Pakistan J. Zool., vol. 48(1), pp. 171-177, 2016.

Effect of Feeding Frequency on Growth Performance, Feed Utilization and Body Composition of Juvenile Nile Tilapia, *Oreochromis niloticus* (L.) Reared in Low Salinity Water

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Article Information
Received 28 May 2015
Revised 10 July 2015
Accepted 11 July 2015
Available online 1 January 2016

Authors' Contributions:
AMD did the experimental work. SSAS maintained fish stocks. HK prepared fish feed. AG analyzed feed components. MHR analyzed fish meet. GA designed the experiment, wrote the manuscript with the help of IBK.

Key words:
***Oreochromis niloticus*, growth, feed conversion, feeding frequency, body composition.**

ABSTRACT

Feeding frequency is well known for the reduction of aquaculture production cost and preventing water quality deterioration as the result of excess feeding. The influence of feeding frequency was studied in the present study, when juvenile Nile tilapia O*reochromis niloticus* (initial body weight 1.0 g) were maintained in rearing tanks 35 liters each (water temperature 29ºC, salinity 14‰, pH 7.7, dissolve oxygen 5.1ml/l and ammonia never exceeded 0.1 ml/l) and were fed diet of 35% protein for 42 days. Fish were fed at four frequencies: two, three, four and five times a day. Each feeding frequency was assigned to three tanks of fish with 10 fish per tank. Results showed that significantly higher weight gain, specific growth rate, feed conversion and protein efficiency ratio were observed at feeding frequency of four to five times daily. Moisture, protein and ash contents of whole body were not affected by feeding frequency. Lipid content of fish fed four and five times daily was significantly higher than that of the fish fed one and two times daily. The condition factor remained consistent at all feeding frequencies and survival was 100% throughout the experiment. These results suggest that under similar culture conditions, the optimum feeding frequency of juvenile Nile tilapia (from initial body weight of 1.0 g to 5.8 g) is four times daily.

INTRODUCTION

The commercial feasibility of any intensively cultured fish species depends on market demand and cost of production. The largest section of the production cost lies in feed, with protein containing the most expensive component (Pandian, 1989; National Research Council, 1993). However, to develop fish culture at commercial level, it is important to establish an appropriate feeding management strategy that is based on identification of the daily feeding patterns or rhythms (Phillips *et al*., 1998; Wang *et al*., 1998). It is well known that the amount and timing of feeding play an important role in growth and feed utilization efficiency (Brett and Groves, 1979; Reddy and Katre, 1979; Hung and Lutes, 1987; Hung *et al*., 1993; Cho *et al*., 2003). Knowledge about the optimum feeding is important not only for regulating the feed intake, growth and chemical composition of fish but also for preventing water quality deterioration as a result of overfeeding (Du *et al*., 2006; Salama, 2008; Ertan *et al*., 2015). On the other hand, overfeeding of fish can overload the stomach and intestine, leading to decreases in digestive efficiency and reductions in feed utilization (Jobling 1986; Hung and Lutes, 1987; Storebakken and Austreng, 1987; Du *et al*., 2006). Thus, the diet amount fed each time, or feeding frequency, may influence diet utilization (Sanches and Hayashi, 2001). This is due to the fact that diet is directly applied to water and the non-uptaken portion will be dissolved and lixiviated. Feed conversion ratio increase and environmental pollution are the results. Since fish juveniles uptake a high daily diet ratio to meet their nutritional requirement and thus ingest adequate amount of diet, and since high feeding frequency results in high daily diet intake ratio and small amounts of diet per feeding (Sanches and Hayashi, 2001),

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0030-9923/2016/0001-0171 $ 8.00/0

a higher frequency may be the most adequate.

Tilapia (*Oreochromis* spp.) are known as commercially important food fishes for aquaculture throughout several regions of the world such as China, South-east Asia, Africa, USA and Latin America/Caribbean (Lim and Webster, 2006; Chowdhury, 2011). According to FAO (2012) global tilapia production, which totaled less than 500,000 metric tons in the early 1990s, topped 3.5 million metric tons in 2011. In 2012 it increased up to 2.7 percent, and further climbed to 3.4 percent in 2013. By 2014, it is expected to approach around 3.9 million tons. In Pakistan, tilapia is known for its good quality meat and it is cheaper as compared to other food fishes. It can play an important role in aquaculture development in the country. Few feeding trials indicated that Nile tilapia (*Oreochromis niloticus*) has potential for aquaculture in Pakistan because of its hardiness and rapid growth (Jamil *et al*., 2004; Khan *et al.*, 2014; Chughtai *et al.*, 2015).

