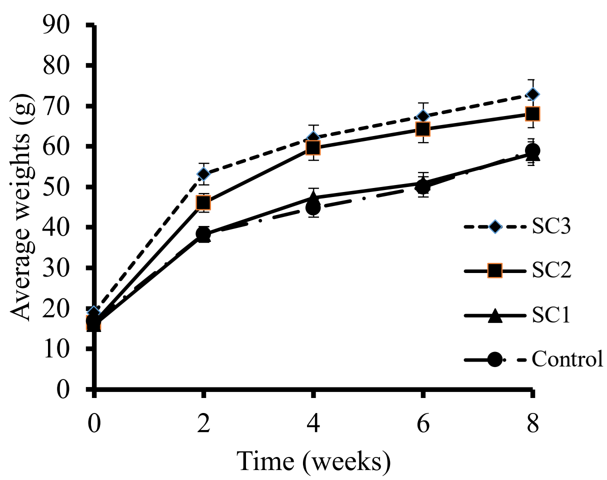

Figure 2. Weight increase of hybrids treated with *S. cerevisiae* probiotics. SC1-SC3 refers to 2, 4 and 6 g/kg, while Control denotes 0 g/kg.

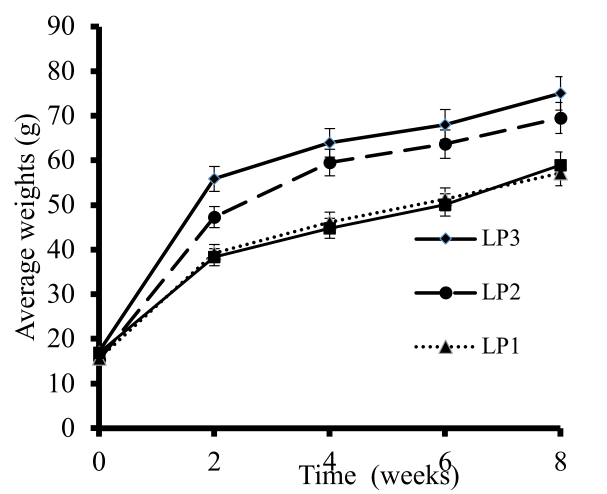

Figure 3. Weight increase in hybrids treated with *L. plantarum* probiotics. LP1-LP3 refers to 2, 4 and 6 g/kg, while Control denotes 0 g/kg.

