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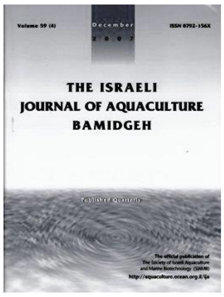
ISSN 0792 - 156X

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

The Israeli Journal of Aquaculture - Bamidgeh, IJA_67.2015.1149, 8 pages



Effect of Feeding Rate on Growth Performance, Feed Utilization, and Blood-Chemistry Indicators of Nutritional Status in Juvenile Gift Strain Tilapia (*Oreochromis Niloticus L.*)

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(Received 7.8.2014, Accepted 23.10.2014)

Key words: GIFT tilapia; feeding rates; growth performance; blood parameters; physiology

Abstract

This study investigated the effect of feeding rates (2-10% of body weight), on the growth performance, feed utilization, and blood-chemistry indicators of nutritional status in juvenile GIFT strain tilapia, (*Oreochromis niloticus)* initial weight 72.73±0.15g. The 56 day trial conducted in cages tested five groups, with three replicates each. Fish were fed twice a day. Data were analyzed using one way ANOVA. Results showed that final body weight, food conversion rate (FCR), weight gain, specific growth rate (SGR), protein retention efficiency (PRE), protein efficiency ratio (PER), feed intake, and condition factor (CF) were significantly affected by the feeding rates ($P<0.05$). The broken line method of linear regression analysis, $y=17638x-217.9$, $R^2=0.945$; $y=280x+860.6$, $R^2=1$, indicated that maximum weight gain occurred at 6.21% feeding rate. Insulin growth factor-one (IGF-I) level, hepatosomatic index (HSI), white blood cell (Wbc), red blood cell (Rbc), hemoglobin (Hb), and hematocrit (Ht) parameters were not significantly affected by the feeding rates ($P>0.05$). Serum indicative parameters of liver-enzyme functions and food metabolism, including blood glucose (GLU), triglyceride (TG), total protein (TP) levels, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzyme activities were assayed. Blood glucose level of the fish was significantly affected by the feeding rates ($P<0.05$). TG and TP levels; AST, ALT and ALP activities were not significantly affected by the feeding rates ($P>0.05$). Feed provided at a rate of 2%-10% of body weight may meet physiological wellbeing of GIFT strain tilapia, however growth performance and other endocrine functions (IGF-I) are affected by lower and higher feeding rates. Our findings could be very useful to tilapia farmers.

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Introduction

One problem facing fish culturists is the need to obtain a balance between rapid fish growth and optimum use of the supplied feed (Aderolu et al. 2010), since fish production is closely related to feed quality, feeding regime, and the rearing environment (Yousif 2002; Chakraborty and Banerjee 2009; Aderolu et al. 2010; Qiang et al. 2012a). Fish growth is governed by genetics, nutritional status, and environmental conditions the effects of which are integrated through a complex endocrine mechanism that directly or indirectly involve several hormones (Li et al. 2006; Correa and Cerqueira 2007; Qiang et al. 2012a). Feeding rates have been identified as an important factor affecting growth, in intensive and extensive aquaculture systems (Lovell, 1998; Abdel-Hakim et al. 2009).

Feeding rates, growth, and food conversion are major variables in commercial aquaculture (Mensah and Attipoe, 2013). In general, tilapia are easily cultured and highly adaptable to a wide range of environmental conditions. In the United States, the most commonly farmed tilapia species are Nile (*Oreochromis niloticus*), Mozambique (*O. mossambicus*), blue (*O. aureus*), and hybrids (Green, 2006). In China, increased culture of GIFT (Genetically Improved Farmed Tilapia) strain is due mainly to its many advantages including rapid growth rate, and disease resistance (Qiang et al. 2013b). In aquaculture the quality and quantity of feed supplied can have a significant effect on aquatic animals and their environment. Protein requirements for tilapia decrease with age and size with higher dietary crude protein concentrations (30-56%) required for fry, and (30-40%) for juvenile tilapia, and lower protein levels (28-30%) for larger tilapia (Winfree and Stickney, 1981; Jauncey, 1982; Siddiqui et al., 1988; Twibell and Brown, 1998; Al Hafedh, 1999). Feeding regime studies on fish including tilapia, have been mainly devoted to tank and pond based systems (Rakocy, 1989; Yousif, 2002; Riche and Garling, 2003; Sumagaysay-Chavoso, 2007; Aderolu et al., 2010), without any attempt to correlate endocrine, liver, and blood indicative constituents that are connected to the growth and wellbeing of the fish. Moreover, little is known of the effects of feeding rates on growth and feed utilization of GIFT strain tilapia under conditions of growth in cages.

