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Compensatory Weight Gain and Muscle Tissue Biochemical Composition of GET Excel Tilapia (*Oreochromis niloticus*) Juveniles

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ABSTRACT

The ability of tilapia (*Oreochromis niloticos*) for compensatory growth response following a period of starvation was investigated in the MSUN-IFRD Laboratory. The effects of food deprivation on growth performance, feed response, survival, and chemical composition of fish muscle of GET Excel tilapia juveniles were studied. The juveniles (1.19 - 1.62g) were subjected to different periods of starvation: 0, 2, 3, 4, and 5 days (representing the control T1 and experimental treatments T2, T3, T4 and T5), after which they were fed for 30 days. Periodic sampling was done every seven days to determine survival and change in weight of tilapia juveniles. Juveniles starved for shorter periods (2-3 days) recovered faster (<18 days) and showed accelerated growth earlier than those starved for a longer period (4-5 days). By the end of the third culture period, the weight gain of juveniles in T2 (1.02g) and T3 (0.98g) has caught up with the weight gain of the control (0.99g). On the other hand, weight gain of T4 (0.77g) and of T5 (0.76g) were significantly lower ($P<0.05$) from the first three treatments. Specific growth rate (SGR) in T2 (2.96% $BW.d^{-1}$) and T3 (2.84% $BW.d^{-1}$) were also comparable ($P>0.05$) with T1 (2.86% $BW.d^{-1}$) after 18 days of culture (DOC). Feed conversion efficiencies (FCE) of T2 (53.68%) and T3 (54.01%) were significantly higher ($P<0.05$) than other treatments after 24 DOC. Mean survival rates (83.3-90.0%) by the end of the culture were not significant ($P>0.05$) in all treatments. Difference in weight gain, SGR, FCR, feed requirements, and protein content of fish muscle were not significant among the treatments ($P>0.05$) when the experiment was terminated. The result showed that GET Excel tilapia juveniles demonstrated a positive compensatory growth and high survival rate after being subjected to up to 5 days of starvation.

Keywords: Recovery growth, feed conversion efficiencies, protein content, starvation, and refeeding techniques.

INTRODUCTION

Fish may experience depletion of energy reserves making them thin and in poor physiological condition. Marked cycles of depletion and repletion of energy reserves often occur in wild fish populations and growth will normally be limited by the availability of food supply (Jobling, 1994). Research has shown that various animals, including some fish, temporarily deprived of feed grow more rapidly when feeding is resumed and catch up with animals that were not so deprived (Kim and Lovell, 1995). This phenomenon is known as compensatory growth. Compensatory growth that leads to the same body size with the control group can be termed "catch-up" growth, and ''overcompensation'' refers to compensatory growth that results in a larger body size than control individuals (Skalski, *et al.*, 2005). The fish in the poorest condition usually showed the greatest response and displayed the most rapid rates of weight gain when adequate feeding conditions are restored (Jobling, 1994).

The mechanisms underlying the compensatory growth phenomenon are not fully understood (Broekhuizen, *et al.*, 1994), however, most animals adapt to food deprivation by reducing metabolic expenditure (Jobling, 1994). During the refeeding period, the metabolic rate may not immediately return to the same level as that of continuously fed animals. Likewise, most animals became hyperphagic or displaying excessive consumption during refeeding. Moreover, low rates of metabolic expenditure with high consumption rate would result in high amounts of nutrients available for growth.

A number of studies on recovery growth conducted on domesticated animals exist but few studies had been conducted on fish. In fish, attention was focused on exploiting the compensatory growth response as a technique to improve fish growth rates and growth efficiency (Jobling, *et al.*, 1993). The hybrid bluegill fed with either mealworms or a commercially prepared fish diet on a repeated two-day starvation and refeeding schedule would significantly outgrow continuously fed controls (Hayward, *et al.*, 1997). When catfish were either fed daily to apparent satiation or restrictively fed for 3, 6, or 9 weeks, it was found that the weight, feed conversion, dressing yield, and body composition values over the duration of the experiment were the same for fish subjected to all feeding regimes (Kim and Lovell 1995). Positive response or compensatory growth was also obtained in sea bass, sea bream, and rainbow trout (Sahin, *et al.*, 2000).