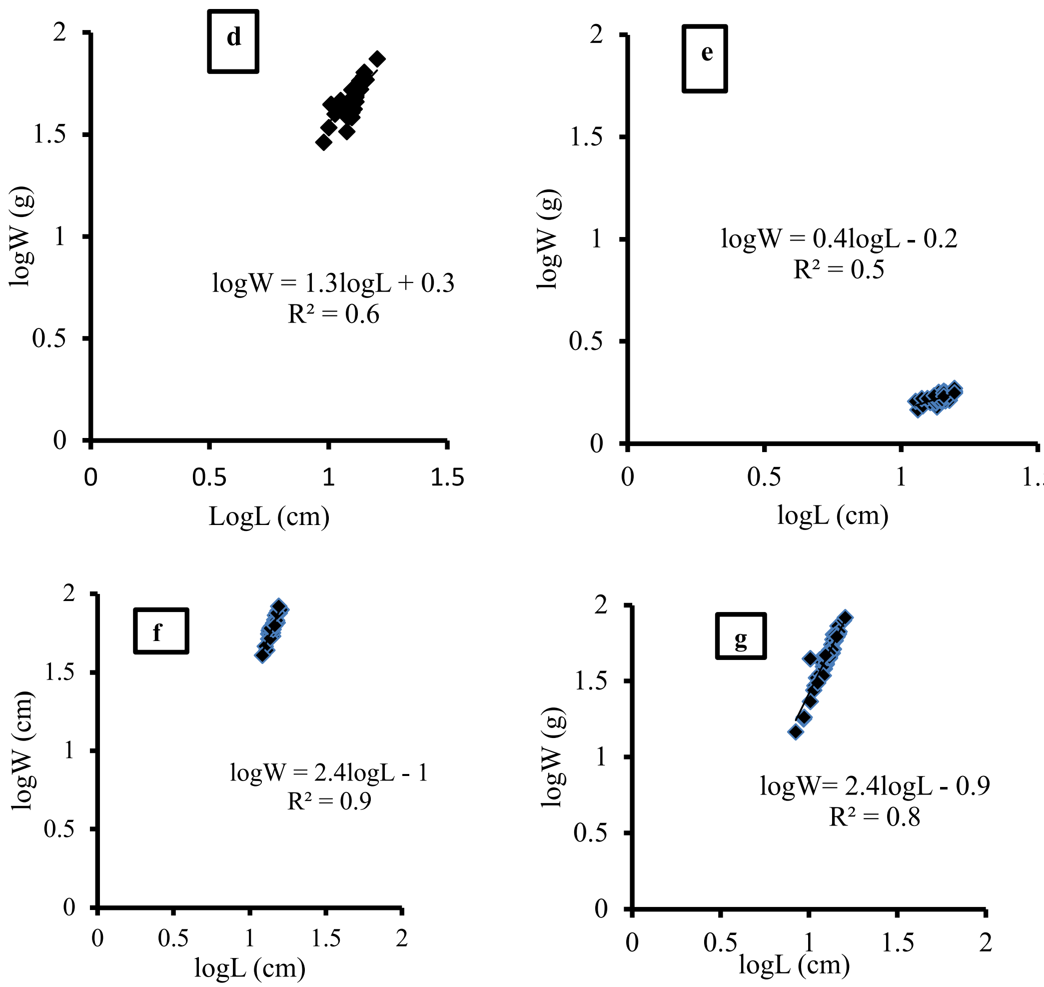

Figure 4. LWR of hybrids treated with (d) 2g, e 4g, and (g) 6 g/kg of *L. plantarum*, and (f) control, respectively.

showed any significant variation within and among treatments (Table 3). The "b" values were not significantly different from the isometric 3 value ($p > 0.05$, t-test).

Discussion

Administration of probiotics as a fish dietary supplement may be a strategy to improve immune response and growth performance. The present study has investigated the latter, which seems to vary with both probiotic and target species. A study on the influence of *S. cerevisiae* on *O. niloticus* has, for example, shown improved growth performance (Osman *et al.*, 2010; Welker and Lim, 2011; Ozório *et al.*, 2012).

However, Abraham *et al.* (2008) reported a negative influence of commercial probiotics on the ornamental fish, *Carassius auratus*. Results of the present study agree with Osman *et al.* (2010) who similarly reported a significant increase in growth performance (de Rodrigáñez *et al.*, 2009) and stimulation of FCR and weight gain in *O. niloticus* fed on higher levels of *S. cerevisiae*. In this study, the best final mean weight, specific growth rates (SGR), weight gain, survival rates and FCR were observed at 6 g/kg of both *S. cerevisiae* and *L. plantarum* while control groups had the lowest growth. Regarding probiotic roles in influencing growth, Tovar *et al.* (2002) associated yeast inclusion in fish feeds with enhancement of amylase secretion in the fish gut. Tawwab *et al.* (2008) links the live yeast addition to fish feeds with improved protein and diet digestion as well as feed utilization.

Probiotics seem to improve digestion through production of digestive enzymes (Welker and Lim, 2011). It is possible that *L. plantarum* and *S. cerevisiae* in the present study enhanced the hybrid growth performance through similar mechanisms. Intestinal digestion and absorption enhancement depend on provision of digestive enzymes like proteases and lipases (Ramirez and Dixon, 2003). Therefore, enhanced FCR and fish growth may be due to increased appetite and improved feed digestibility (Osman *et al.*, 2010). The same may have