Since fish feed comprises the largest expense in aquaculture production, and feeding rate is an important factor affecting fish growth, determination of optimal feeding rate is imperative to the success of any aquaculture operation (Marimuthu et al. 2011). The objective of the present study therefore was to assess the effects of feeding rates on growth performance, feed utilization, and hematological and serum indicative parameters of liver enzyme-function responses in GIFT tilapia juvenile grown in cages.

Materials and Methods

Fish and acclimatization process. Healthy GIFT tilapia juveniles, bred at the Yixing farm of the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences in China, were tested in this trial. Prior to the commencement of the experiment, the fish were acclimatized for a month in a 12m^2 concrete tank. During the acclimation process, continuous aeration was ensured and fish were fed three times daily to near satiation with floating feed (35% crude protein) to attain the desired size for the experiment.

Experimental design and management. Fifteen cages (1m^3) made of nylon mesh fabric, were stretched and immersed to a depth of 0.5m in a large concrete pool (390m^3 water, depth of 0.8m and stocked with the acclimated GIFT tilapia juveniles. MANOVA (Multiple Analysis of Variance) results for initial stocking weight (72.73±0.15) showed that there was no significant difference (P>0.05) between the weight of the fish in the treatment groups or replicates (Table 2).

The fish (80 per cage) were fed the experimental diet (Table 1), in triplicate at a feeding rate of 2%, 4%, 6%, 8% and 10% body weight, corresponding to F1, F2, F3, F4 and F5 respectively. The animals were fed the prescribed rations twice a day; (08:00h and 17:00h), 7 days a week, for two months. The experiment was carried out in the open air under natural photoperiod (12hr light:12hr dark) at Yixing farm. Fish were sampled on a biweekly basis, for readjustment of quantity of feed. The total amount of feed consumed was subsequently calculated as summation of given feed during the experimental period. Water quality parameters were monitored daily during the feeding trial; temperature ranged from 28-29 °C, dissolved oxygen ranged from 8.0-6.0 mg/L, and ammonia nitrogen ranged from 0.02-0.05 mg/L.

Table 1. Approximate food composition of experimental diet*
(*Tian Bang Freshwater fish feed industry, Ningbo, China.)

Items	*%/Kg (dry feed)*
Crude protein	40
Fiber	8.0
Ash	18
Moisture	12
Calcium	1.0
Total phosporus	1.0
NaCl	3.0
Lysine	2.1

Sample collection and analytical methods. At the end of the 8-week feeding trial, fish in each cage were individually weighed and sampled for tissue analysis 24 hr after the last feeding. Three fish from each replicate cage (9 fish per treatment) were used for whole body composition analysis. Blood samples were drawn from the caudal vein of 9 fish per treatment (3 fish per replicate cage) with heparinized needles. Whole blood was used for white blood cell count, red blood cell count, hematocrit, and hemoglobin content measurement, and blood serum separated after centrifugation (3000×g, 15 min, 4°C). Red blood cells, white blood cells, hemoglobin and hematocrit, were then measured using Auto Hematology Analyzer (BC-5300Vet, Mindray, P.R. China), with test kit bought from Shenzhen Mindray Medical International Co. Ltd. in China.

