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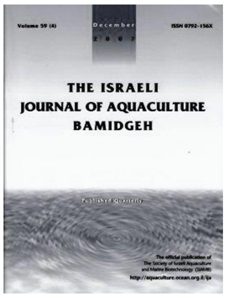

Effect of Feeding Frequency on Growth Rate, Body Composition and Gastric Evacuation of Juvenile GIFT Strain of Nile Tilapia (*Oreochromis niloticus)*

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Key words: tilapia, feeding frequency, gastric evacuation, growth benefit, body composition

Abstract

The specific objectives of this study were to determine the optimum feeding frequency in juvenile GIFT (Genetically Improved Farmed Tilapia) strain of Nile tilapia (*Oreochromis niloticus*), for growth rate, body composition, and gastric evacuation, as well as to investigate the passage of a single meal through the stomach in order to establish gastric evacuation rate. Six treatment groups of juvenile GIFT were fed to satiation with extruded feed, at 6 feeding frequencies: four, three, and two meals a day, and four meals, three, and two meals, every 2 days, for 6 weeks respectively. Each treatment (n=20) was replicated three times. As feeding frequency decreased, the growth rate of the juvenile GIFT decreased gradually. The feed intake of fish fed two meals a day was significantly lower than that of fish fed three meals a day ($P < 0.05$), whereas the feed efficiency ratio of fish fed two meals a day was significantly higher than that of fish fed three meals a day ($P<0.05$). As feeding frequency decreased, moisture content of fish body increased and the fat and protein contents decreased gradually. Hepatosomatic indices of fish fed two, and three meals a day, were not statistically different to each other or to the remaining groups. The livers of all fish were normal. Gastric evacuation of the fish was best evaluated with the square root model. It was estimated that gastric feed contents gradually decreased and reached the pre-feeding level within 15 h. Evacuation of 80% would require 9 h which appeared to correspond with the return of appetite. Our results suggest that two meals a day are optimal for growth performance of juvenile GIFT Nile tilapia.

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Introduction

Tilapias (*Tilapia*) are among the most important aquaculture species in the world exhibiting high adaptability and tolerance to a wide range of environments (Pullin, 1985). Production of farmed tilapias is over 1.2 million in China, accounting for approximately 50% of global production. GIFT strain of Nile tilapia is a genetically improved Tilapia species, and is popular because it grows quickly, and tolerates a wide range of salinity and temperatures (Ponzoni et al., 2005). It is also highly marketable and affordable.

The growth of fish at all stages is largely governed by the kind of food, ration, feeding frequency, food intake, and its ability to absorb nutrients. Feeding frequency is an important aspect for the survival and growth of fish, in the early stages (Villarroel et al., 2011; Mollah et al., 1982). Optimizing feeding regimes may improve the efficiency of production by enhancing growth and reducing feed conversion ratio (Dwyer et al., 2002). Appropriate feeding regimes may also minimize feed wastage, leading to improvements in water quality and/or reductions in size heterogeneity (Dwyer et al., 2002; Tucker et al., 2006). Poorly timed or over-feeding regimes may lead to increased cannibalism (Folkvord et al., 1993) or higher costs of production. Understanding digestion rate and its relationship to gastric evacuation rate (GER) may facilitate prediction of appetite return under a given set of conditions and diets. Demonstrating a consistent relationship between gastric evacuation and appetite return will allow prediction of optimal feeding frequency (Grove et al., 1978). Making food available at an appropriate rate corresponding to return of appetite can maximize intake and increase feed efficiency (Windell et al., 1972; Lee et al., 2000). Fish culturists can use GER and gastric evacuation time (GET) to develop appropriate feeding strategies for increased efficiency.

Most studies on feeding frequency have emphasized growth rates and food conversion efficiencies (Villarroel et al., 2011; Panagiotis et al., 2003, Hogendoorn, 1981; Tsevis et al., 1992). Some have focused on the effects of feeding frequency on the body composition, and gastric evacuation (Tekinay, 2003) but information relating to tilapia is scarce. This study was conducted to determine the optimum feeding frequency by evaluating parameters of gastric evacuation from the stomach of juvenile GIFT strain of Nile tilapia.