Results on compensatory trials suggest complete recovery following food restriction that improved feed efficiency and feed conversion. But some studies indicated that the overall time taken to reach market size was longer on the restricted-refeeding than on normal production feeding cycle (Jobling, 1994). Results on compensatory growth may depend upon factors such as the severity and duration of the restriction, the size and age of the fish, and the rearing environment.

This study evaluates the applicability of compensatory growth phenomenon through starvation and refeeding technique on the culture of GET (Genetically Enhanced Tilapia) Excel tilapia juveniles. The study aims to evaluate the effects of starvation and refeeding on the growth, survival, and nutrient composition of GET Excel tilapia juveniles subjected to different periods of food deprivation followed by refeeding.

Restricted feeding regimes can be considered promising tools for increasing the efficiency of fish production. Development of feeding regimes that take advantage of compensatory growth is of practical importance in the light of rising operational costs of aquaculture. By not feeding or by limiting the feed, fish farmers can save money from reduced feed and labor costs. This technique can also be used during inevitable circumstances such as during severe weather conditions, limited feed supply, and holidays when fish are either fed infrequently or not at all.

MATERIALS AND METHODS

Experimental animal

The juveniles of GET Excel tilapia, *Oreochromis niloticus,* used in the experiment were taken from the Commercial Tilapia Nursery of MSU at Naawan. A total of 350 uniformly sized (1-2 g individual weight) tilapia juveniles were selected from pooled samples of F_1 offspring from 30 breeders. The juveniles were placed in aquaria, acclimated for three days to laboratory condition, and were fed to satiation prior to the start of the experiment.

Experimental set-up and design

The experiment was conducted at the MSU-IFRD Wet Laboratory. Eighteen aquaria with 60-L capacity were filled with 45 L of filtered freshwater with aeration supplied by airstones placed at the center of each aquarium. The aquaria were cleaned daily and water was carefully drained up to 80% of its volume prior to feeding in the morning at 0600h. Dissolved oxygen and temperature were monitored using WTW Oxymeter with built-in digital thermometer before and after water management at 0600 h and at 1600h. Water samples were taken every morning before and after water management for ammonia content analysis following the standard phenate method using the Shimadzu UV-Vis spectrometer at 640 nm wavelength (APHA, 1995).

Groups of 20 tilapia juveniles were transferred to 15 aquaria filled with 45 L freshwater. Initial weight of the fish was taken by group-weighing all 20 fish from each replicate using a top loading digital balance (Ohaus) prior to stocking. Average weight of the fish represented the average initial wet weight (ww) of the fish in each replicate. The study consisted of five treatments: continuously fed fish as control (T1) and four treatments consisting of two (T2), three (T3), four (T4), and five (T5) days of starvation following satiation feeding in three replicates using a completely randomized design. The fish were refed after the starvation period of each treatment and reared for 30 days.

Sampling for growth was done every seven days, that is, at the end of six days of culture (DOC) corresponding to one period. Growth sampling was done by group weighing of all fish from each replicate. These data were used to adjust feed ration and to evaluate growth differences among treatments.

Feed and feeding regime

Crumbled tilapia feed with moisture content of 10% was introduced to the juveniles after starvation. The juveniles were fed twice daily to satiation with pre-weighed feed (5g); half of the ration in the morning after water management (0600h) and half at 1600 h. The feed was gradually introduced until the juveniles ceased to eat. Uneaten feed was collected one hour after introduction to determine the actual consumption in each treatment.