Blood serum was used for insulin-like growth factor I (IGF-I), glucose (GLU, triglyceride (TG), total protein (TP) levels; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activity analysis. All the samples were quick frozen and kept at -80°C until analysis. Insulin-like growth factor I (IGF-I) levels were measured by homologous radioimmunoassay (RIA), according to Qiang et al. (2012b), using test kits bought from Shanghai Lengton Bioscience Co., Ltd. in China. Serum glucose (GLU), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities, triglyceride (TG), and total protein levels (TP), were measured by colorimetric method, using Mindray Auto Bio-chemical Analyzer (BS-400, Mindray, P.R. China) and test kit from Mindray Bio Medical Co., Ltd. in China.

Crude protein, crude lipid, moisture, and ash in whole body samples were determined following standard methods (AOAC, 1995). Crude protein (N×6.25) was determined by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (1030-Auto-analyzer, Tecator, Hoganos, Sweden). Crude lipid was determined by the ether-extraction method using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Moisture was determined by oven drying at 105 °C until a constant weight was achieved. Ash content was measured after placing samples in a muffle furnace at 550 °C for 24 h.

Calculations and statistical analysis: The parameters for assessment of growth performance were calculated thus:

Feed intake=weight gain/feeding efficiency.

Survival (%) =100 × Final number/Initial number

Weight gain (WG %) $=100\times(W_f - W_I)/W_I$

where, W_I is the initial weight, and Wf is the final weight

Specific growth rate; SGR (%/d) = $[(\ln W_2 - \ln W_1) / (t_2 - t_1)]\times 100$

Where, W_1 and W_2 are body weights (g) at starting (t_1) and ending time (t_2)

Feed conversion ratio (FCR) = dry feed fed (g)/ Wet weight gain (g)

Protein efficiency ratio (PER) = Wet weight gain (g)/protein intake (g)

Protein retention efficiency (PRE) = 100 × protein gain (g) / protein intake (g)

Hepatosomatic index (HSI %,) = 100 x liver weight (g) /body weight (g)

Condition factor (CF) = $[(W/L^3) \times 100]$, where W is the wet weight of the fish and L is the standard length.

Results were expressed as mean±SD. Data were subjected to one-way analysis of variance. When significant differences occurred, the group means were further compared with Duncan's multiple-range tests. All statistical analyses were performed using the SPSS 19 (SPSS, IL, USA).

Results

Growth performance and feed utilization of GIFT juvenile tilapia fed graded levels (2-10%) for 56 days are shown in Table 2. Results showed that final body weight, FCR, weight gain, SGR, PRE, PER, feed intake, and CF were significantly different (*P*<0.05) among the various rationed groups. Final body weight, FCR, weight gain, SGR, feed intake, and CF, tended to increase with increased feeding rate (*P*<0.05). Serum IGF-I and HSI tended to increase with increased feeding rate; but no significant differences (P>0.05) were observed among the various rationed groups. On the contrary, PRE and PER significantly decreased with increased feeding rate (*P*<0.05). Percentage survival also decreased with increased feeding rate, but no significant differences were observed among the groups (P>0.05). The relationship between weight gain and feeding rate was best expressed by the broken line method using linear regression analysis ($y = 17638x-217.9$, $R^2 = 0.945$; $y=280x+860.6$, $R^2=1$) indicating that the maximum weight gain occurred at a feeding rate of 6.21% of body weight of dry feed. (Fig.1). The poorest FCR (1.87), the lowest PRE (22.15) and PER (1.34) were observed in fish fed dry feed at a rate of 10% of body weight. Mean percentage survival was generally high in all treatments, ranging from 93.3-98.3% and was not affected by feeding rates (*P*>0.05).