Although some work has been done on the feeding patterns of Nile tilapia (Macintosh and Little, 1995; Tung and Shau, 1991; Vera Cruze and Mair, 1994; Sanches and Hayashi, 2001), data regarding feeding frequency for this species in Pakistani waters is scarce. It was in view of this paucity of information that the present study was carried out to investigate the influence of feeding frequency on the growth, feed efficiency and body composition of juvenile Nile tilapia for a period of 42 days. In this study, a low-cost feed was formulated using locally-available ingredients and was supplied to the fish at different feeding frequencies for 42 days.

MATERIALS AND METHODS

Experimental diet

Fish feed (20.2 kJ g^{-1} digestible energy) was formulated on dry matter basis (g 100^{-1}) in one batch to supply calculated protein levels of 35% with fishmeal providing the majority of dietary protein (Table I). A mixture of minerals and vitamins were added to the ingredients (rice bran, wheat bran, mustered oil cake and wheat flour) of diets. All these ingredients were purchased from the local markets and were ground to 500µm and mechanically mixed for15 min to ensure homogeneity. Fish oil was added and then mixed again for 15 minutes. Water (250 mL kg^{-1} dry ingredients mixture) was added and mixed for another 15 minutes to attain a consistency appropriate for pelleting. The wet mash was pelleted with a California Laboratory Pellet Mill (model CL-type 3, California Pellet Mill Company, San Francisco, CA, USA.) using a 2-mm die. No heating or steam was used in the pelleting process and the wet pellets were air-dried at room temperature for 20 hours. The experimental feeds were then stored at –20°C for feeding trials.

Table I.- Formulation and chemical analysis of the experimental diet.

Ingredients (%)	g 100 g^{-1} diet (dry)
Fish meal	34.5
Wheat brawn	16.0
Rice brawn	11.0
Mustered oil cake	13.0
Wheat flour	20.0
Vitamin-mineral premix[1]	2.5
Fish oil	3.0
Proximate composition[2]	
Moisture	7.0±0.5
Crude protein[3]	34.6±0.5
Crude lipid	5.8±0.6
Crude fiber	5.9±0.4
Ash	7.0±0.7
NFE[4]	46.7±0.3
Energy (kJg^{-1})	20.2±0.6
P/E (mg crude protein kJ^{-1})	17.1±0.5

[1]Vitamin and mineral mixture contained the following ingredients (g 100 g^{-1} diet): Ascorbic acid (vit C), 15.2; thiamin HCl (vit B_6), 1.1; inositol, 39.5; calcium, 1.25; zinc, 1.0; retinol (vit A), 1.5; phosphorus, 3.5; choline chloride, 3.5; magnesium, 2.0; copper, 1.0; pyridoxine (vit B_6), 1.3; phospholipids, 3.5; α-tocopherol acetate (vit E), 5.5; folic acid, 0.4; cholecalciferol (vit D_3), 7.5; cyanocobalamine (vit B_{12}), 0.006; riboflavin (vit B_2), 1.5; menadione sodium bisulphite (vit K_3), 0.03; manganese, 2.0; iodine, 2.0; sodium, 1.0; iron, 1.0; nicotinic acid, 4.3; biotin, 0.35.
[2]Dry matter basis (%): mean ± SE, number of determination =3.
[3]Measured as nitrogen × 6.25.
[4]Nitrogen-free extract = 100 – (% protein + % fat + % ash + % fiber).

Experimental design

Juveniles of Nile tilapia, *Oreochromis niloticus* (mean weight 1±0.01g and mean length 3.8±0.02 cm) collected from Government Fish Hatchery, Chilya, Thatta, Sindh were held in seawater for fifteen days before starting the experiment. After the acclimatization phase, fish were randomly distributed in twelve (12) plastic experimental indoor tanks (10 fish per tank). The water carrying capacity of each tank was 35 liters. Aeration was provided by aerators throughout the entire experiment which lasted 42 days. Fish were subjected to a natural photoperiod and all tanks had similar light conditions. Physico-chemical parameters *i.e.*, temperature, salinity, pH and dissolved oxygen were monitored daily.