The feed, as well as the uneaten feed, was analyzed for dry matter after complete removal of moisture by oven-drying (at 100°C) for at least 24 h or until the weight of the feed was constant. This was done to correct the natural moisture content of the feed and the water content of uneaten feed resulting from soaking in the water. Feed consumed (in dry matter form) was computed as:

Feed Consumed = Introduced feed - Uneaten feed

Average weight of the juveniles was taken at each sampling period and upon termination of the experiment to determine the final weight gain and the specific growth rate (SGR). Each replicate was examined for any dead fish during each feeding cycle which were then taken out of the aquaria and weighed. Survival rate was determined in each treatment at all sampling periods.

Biochemical composition of fish tissue

Apart from the experimental treatments, separate groups of 20 juveniles taken from the same batch were stocked in three aquaria to evaluate the initial biochemical composition of fish tissue and the subsequent changes as a result of different starvation periods. Samples were taken from each aquarium starting on day zero (D0) and each time when feed was introduced to a previously starved juvenile group, i.e., D2, D3, D4, and D5.

Evaluation for final biochemical composition, specifically crude protein and crude fats, of fish tissue was made in each replicate after the experiment was terminated. Determination of crude protein was made using the Kjeldahl method while crude fats and moisture content were determined from the Soxhlet method (Hamilton and Simpson, 1970).

Data Analysis

Growth in terms of weight gain, specific growth rate (SGR), survival rate, and percent feed conversion efficiency (% FCE) were calculated at each sampling period and at the termination of the experiment, following the equation for SGR (Ricker, 1975) and FCE (Mihelakakis, *et al*., 2004) as follows:

$$SGR = [(\ln BW_f - \ln BW_i)/t] \times 100$$

$$\%FCE = [(BW_f - BW_i)/TF] \times 100$$

where W_f is the average final weight, W_i is the average weight of the sample taken at initial stocking, t is the experimental period in days and TF= total amount of feed introduced to each tilapia juvenile group in the five treatments.

Data were analyzed using one-way ANOVA included in the SPSS (ver.13) software package, where applicable. The data were considered significant at 5% level and Tukey's HSD was used to determine the source of significant difference between treatments, if significant.

RESULTS

Growth performance and survival

Juvenile tilapia subjected to various lengths of starvation period were found to recover fast enough once feeding was continued and exhibited an accelerated growth response. Figure 1 shows that fish in T2 (starved for only 2 days) increased in weight faster than in T3 (starved for 3 days) after only six days. No weight determination was done in T4 (starved for 4 days) and T5 (starved for 5 days) during the first sampling period as the sampling schedule was too close to the refeeding time of these treatments. It is apparent in Fig. 1 that fish growth in all treatments increased steeply during the third and fourth periods, with T2 and T3 showing comparative weight gains with that of the control (T1). The results also show that weight gain in T2 and T3 became progressively closer to the value of the control ($P>0.05$). All three treatments had significantly higher growth performance than T4 and T5 during the second and third sampling periods ($P<0.05$). Upon the termination of the experiment, weight gain of juveniles in T2 and T3 even exceeded that of T1, although the differences among treatments was not significant ($P>0.05$). Highest final weight gain (1.91g) was obtained in juvenile tilapia grown in T2 and lowest in T5 (1.47g) where the fish were exposed to the longest period of food deprivation (Table 2).