Table 2. Effect of feeding rates on growth and serum IGF-I levels in GIFT tilapia juveniles

Item	*Feeding Rate*				
	2%	*4%*	*6%*	*8%*	*10%*
Initial body weight (g)	72.79±0.13	72.78±0.08	72.85±0.04	72.74±0.10	72.73±0.15
Final body weight (g)	206.6±9.29[a]	356.2±2.05[b]	492.3±28.8[c]	482.2±14.7[c]	486.9±17.7[c]
FCR	0.90±0.05[a]	1.10±0.07[b]	1.03±0.01[b]	1.41±0.07[c]	1.87±0.01[d]
Weight gain(g)	183.9±12.6[a]	389.4±12.3[b]	575.8±39.2[c]	562.8±19.5[c]	569.5±25.8[c]
Survival (%)	98.3±1.44	96.7±1.44	93.3±5.20	94.2±2.89	93.3±2.89
SGR(%/day)	1.49±0.06[a]	2.27±0.01[b]	2.73±0.08[c]	2.70±0.04[c]	2.72±0.05[c]
PRE	46.78±2.72[d]	37.38±2.48[c]	39.99±0.38[c]	28.53±1.53[b]	22.15±0.15[a]
PER	2.78±0.1[d]	2.28±0.15[c]	2.44±0.02[c]	1.78±0.10[b]	1.34±0.01[a]
Feed intake (g/fish)	109.5±0.12[a]	286.2±17.40[b]	393.3±24.23[c]	528.3±21.04[d]	706.3±25.05[e]
IGF-I (ng/ml)	11.92±3.16	11.96±3.52	13.80±3.45	13.01±3.98	13.21±2.98
CF	3.01±0.22[a]	3.13±0.19[ab]	3.26±0.17[b]	3.27±0.18[b]	3.28±0.17[b]
HSI	3.11±0.32	3.18±0.30	3.19±0.32	3.15±0.37	3.12±0.38

[a]Values are presented as mean±SD (n=3); values with different superscripts in the same row differ significantly(*P*<0.05); values without different superscripts in the same row do not differ significantly（*P*>0.05）FCR, feed conversion ratio; SGR specific growth rate; PER, protein efficiency ratio; PRE, protein retention efficiency; IGF-I, insulin growth factor I; CF, condition factor; HSI, Hepatosomatic index. Total number of fish per group (N) =80.

The proximate composition of whole body of GIFT strain tilapia is presented in Table 3. No significant differences (*P* >0.05) were found in the whole body moisture, protein, lipid, and ash contents of the fish in all treatment groups.

Table 3. Effect of feeding rates on whole body composition of GIFT tilapia juveniles

Item	*Feeding Rate*				
	2%	*4%*	*6%*	*8%*	*10%*
Mositure (%)	73.8±2.14	72.3±0.65	72.1±1.45	72.5±0.67	72.4±0.84
Protein (%)	15.7±0.83	16.5±0.76	16.9±0.51	16.8±0.43	16.70±0.46
Lipid (%)	7.89±1.26	7.27±0.13	7.32±1.63	7.43±0.52	7.41±0.41
Ash (%)	3.56±0.21	3.24±0.42	3.19±0.34	3.28±0.24	3.48±0.20

[a]Values are presented as mean±SD (n=3); values without different superscripts in the same row do not differ significantly (*P*>0.05). Total number of fish per group (N) =80.

White blood cell count, red blood cell count, hemoglobin and hematocrit content of GIFT tilapia fed graded levels of the experimental diet are presented in Table 4. Hematological parameters were not significantly affected by the predetermined feeding rates (P>0.05); however, the lowest level of white blood cell count was observed in F3.

Table 4. Effect of feeding rates on hematological parameters of GIFT tilapia juveniles

Item	*Feeding Rate*				
	2%	*4%*	*6%*	*8%*	*10%*
Wbc（1×10^9/L)	187.54±16.82	193.13±17.32	186.57±14.30	192.18±15.27	191.18±15.27
Rbc (1×10^{12}/L)	1.84±0.34	1.86±0.19	1.82±0.22	1.83±0.30	1.81±0.30
Hb(g/L)	96.44±10.70	98.56±14.82	100.33±7.31	95.66±11.23	93.56±13.03
Ht (%)	33.24±5.06	35.92±6.18	34.77±5.26	35.60±5.48	36.30±4.86

[a]Values are presented as mean±SD (n=3 values without different superscripts in the same row do not differ significantly (*P*>0.05). Wbc=White blood cell; Rbc=Red blood cell; Hb=Hemoglobin; Ht=Hematocrit. Total number of fish per group (N) =80.