Materials and Methods

Experimental diet and environment. GIFT strain of Nile tilapia fry were obtained from a local hatchery (Nanning, Guangxi, China). For one week prior to the experiment, the fish were acclimated by giving them the experimental diet three times a day.

At the start of the experiment, the fish were weighed and randomly distributed into eighteen 0.8-1.0 l nylon net boxes, 30 fish/box. Mean body weight per juvenile individual was 3.7±0.2 g. Those boxes were placed in two 6*1.5*1.3m cement ponds, 9 boxes/pond. A round white feeding plate (r=8.5cm, h=3cm) was fixed in each box for feces collection. Filtered water was supplied at a flow rate of 5 l/h to each pond. The feeding trial was conducted under natural photoperiod. Water parameters included: salinity 12 ppt, temperature 30±1.5 °C, pH 7.0±0.1, ammonia nitrogen 0.56±0.01 mg/l, and dissolved oxygen level>6.0 mg/l.

Experimental diets. The experimental diets were formulated to satisfy the nutrient requirements of juvenile Tilapia. Ingredients of the experimental diets were pelleted with mechanical pressure. The different feeds were stored in a freezer at -20°C until use (Table 1).

Table 1 Experimental diet and its ingredients[1]

Ingredient	*Ratio* (%)
Wheat middling	20.0
Peanut meal	25
Rapeseed meal	12
Rice bran	10
Soybean meal	0
Crude rice bran	8.9
Peru Fishmeal	20
Soybean oil	2.1
Salt	0.1
Ca $(H_2PO_4)_2$	0.6
Choline	0.1
Min.premix.[2]	1
Vit. Premix.[3]	0.2
Nutrition Components%	
Moisture	9.2
Crude protein	36.9
Crude fat	5.5
Crude ash	9.1
Crude fiber	7.0
Carbohydrates [4]	32.4
GE [5](MJ/kg)	16.4

[1] Average of two replicates.
[2] Mineral added to supply the following (per kg diet): manganous sulphate (32.5% Mn), 50 mg; ferrous sulphate (20.1% Fe), 40 mg; copper sulphate (25.4% Cu), 5 mg; zinc sulphate (22.7% Zn), 90 mg; sodium selenite (45.6% Se), 1 mg; cobalt chloride (24.8% Co), 3.0 mg; sodium fluoride (42.5% F), 5 mg.
[3] Vitamin added to supply the following (per kg diet): vitamin A, 10000 IU; vitamin D3, 4000 IU; vitamin E, 400 IU; vitamin K3, 50 mg; thiamine HCl, 60 mg; riboflavin, 70 mg; d-Ca pantothenate, 200 mg; biotin, 2.0 mg; folic acid, 20 mg; vitamin B12, 0.20 mg; niacin, 300 mg; pyridoxine HCl, 20 mg; ascorbic acid, 300 mg; inositol, 400 mg; choline chloride, 2000 mg; butylated hydroxy toluene, 20 mg; butylated hydroxy anisole, 20 mg.
[4] Carbohydrates = 100 − (protein + lipid + ash).
[5] GE: protein=16.9 kJ/g, lipid=37.8 kJ/g, carbohydrate=16.9 kJ/g.

Growth trial and gastric evacuation. Two independent trials were performed to evaluate the probability of relating gastric evacuation to optimal feeding frequency. In a growth trial, fish in eighteen net boxes were divided into six feeding regimens. Each regimen had three replicates. Throughout the 6 week experiment, the fish were hand-fed to apparent satiation at one of six feeding frequencies: two meals a day, two meals every 2 days (at 0900 and 1800 h), three meals a day and three meals every 2 days (at 0900, 1330 and 1800 h), four meals a day and four meals every 2 days (at 0900, 1200, 1500 and 1800 h).

At the completion of the feeding experiment, the fish were not fed for 24 h. The next morning they were fed the experimental diets to visual satiety once at 0900 h. All other conditions were the same as in the feeding trial. Five fish were sampled half an hour after the 0900 feeding, and then anesthetized with ice water and sacrificed for stomach dissection. The stomach contents were removed by gentle pressure into separate pre-weighed containers, and then weighed. At hourly intervals following the 0930 sampling, the same procedure as that described above was performed and feed contents from the stomach were collected.