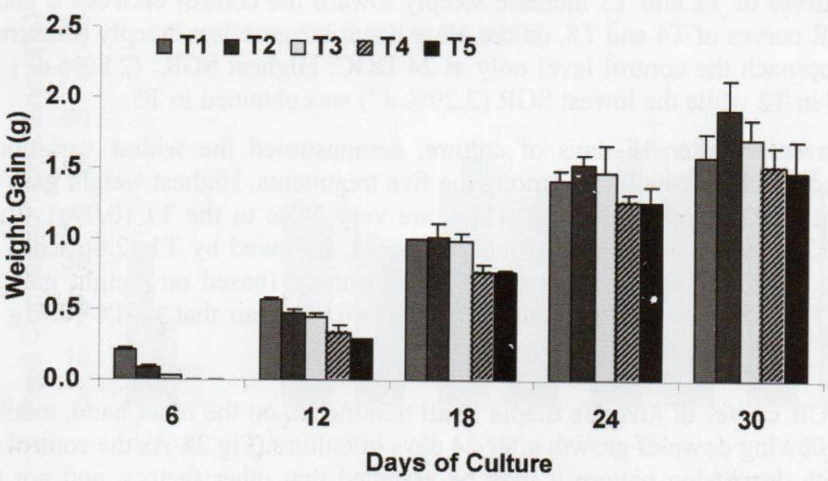

Figure 1. Periodic weight gain of GET Excel tilapia juveniles under various feed deprivation schemes. Error bars are means ± SD of three replicates.

Table 2. Growth performance, feed utilization, feed economics, and survival of GET Excel tilapia subjected to different starvation periods after 30 days of culture. The treatments (starvation time) were T1 (control), T2, T3, T4, and T5 refers to 0, 2, 3, 4, and 5 days feed deprivation, respectively. Values are mean ±SD of three replicates.

	Treatments				
Parameters	1	2	3	4	5
Initial body wt. (g)	1.47 ±0.04	1.45 ±0.03	1.47 ±0.02	1.47 ±0.05	1.48 ±0.02
Final body wt. (g)	3.05 ±0.19	3.36 ±0.18	3.17 ±0.13	2.98 ±0.18	2.94 ±0.23
Weight gain (g)	1.58 ±0.17	1.91 ±0.21	1.71 ±0.14	1.510 ±0.15	1.470 ±0.22
Specific growth rate (SGR, %.day-1)	2.42 ±0.19	2.80 ±0.25	2.57 ±0.17	2.35 ±2.35	2.29 ±0.24
Food conversion efficiency (FCE, %)	36.48 ±4.33	47.72 ±4.68	45.62 ±3.78	40.50 ±3.83	41.55 ±5.81
Kg feed per Kg juveniles	2.77 ±0.35	2.11 ±0.21	2.20 ±0.18	2.48 ±0.23	2.42 ±0.34
Amount of feed (P)	59.25 ±7.49	45.14 ±4.53	47.12 ±3.79	53.15 ±4.82	52.18 ±7.35
Survival (%)	83.33 ±7.64	86.67 ±2.89	86.67 ±2.89	90.00 ±2.89	83.33 ±2.89

Specific growth rates (SGR) of juveniles following starvation are shown in Fig. 2. The SGR curves of T2 and T3 increase steeply toward the control between 6 and 19 DOC. The SGR curves of T4 and T5, on the other hand, increase less steeply (thus, more slowly) and approach the control level only at 24 DOC. Highest SGR (2.80%.d^{-1}) was again obtained in T2 while the lowest SGR (2.29%.d^{-1}) was obtained in T5.

The juveniles after 18 days of culture, demonstrated the widest variation in weight gain and specific growth rate among the five treatments. Highest weight gain was observed in T2 (1.02g) and T3 (0.98g) which are very close to the T1 (0.99g) value. Highest SGR (2.96%.d^{-1}) was likewise obtained in T2, followed by T1 (2.86%.d^{-1}) and T3 (2.84%.d^{-1}). ANOVA showed that growth performance (based on weight gain and SGR) in T1, T2 and T3 was significantly better ($P<0.05$) than that in T4 (0.77g and 2.34%.d^{-1}) and T5 (0.76g and 2.31%.d^{-1}).

The SGR curves of juvenile tilapia in all treatments, on the other hand, exhibit a downtrend or slowing down of growth after 24 days of culture (Fig 2). As the control also manifested such decreasing pattern it may be assumed that other factors, and not feed deprivation, may have intervened with the positive growth trends exhibited by the fish up to the fourth sampling period. Except for a few individuals, tilapia juveniles subjected to varying periods of starvation survived toward the end of the experiment. Mean survival rates in all treatments was highest in T4 (90.00%) and lowest in both T1 and T5 (83.33%). These differences, however, were not significant ($P>0.05$), suggesting that starvation of up to five days did not threaten the survival (nor growth) of tilapia juveniles.