Glucose (GLU), triglyceride (TG), total protein, aspartate aminotransferase (AST), alanine aminotransferase(ALT), alkaline phosphatase (ALP) level, and activity of GIFT strain tilapia apportioned the experimental diet, are presented in Table 5. The results show that blood glucose (GLU) level was significantly affected by feeding rate (P<0.05). The highest and lowest levels of blood glucose were observed in F1 and F5 respectively. There was no significant difference (P>0.05) among the treatment groups for levels of triglycerides, (TG), total protein (TP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) enzyme activities.

Table 5.Effect of feeding rates on serum indicative parameters of liver function, protein and energy metabolism in GIFT Tilapia juveniles

Item	*Feeding Rate (%)*				
	2%	*4%*	*6%*	*8%*	*10%*
GLU(mmo/L)	5.46±1.72[c]	5.30±0.77[c]	4.15±0.83[b]	2.60±0.33[a]	2.51±0.33[a]
TG (mmo/L)	2.43±0.48	2.45±0.64	2.41±0.53	2.59±0.67	2.58±0.55
TP (g/L)	30.92±3.78	31.90±2.17	32.88±3.53	32.13±1.64	32.13±1.84
AST (U/L)	132.12±70.9	120.37±38.8	131.87±40.3	127.40±13.4	136.42±14.1
ALT(U/L)	6.28±6.09	5.27±2.43	5.48±4.98	5.75±2.12	5.87±2.22
ALP(U/L)	33.23±4.61	31.78±6.76	29.87±4.94	32.92±3.54	31.92±3.64

Values are presented as mean±SD (n=3); values with different superscripts in the same row differ significantly(P<0.05). Total number of fish per group (N) =80.

Discussion

In the present study the final body weight, FCR, weight gain, SGR, PRE, PER, feed intake and CF of GIFT strain tilapia juvenile were significantly affected by the feeding rates. Mean weight gain and SGR of GIFT tilapia fed 2% and 4% body weight were lower than those fed 6-10%. SGR varies between and among species in relation to culture conditions (Qiang et al. 2012a). The best growth performance in this study was attained at 6-8% body weight and was confirmed by IGF-I levels in F3 and F4 groups as compared to F1 and F2 (Table 2). Insufficient feeding could have caused the poor growth performance in F1 and F2. When fish are fed to satiation, they tend not to eat again until their stomach is almost completely evacuated; hence the poor feed utilization of the F5 group. Relationship between fish final body weight and feeding rate was best expressed by broken line method of linear regression analysis, $y = 17638x-217.9$, $R^2 = 0.945$; $y=280x+860.6$, $R^2=1$; which indicated that maximum weight gain occurred at 6.21% feeding rate (Fig.1).

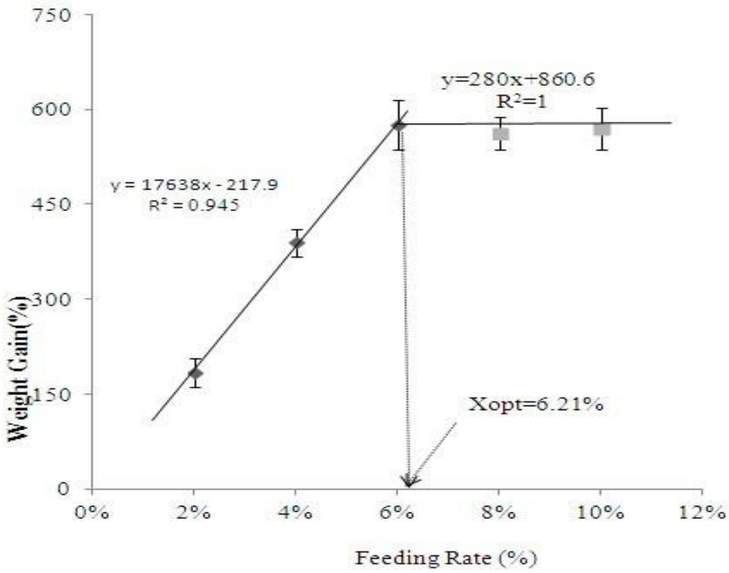

Fig. 1. Relationship between weight gain (WG) and predetermined feed rates based on broken line method of linear regression analysis, where Xopt represents the optimal feeding level for the maximum WG of GIFT strain tilapia.