Feeding protocol

Experimental diet containing 35% protein concentration was tested to find out the optimum feeding frequency of Nile Tilapia (*Oreochromis niloticus*). During this experiment, diet was supplied to the experimental juveniles with the frequency of two, three, four and five times per day. Fish were hand-fed on daily ration of 3% wet body weight per day for 42 days. The daily feed supplied was recorded and uneaten feed was collected two hours after the start of feeding. The amount of food to be provided being adjusted following weekly sampling for the determination of gain in weight and length per treatment which lasted 42 days. Each tank was completely drained and thoroughly scrubbed on the day of sampling.

Measurement and analysis

At the end of study five fish were removed from each tank, killed and pooled for whole body composition analysis. Fish whole-body samples were taken out of the –20 °C cold store and thawed at room temperature using a fan. Subsequently, all these samples were homogenized, dried and then ground into a powder before chemical composition analysis.

At the end of the experiment, three replicate samples with10 fish per replicate were taken and kept frozen at –20 °C for subsequent analysis of the fish whole body composition.

The moisture, protein, lipid and ash contents of experimental diets and samples were analyzed according to the standard methods (Association of Official Analytical Chemists 2000). Moisture was determined by drying in an oven (Labostar-LG 122, Tabai Espec, Osaka, Japan) at 105 °C for 24 h; ash by burning in a muffle furnace (Isuzu Seisakusho, Tokyo, Japan) at 550°C for 18 h; crude protein by the Kjeldahl method (N × 6.25) using an automatic Kjeldahl System (Buchi 430/323, Flawil, Switzerland); crude fiber by acid detergent fiber analysis; and crude lipid by the chloroform/ methanol (2:1, v/v) extraction procedure (Folch *et al*., 1957). The carbohydrate content was calculated by subtracting the content of lipids, total protein and ash from the dry weight, and gross energy estimation was made using an automatic bomb-calorimeter (Parr Instrument, model1265, Moline, IL, USA). All chemical analyses were performed in triplicate and averaged.

Calculation of growth parameters

At the end of the experiment, all fish from each tank were individually weighed and their total length was measured for calculation of the condition factor [CF = (100 × body weight in g)/ (TL in cm) ^{3}]. Growth and feed efficiency were monitored in terms of the final weight, weight gain (expressed as the percent of initial body weight at the end of the experiment), specific growth rate (SGR) (ln final body weight – ln initial body weight/time, expressed as % per day), feed conversion ratio (FCR) (feed fed/wet weight gain), protein efficiency ratio (PER) (wet weight gain/protein intake).

Statistical analysis

The data on fish growth, feed utilization efficiency and whole fish body constituents were subjected to one-way analyses of variance (ANOVAs) to determine whether there was a significant difference (P<0.05) among fish fed at different protein levels. Differences between means were assessed at the 5% probability level using Duncan's multiple range test, as described by Robbins *et al.,* (1979) and Steel and Torrie (1980). The data are presented as mean±SE of the replicate groups.

RESULTS

Water quality

The water temperature was maintained at 29±0.5°C (mean±SD). Salinity was 14.0±0.7‰ and pH ranged from 7.6 to 7.9 with a mean of 7.7±0.05 throughout the study period. Dissolve oxygen was 5.1±0.1 ml/l. Ammonia never exceeded 0.1±0.004 ml/l.

Growth performance

Growth performance of Nile tilapia juveniles was significantly affected by feeding frequency (Table II). Body weight gain and SGR of the fish fed four and five times daily were significantly (P<0.05) higher than of those fed the two and three times daily. Weight gain and SGR tended to plateau at around 480.0 g and 4.19% day^{-1} respectively.

Feed conversion and condition indices

Feed intake, expressed on a dry matter basis, increased slightly with an increase in feeding frequency. Fish fed the four and five times daily showed significantly higher (P<0.05) feed intake than the other groups (Table II). The same trend was observed in feed conversion ratio (FCR) and PER. There were no significant differences in condition factor (CF) between all the groups where survival remained 100%.