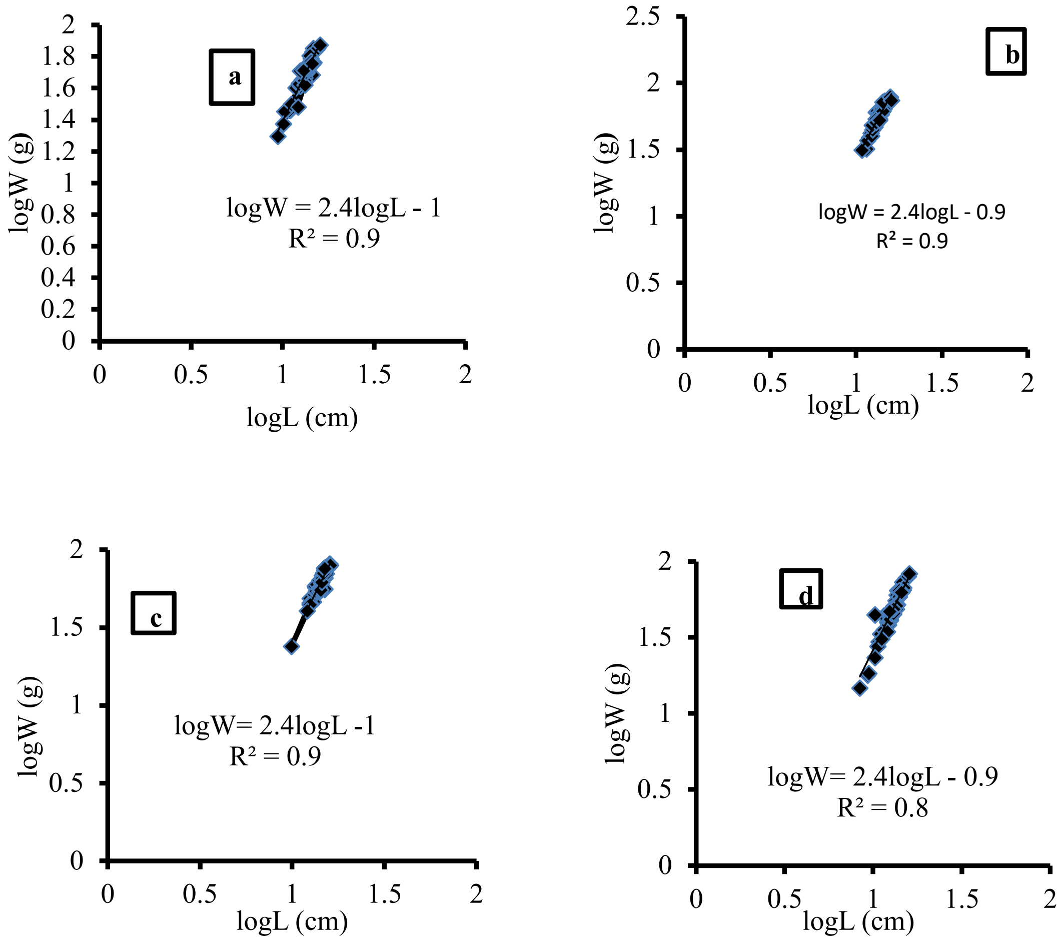

Figure 5. LWR of hybrids treated with (a) 2g, (b) 4g, (d) 6 g/kg of *S. cereviasie*, and (c) control, respectively.

accounted for the results of the present study where hybrids where fed on *L. plantarum and S. cerevisiae*. Improved hybrid growth may also be due to production of vitamins, dietary detoxification and breakdown of complex feed components (Irianto and Austin, 2002).

Talpur *et al.* (2013) showed that *L. plantarum* improves water quality in crab farms. The findings concur with the present results in which water quality parameters were in supportive ranges for fish growth, perhaps as a consequence of the presence of *L. plantarum* and *S. cerevisiae* probiotics. Nevertheless, probiotics did not lead to significant environmental quality improvement in brackish water used to rear monosex Nile tilapia (Khatun and Saha, 2017). The inhibitory capacity of *Bacillus* species (Maia *et al.*, 2013) and yeast (Lukwambe *et al.*, 2015) probiotics to harmful bacteria and algae development have been shown in shrimp ponds. The same might have led to superior growth performance of hybrids fed on *L. plantarum and S. cerevisiae* probiotics in tanks in this study.

A significant influence of both *L. plantarum* and *S. cerevisiae* was realized in SGR, FCR, weight gain and average final weight among the treatments. These results correspond to Tawwab *et al.* (2008) who reported the significant effect of *S. cerevisiae* supplemented feeds on weight gain and SGR in tilapia. Conversely, the treatments did not vary significantly from each other at 4 and 6 g/kg feed levels. SGR rates improved as *L. plantarum* and *S. cerevisiae* levels were raised in the feeds. This suggests that the SGR of hybrids was dependent on levels of probiotic supplements in feeds. Therefore, both *L. plantarum* and *S. cerevisiae* may have provided similar growth promoting factors to hybrids in this study. Consequently, *S. cerevisiae* can be used as a replacement to both live food and fish meal (Oliva-Teles and Gonçalves, 2001) to improve SGR of the hybrids.