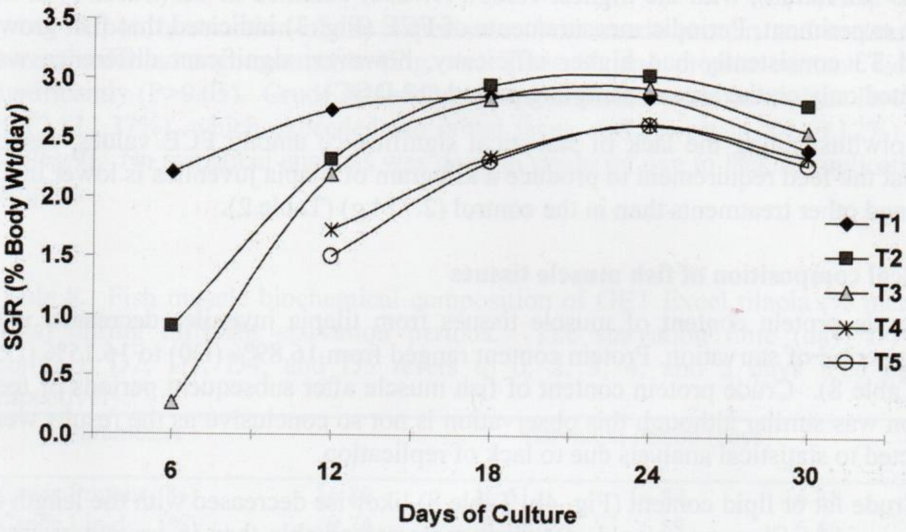

Figure 2. Periodic specific growth rate of GET Excel tilapia under various feed deprivation schemes.

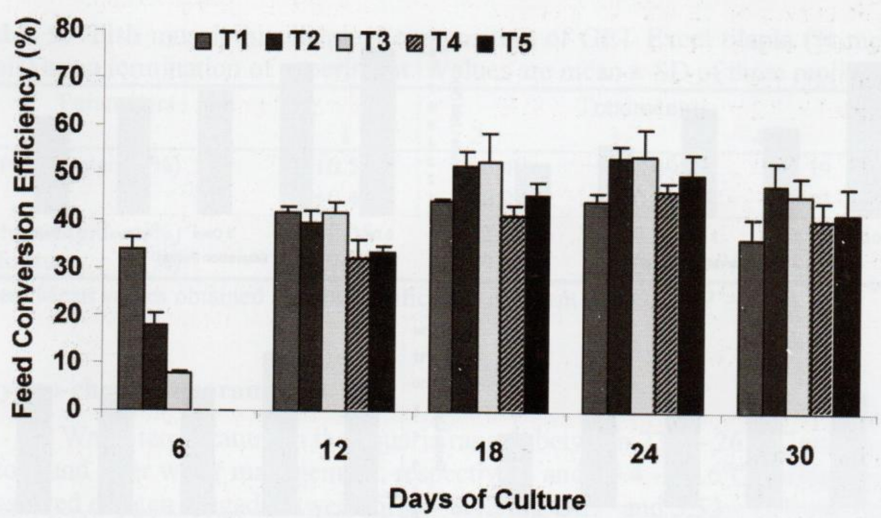

Figure 3. Periodic feed conversion efficiency of GET Excel tilapia subjected to different starvation periods. Bars vertical are means of ± SD of three replicates.

Feed conversion efficiency

Starved tilapia juveniles exhibited higher efficiency in feed utilization following starvation for 2-5 days (Fig. 3). Mean FCE values were higher in starved fish than in the control (no starvation), with the highest value (47.72%) obtained in T2 (Table 1) at the end of the experiment. Periodic measurements of FCE (Fig. 3) indicated that fish grown in T2 and T3 consistently had higher efficiency, however, significant difference was demonstrated only on the second sampling period (12 DOC).