Body composition

The moisture, protein and ash contents of fish whole body were not significantly (P>0.05) affected by feeding frequency (Table III). The lipid content of fish fed four and five times daily was significantly (P<0.05) higher than that of fish fed two and three times daily. The lipid contents of whole body increased significantly (P<0.05) with increasing feeding frequency (Table III).

Table II.- The growth rate and feed utilization of juvenile Nile tilapia fed at different frequencies for 42 days.

Parameters	Feeding frequency			
	2	3	4	5
Final weight (g)	3.5±0.1[a]	4.6±0.3[b]	5.8±0.5[c]	5.9±0.4[c]
Weight gain, % of initial weight[1]	250.0±1.6[a]	360.0±2.5[b]	480.0±2.2[c]	490.0±1.0[c]
Specific growth rate[2]	2.98±0.01[a]	3.63±0.03[b]	4.19±0.03[c]	4.23±0.01[c]
Feed intake[3] (g fish^{-1})	10.5.±1.3[a]	13.3±1.5[b]	16.6±1.1[c]	17.0±1.0[c]
Feed conversion ratio[4]	4.2±0.2[a]	3.69±0.1[a]	3.46±0.5[b]	3.47±0.2[b]
Protein efficiency ratio[5]	1.28±0.04[a]	1.48±0.01[a]	2.13±0.02[b]	2.39±0.01[b]
Condition factor[6]	2.5±0.03 [a]	2.6±0.04 [a]	2.7±0.03 [a]	2.6±0.04 [a]
Survival (%)	100	100	100	100

Values (means±SE, n = 3 and each n consists of 10 fish per replicate) in the same row with different superscripts are significantly different (P>0.05). Initial body weight and length of the fish was 1±0.05 g and 3.5 cm ± 0.01 respectively.
[1]Weight gain, % of initial weight = 100 × [final body weight – initial body weight / initial body weight].
[2]Specific growth rate = 100 × [ln final body weight – ln initial body weight / time in days].
[3]Feed intake = total feed fed as % body weight – total uneaten feed.
[4]Feed conversion ratio = total feed fed (g) / total wet weight gain (g).
[5]Protein efficiency ratio = wet weight gain / protein (N × 6.25) intake.
[6]Condition factor (CF) = 100 × (weight / length^3).

Table III.- Whole body composition (% dry weight basis) of Nile tilapia juvenile fed at different frequencies for 42 days.

Parameters	Feeding frequency			
	2	3	4	5
Moisture	71.2±1.9[a]	71.0±1.7[a]	71.3±1.5[a]	71.2±1.5[a]
Protein	52.8±2.1[a]	52.9±1.5[a]	53.1±1.2[a]	53.0±1.4[a]
Lipid	33.6±1.8[a]	33.5±1.4[a]	35.1±1.3[b]	35.4±1.5[b]
Ash	12.2±1.5[a]	12.4±1.3[a]	12.6±1.1[a]	12.4±2.0[a]

Values (mean±SE, n =3 and each n consists of 10 fish per replicate) in the same row with different superscripts are significantly different (P>0.05). Chemical composition of initial body was: moisture 70.0%, protein 51.5%, lipid 33.0% and ash 11.2%

DISCUSSION

In the present study, juveniles of Nile tilapia were shown to require feeding frequency of four times or five times daily for the highest growth performance, associated with significantly higher weight gain and better feed conversion. Since growth and feed conversion are influenced by feedings and higher feedings are counterproductive (Brett and Groves, 1979; Reddy and Katre, 1979; Hung and Lutes, 1987; Hung *et al.*, 1993; Ng *et al.*, 2000). Therefore, feeding to fish should be reduced to a minimal level. This minimal level corresponds to four times a day in the present study. Feeding above four times daily was over the satiety level of the fish and some food waste might occur. Moreover, when feeding was with two and three times a day, fish showed the lowest growth which might have been due to the nutrient requirement for maintenance. It appears that a large proportion of nutrient in the diet was used to maintain life, and only a small proportion was available for growth. The finding that higher growth of Nile tilapia accompanied with higher food conversion similar to the findings in other fishes, such as sturgeon *Acipenser transmontanus* (Hung and Lutes, 1987), rainbow trout *Oncorhynchus mykiss* (Storebakken and Austreng, 1987), striped bass *Morone saxatilis* (Hung *et al.*, 1993), tilapia *Tilapia aureus* (Papoutsoglou and Voutsinos, 1988), clownfish *Amphiprion percula* (Johnston *et al.*, 2003), bagrid catfish *Mystus nemurus* (Ng *et al.*, 2000) and grass carp *Ctenopharyngodon idella* (Du *et al.*, 2006). Regardless of the existence of these fish species and categories, many other authors reported that feeding of four times a day is the best for tilapia fry (Abucay and Mair, 1977; Jobling, 1983; Macintosh and De Silva, 1984; Poma and Green, 1990; Tung and Shiau, 1991; Chambers, 1984; Vera Cruz and Mair 1994; Ridha and Lone, 1995; Cui *et al.*, 1994). These observations are in conformity with the results of the present study.