The best FCR, PRE, and PER in this study were recorded for 2-6% body weight feed. FCR, PRE, and PER decreased when fish were fed dry feed at 8-10% of body weight; utilization of smaller amounts would have been more efficient. IGF-I in fish has been linked with growth (Qiang et al. 2012b); it increases in blood of temperate fish during the growing season (Mingarro et al. 2002), and is stimulated by high temperatures, dietary protein levels (Qiang et al. 2012b) and photoperiod (McCormick et al. 2007). In the present study the faster growing group had higher IGF-I levels: 6-8% body weight feed per day for 50-100g monosex tilapia (on average 7%; 75g) was recommended (Roys, 2014). This was close to the optimal feeding rate (6.21%; 73%) for mixed-sex GIFT strain tilapias. The differences could be attributed mainly to the differences in tilapia strains and the culture environment. Whole body composition of GIFT strain tilapia was not affected by the feeding rates in this study.

The health status of fish is reflected in blood parameter levels such as red blood cells, white blood cells, hemoglobin, and hematocrit (Kpundeh et al. 2013). Hematological parameters of GIFT strain tilapia juveniles were not significantly affected by the feeding rates (Table 4) and were within the range reported for healthy juvenile Nile tilapia, *Oreochromis niloticus* (Welker et al. 2007). Blood glucose (GLU) levels of the fish were significantly affected by the predetermined feeding rates. Higher levels were recorded for fish fed lower rates of dry feed, indicating that the protein in the diet could have been converted into energy and used for metabolic activities. Levels of TG, TP and the activities of AST, ALT and ALP in fish blood were not affected by the feeding rates (Table 5), this could be due to the high protein level (40% crude protein) in the experimental diet, which further validated the optimal feeding rate (Fig. 1) and the hematology of the fish. These results support the observation that a ration of dry feed at 2% of body weight was enough to maintain normal bodily processes. Feeding GIFT strain tilapia dry feed at a 2-10% of body weight ration per day, supplies the needs for physiological wellbeing. Feeding GIFT strain tilapia juvenile 40% dietary protein diet beyond 6.21% body weight per day will incur feed wastage and poor FCR and increase production cost. This paper has demonstrated optimal feeding levels to promote physiological wellbeing and optimal growth performance for the culture of tilapia.

Conclusion

In conclusion, feeding GIFT strain tilapia 2-10% body weight of a 40% dietary protein diet can influence growth performance significantly. Growth rates of GIFT strain tilapia increased with feeding rate. Hematological parameters (red and white blood cells, hemoglobin, and hematocrit content) and serum biochemical parameters including total protein, cholesterol, and triglyceride, unlike blood glucose, were not significantly affected by the feeding rates. Fish whole body composition was also not significantly affected by the feeding rates. The optimal feeding rate for GIFT strain tilapia juveniles should be 6.21% body weight feed per day for maximum weight gain.

Acknowledgements

This study was supported by Special Fund for Agro-scientific Research in the Public Interest (200903046-02). The authors are grateful to Mr. Zhu Zhixiang for providing biological material, site, and other technical assistance. The authors are thankful to Dr. Lui Bo and his crew for their assistance in the sampling process. The authors also wish to express their appreciation to the Ministry of Fisheries and Marine Resources, Sierra Leone, for their support.

Declaration of Interest

There are no conflicts of interest related to the research study and preparation of the manuscript. The authors are exclusively responsible for any kind of conflict of interest.

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