FCR varied significantly between controls, and the 4 and 6 g/kg feed levels for both *L. plantarum* and *S. cerevisiae* respectively. Similar results were documented for tilapia fed on 40 % crude protein diets supplemented with *S. cerevisiae* (Flores *et al.*, 2010). Similarity in protein content of the basal feed in this study may also explain significance in FCR for the hybrids. Live yeast supplementation enhances food utilization and nutrient decomposition in the gastro-intestinal tract (Wang and Xu, 2006; Tawwab *et al.*, 2008), and improves protein digestion (Flores *et al.*, 2003) in tilapia. Furthermore, dietary probiotic additives supply essential nutrients (Welker and Lim, 2011). All these factors could explain the better FCR obtained for hybrids in the present study, as *L. plantarum* could be working through similar mechanisms as *S. cerevisiae*. However, there was no significant influence of the 2 g/kg feed which indicates that this level may be too low to have any effect.

Studies by Flores *et al.* (2003) and Mohammadi *et al.* (2016) showed that yeast probiotics improve survival in both hybrid striped bass and Nile tilapia. The findings corresponded with the results of the present study where survival increased with an increase in *L. plantarum* and *S. cerevisiae* levels. Similar survival rates (90.67±1.15 %) were reported for *Penaeus monodon* in Bangladesh (Hossain *et al.*, 2013).

LWR studies are used to indicate fish wellbeing and how the fish interacts with its environment (Mansor *et al.,* 2012). In addition, these studies are widely used in fisheries management because they can contribute to explaining stock condition. Further, the "b" value in the regression equation specifies heavy, light and isometric fish growth (Ricker, 1973; Mansor *et al.*, 2010) where "b" < 3 and "b" > 3 experience both negative and positive allometric growth (Weatherley and Gill, 1987; Mansor *et al.*, 2010), respectively. According to Mansor *et al.* (2012), the condition factor "K" indicates favourability of the environment in which the fish reside. Therefore, higher "K" values indicate that the environment is more favourable for fish rearing.

Results of the "b" values reported for Nile tilapia (Offem and Omoniyi, 2007) in Nigeria were similar to results from the present study, which showed negative allometric growth (Ricker, 1973; Silva *et al.*, 2015). However, they differ from the values of 2.908 (Britton and Harper, 2008) and 3.415 (Ahmed *et al.,* 2011) for Nile tilapia from Atbara River and Khashm El-Girba reservoir. The difference may be due to geographical location variances. In addition, hybrids were reared in saline water tanks in the present study. Hybrids of Nile tilapia♀ and Rufiji tilapia♂ grow well in all water salinities ranging from 0-35 units with the best growth rates between 15-25 units (Mapenzi and Mmochi, 2016b). Negative allometric growth means their bodies did not grow proportionally (Silva *et al.*, 2015). The allometry may also be affected by differences in oxygen supply in tanks. Moreover, fish with b < 3 are slender (Silva *et al.*, 2015) and have extended bodies (Otieno *et al.*, 2014). This was observed for the hybrids in the present study in which the "b" values for fish fed with *S. cerevisiae* and *L. plantarum* were below the isometric value of 3. Therefore, the cube law was not obeyed by the hybrids; similar to the results of Otieno *et al.* (2014).

The "K" values of the hybrids in this study are similar to the ranges of 1.97-2.38 for males and 2.02-2.63 for females found in Nile tilapia in Lake Naivasha, Kenya (Otieno *et al.*, 2014). Ighwela and Ahmed, (2011) recommended that "K" values greater than 1 indicate that the fish are in good condition. Furthermore, good "K" values can be attributed to better feeding intensity (Ndimele *et al.*, 2010) and water quality parameters (Musa *et al.*, 2016). This can also explain better "K" values for hybrids obtained in this study.

Conclusions

The inclusion of *L. plantarum* and *S. cerevisiae* had a significant influence on growth performance of the hybrids in the present study. The probiotic dietary supplementation may constitute a valuable approach for enhancing better fish growth in both semi-intensive and intensive farming systems where stress related diseases and fish losses are prevalent.

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