The proper feeding frequency for maximum growth of fish may differ by fish species, fish size,

dietary protein and energy contents (Minton, 1978; Wang *et al.*, 1998; Company *et al*., 1999; Lee *et al*., 2000). Generally, fish fry one fed smaller meals with greater frequency while once per day is usually sufficient for broodstock and older fish. Usually, the quantity of feed required for optimal growth diminishes as fish age (Schnai *et al.*, 2005). For instance, Pullin and Lowe-McConnell (1982) recommended feeding tilapia, *O. niloticus* 12 times daily when their weight is 20 g but only twice daily when they weigh 200 g. Rainbow trout (0.3 g) grows best when fed eight times per day, but similar results were obtained when 15 g fish were fed three times per day (Piper, 1982).

The influence of feeding frequency on feed intake is also variable among fish species (Bascinar *et al.*, 2007; Dwyer *et al.*, 2002; Webster *et al.*, 2002). In this study, as the total daily amount of feed supply was constant with various feeding frequencies improvement in weight gain, growth rate and feed conversion was found in Nile tilapia juveniles with higher feeding frequency. Evidence to support this is available in other studies showing that two, three or four feedings a day were sufficient for maximum growth of a variety of fish species, for instance sea bass *Dicentrarchus labrax* (Tsevis *et al.*, 1992), grouper *Epinephelus akaara* (Kayano *et al.*, 1993), ayu *Plecoglos susaltivelis* (Cho *et al.*, 2003), common carp *Cyprinus carpio* (Charles *et al*., 1984), channel catfish *Ictalurus punctatus* (Andrews and Page, 1975) and Nile tilapia *O. niloticus* (Vera Cruz and Mair, 1994), rainbow trout *Oncorhynchus mykiss* (Grayton and Beamish, 1977). On the other hand, some researches contradict from these findings. For instance, Guerrero (1975) suggested two times per day frequency for tilapia sex reversal. But growth performance of tilapia might be inhibited during his study. Tung and Shiau (1991) while studying on the effect of feeding frequency on Nile tilapia juveniles in captivity. He found that feeding frequency of six times a day was the best for good growth for the fish. However, these researchers conducted experiments for only two and six times per day feedings and they did not mention intermediary frequencies. Similarly, Jobling (1983) and Cowey (1992) investigated that increase in feeding frequency from one to two times per day resulted an increase in growth of arctic char (*Salvelinus alpinus*) though his research was away from higher frequencies.

Taking into consideration the effects of various feeding frequencies on the Nile tilapia body composition, protein content in fish remained at a comparatively stable level. It comes into view that the lowest frequency could provide dietary protein at or slightly above the maintenance level of the fish as suggested by Hung and Lutes (1987), Mehboob *et al.* (2003) and Cho *et al.* (2003). This further suggests that body lipid is the preferred energy reserve for deposition or mobilization over protein in juvenile tilapia which is long-lasting by the lower lipid contents of fish whole body were found at lower feedings.

The findings of this study suggest that the optimum feeding frequency of juvenile Nile tilapia, *O. niloticus* growing from 1 g to 5.8 g was four meals a day. At this optimum feeding, improved weight gain, greater food intake, higher feed conversion and superior protein retention efficiency are factors apparently responsible for the faster growth rate and lipid reserves in juvenile Nile tilapia under the present experimental conditions.

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