Analytical methods. After not being fed for 24 h, 9 fish were randomly sampled at the beginning and at the end of the feeding trial then stored at -20°C for proximate analysis of fish body. Proximate analysis of the diets and fish were determined according to the AOAC (1990) method. Livers from three randomly chosen fish in each replicate were removed and pooled to determine the liver lipid content (Folch et al,. 1957).

Performance calculations. Performance indices were calculated as follows: Feed effectiveness ratio (FER) = (fish weight gain ×100) / feed intake; Special growth ratio (SGR) = $100\times(\ln W_f-\ln W_i)/t$ (W_i and W_f are the initial and final weight (g) of fish, t = number of experiment days); Hepatosomatic index (HSI) = (liver weight ×100) / body weight; Relative stomach content was expressed as wet weight (g) of feed X 100/weight of fish (g).

Exponential, rectilinear and square root models were used to describe GER (Jobling, 1987). The mathematical model that best described GER of juvenile tilapia in the study was selected by comparing R^2, SDR and RSS.

Exponential function: $Y=A\exp^{(-Bx)}$, Rectilinear function: $Y=A-Bx$, Square root function: $Y^{0.5}=A-Bx$.

Statistical Analysis. The data were subjected to one-way ANOVA to test the effects of feeding frequency at a significance level of 0.05. Duncan's multiple range test was used to rank the groups (Duncan, 1955). The data are presented as mean±S.E. of three replicate groups. All statistical analyses were carried out using the SPSS 16.0 program.

Results

Effect of feeding frequency on feed intake, specific growth ratio, and feed effectiveness ratio. No mortality or physical abnormalities were observed in any group throughout the experiment. Performance indices of juvenile tilapia with different feeding frequencies are presented in Table 2. As feeding frequency decreased, the feed intake and specific growth ratio of juvenile tilapia gradually decreased. The growth rates of fish fed four meals a day, three meals a day and two meals a day were significantly higher than that of fish fed four meals every 2 days , three meals every 2 days and two meals every 2 days (P <0.05). The feed intake of fish fed two meals a day, four meals every 2 days, three meals every 2 days and two meals every two days was significantly lower than that of fish fed three meals a day and four meals a day (P <0.05). The feed efficiency ratio of fish fed two meals a day was significantly higher than that observed in any group throughout the experiment. As feeding frequency decreased, the feed intake and specific growth ratio of juvenile tilapia gradually decreased. The growth rates of fish fed four, three, and two meals a day, were significantly higher than that of fish fed four meals, three, and two meals every 2 days (P <0.05). The feed intake of fish fed two meals a day, four meals every 2 days, three meals every 2 days and two meals every two days were significantly lower than that of fish fed three meals a day and four meals a day (P <0.05). The feed efficiency ratio of fish fed two meals a day was significantly higher (*P<0.05*) than in other treatments (Table 2).

Table 2. The feed intake (FI), specific growth ratio (SGR) and feed effectiveness ratio (FER) in fish with different feeding frequencies *

*Feeding frequency***	*Initial body weight (g) (IBW)*	*final body weight (g) (FBW)*	*Feed intake(g) (FI)*	*Special growth ratios % (SGR)*	*Feed effectiveness ratios % (FER)*
F1	3.72±0.05	48.03±3.95^{a}	41.98±3.16 ^{a}	6.09±0.22^{a}	105.6±3.47^{b}
F2	3.72±0.03	47.61±3.67^{a}	40.07±3.75 ^{a}	6.07±0.17^{a}	109.5±9.37^{b}
F3	3.71±0.04	46.83±11.89^{a}	34.13±2.95^{b}	5.99± 0.57^{a}	126.3±10.08^{a}
F4	3.71±0.05	28.96±3.16^{b}	37.20±1.54 ^{b}	4.88 ±0.22^{b}	61.9±6.54 ^{c}
F5	3.72±0.03	28.84±2.59^{b}	35.09±1.80 ^{b}	4.87 ±0.23^{b}	71.6±4.82^{c}
F6	3.71±0.05	25.81±0.29^{b}	26.80±3.66 ^{c}	4.62 ±0.02 ^{b}	82.4±11.75^{c}

*Values with different lowercase superscript letters within the same column indicate significant difference (*P*<0.05). **F1=Four meals/day, F2=Three meals/day, F3=Two meals/day, D4=Four meals/2days, F5=Three meals/2days, F6=Two meals/2days.

Effect of feeding frequency on body composition and Hepatosomatic index (HSI). As feeding frequency decreased, moisture content of fish body increased. Fat and protein contents decreased gradually. There were significant differences in the characteristics of body composition between the fish fed four meals a day and three meals a day compared to the fish fed two meals every 2 days (P <0.05). Hepatosomatic indices (HSI) of fish fed two meals a day and three meals a day were not significantly different (P >0.05) to the remaining groups. Livers were normal (Table 3).

Table 3 Proximate analysis of nutrient contents by body and Hepatosomatic indices (HIS)in fish with different feeding frequencies(%)*

Feeding frequency	*Moisture*	*Protein*	*Crude lipid*	*Ash*	*HSI*
Initial	74.99±0.14^{cf}	13.87 ±0.18 ^{a}	4.32 ±0.32 ^{a}	4.06±0.28 ^{a}	1.73±0.34^{ab}
F1	72.57 ±1.26^{ae}	16.70 ±0.70^{b}	6.45±0.33 ^{bc}	4.28 ±0.23^{a}	1.87±0.37^{b}
F2	71.63 ±1.82^{a}	16.41 ±1.45 ^{b}	7.13 ±0.61^{c}	4.84 ±0.60^{b}	1.63±0.25^{ab}
F3	73.91±0.97 ^{ce}	16.13 ±0.75 ^{bc}	6.00±0.32 ^{bd}	3.95±0.12 ^{a}	1.46±0.08^{ab}
F4	73.64±1.67 ^{ace}	15.89±0.89 ^{bc}	6.20±0.65 ^{b}	4.27 ±0.19^{a}	1.78±0.23^{b}
F5	75.07 ±0.30 ^{cf}	15.41 ±0.53 ^{bc}	5.68±0.39 ^{bd}	3.84 ±0.08 ^{a}	1.28±0.25^{a}
F6	76.12 ±0.90 ^{bf}	14.76 ±0.40^{ac}	5.28 ±0.67 ^{d}	3.84 ±0.04^{a}	1.57±0.33^{ab}

*Values with different lowercase superscript letters within the same column indicate significant difference (*P*< 0.05).

Gastric evacuation. Gastric content weight related to GET of juvenile tilapia fed a single meal at 0900 h are shown in Fig. 1.

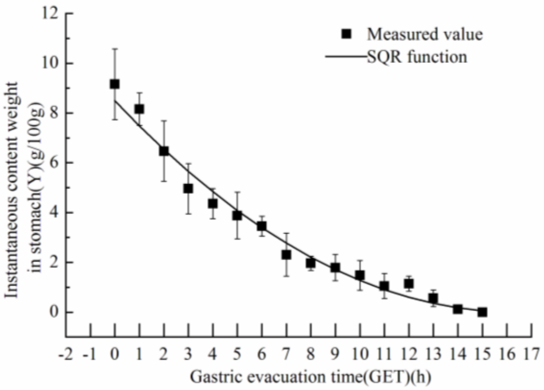

Fig. 1 Changes in gastric evacuation time.

Stomach contents gradually decreased and reached almost the same level as pre-feeding level in 15 h post feeding. Gastric evacuation rate in this study was best described by the square root function for the highest R^2 and lowest RSS and SDR as compared with exponential and linear functions (Table 4).

Table 4. 3 mathematical models of gastric evacuation of fish

Weight (g)	*Mathematical model*	*A*	*B*	*R2*	*SDR*	*RSS*
4.2±0.24g	Square root model	2.914	0.178	0.998	0.132	0.283
(31.5±0.5℃)	Exponential model	9.44	0.825	0.989	0.2888	1.334
	Linear model	7.390	0.562	0.904	0.875	11.413

Discussion

The influence of feeding frequency on fish growth has received much attention (Panagiotis et al., 2003, Tekinay A.A., 2003, Boujard et al., 1992; Hayward et al., 1997; Lee et al., 2000). Different feeding regimes and photoperiods can significantly affect the growth of newly weaned snapper (Villarroel et al., 2011, Tucker et al., 2006). In this study, no mortality was observed in any group. Similar results have shown no significant effect of feeding frequency on survival rates (Goldan et al., 1997). Feeding frequency had a significant effect on food intake and growth in juvenile tilapia, as feeding frequency increased, the feed intake and specific growth ratios gradually increased. Our investigation indicated that high growth performance of juvenile GIFT strain of Nile tilapia corresponded to high feed intake. The specific growth ratios and feed effectiveness ratios of fish significantly decreased when feeding frequency was four meals, three, and two meals every 2 days. It also suggested that when feeding frequency was too low, nutrient requirements of normal growth for juvenile GIFT strain of Nile tilapia could not be satisfied. Studies conducted on other fish species have also shown that feed intake and growth generally increase with feeding frequency up to a given limit (Andrews et al., 1975; Grayton et al., 1977; Tsevis et al., 1992). The daily feed intake of fish changes with feeding frequency and improves the growth of fish (Ishiwata, 1969a).

The specific growth rate of fish fed two meals a day was close to that of three and four meals a day, and significantly higher than that of four, three, and two meals every 2 days ($P < 0.05$). The feed effectiveness ratio of fish fed two meals a day was significantly higher than that of the other feeding frequencies ($P < 0.05$), thus increased feed effectiveness ratio was the primary reason for the high growth performance. Analysis of body composition demonstrated that feeding frequency on body fat and protein contents of the fish increased gradually, and the moisture content decreased, as feeding frequency increased. Hepatosomatic indices of fish fed two meals a day and three meals a day were not significantly different to the other groups. Feeding frequency is highly correlated with gastric evacuation time (Holmgren et al., 1983; Lee et al., 2000), and appetite return is closely related to the rate of gastric evacuation (Grove & Crawford, 1980; Huebner et al., 1982; Lee et al., 2000). In rainbow trout complete evacuation takes 15 h whereas 80–90% evacuation requires 6 h, which corresponds to the return of appetite (Grove et al., 1978).

In our study, stomach contents gradually decreased and reached almost the same level as pre-feeding level 15 h after feeding (Fig. 1). The evacuation curves constructed from the data collected in the experiment predict that 9 h are required to attain 80% evacuation in juvenile GIFT strain of Nile tilapia (weight 48 g) corresponding to the return of appetite. In fish fed four, three and two meals every 2 days, with intervals beyond 24 h, stomach evacuation occurred and point of appetite return passed, resulting in decreased feed intake and growth performance. However, fish fed two meals a day with an interval of 9 h reached 80% evacuation and their appetite returned before the next feeding. Fish fed two meals a day had higher SGR and FE compared with other feeding frequencies. Two independent trials were performed. Results of both showed that gastric evacuation facilitated estimation of optimal feeding frequency of juvenile GIFT strain of Nile tilapia.

In another study, tilapia O. *aureus,* 80% evacuation took 6 h (Riche et al. 2004). This discrepancy may be due to different protein or energy content in the feed, different tilapia species, or different fish size. Further research is required to evaluate the relationship between gastric evacuation and return of appetite in other tilapia species for different sized fish.

A number of mathematical models exist to describe GER (Jobling, 1981, 1983, 1986a; Persson, 1986). The most widely used are the exponential, rectilinear, and square root models. All of them describe a curvilinear relationship, but differ in their conclusions (Jobling, 1981; Persson, 1986; Jobling, 1987). It was suggested that evacuation of dry pelleted feed is best described by a square root or linear function. The exponential function is more appropriate for pellet fed tilapia reared in warmer temperatures (Brodeur, 1984).

In the present study, GER of 48 g juvenile tilapia is best described by a square root model. The differences in the studies may be due to the fact that GER is a function of temperature, fish weight, meal size, dietary composition, and energy.

From our findings we found that giving two meals a day was more effective than other feeding frequencies for improving growth performance of juvenile GIFT strain Nile tilapia which grew from 3.7 to 48.0 g fed on diets containing high levels of protein and energy. Gastric evacuation rate is one available method for estimating the optimal feeding frequency of juvenile GIFT strain of Nile tilapia.

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