Notwithstanding the lack of statistical significance among FCE values, results suggest that the feed requirement to produce a kilogram of tilapia juveniles is lower in T2 (2.11 kg) and other treatments than in the control (2.77 kg) (Table 2).

Biochemical composition of fish muscle tissues

Crude protein content of muscle tissues from tilapia juveniles decreased with increasing period of starvation. Protein content ranged from 16.89% (D0) to 16.15% (D5) (Fig 4a, Table 8). Crude protein content of fish muscle after subsequent periods of feed deprivation was similar although this observation is not so conclusive as the results were not subjected to statistical analysis due to lack of replication.

Crude fat or lipid content (Fig. 4b, Table 8) likewise decreased with the length of starvation period. Changes in crude fat were more remarkable than in protein as fat is easily lost during periods of feed deprivation. Crude fat decreased from 9.24% on day 0 to 5.42% on the fifth day.

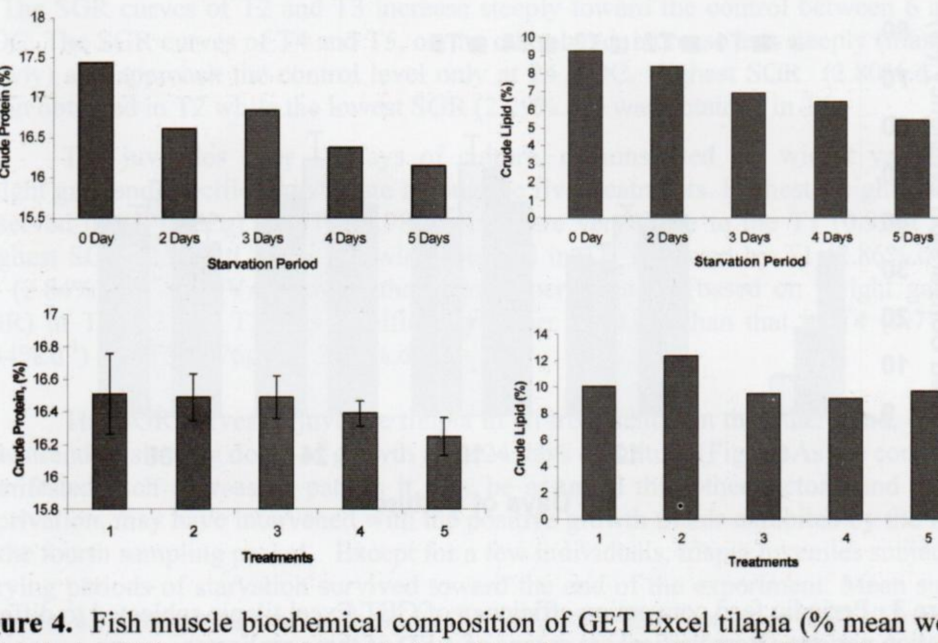

Figure 4. Fish muscle biochemical composition of GET Excel tilapia (% mean wet wt. basis) during different starvation periods (a, b) and upon termination of experiment (c, d). Vertical bars are means of ± SD of three replicates

Moisture content slightly increased as the period of starvation lengthened. Moisture content ranged from 80.34% (D0) to 81.37% (D5). Crude protein, crude fat, and moisture content during this period were not subjected to statistical analysis due to lack of replications of the samples.

Upon termination of the experiment, both crude protein and moisture content were similar among treatments (Fig. 4c, Table 9) and protein content did not vary significantly (P>0.05). Crude fat level returned to normal with the highest level observed in T2 (12.37%), which exceeded the initial level, and lowest in T4 (9.12%) (Fig. 4d, Tables 9). No statistical analysis was done in crude fat due to lack of replications of the samples.

Table 8. Fish muscle biochemical composition of GET Excel tilapia (% mean wet wt. basis) during different starvation periods. The starvation time (day, D) were D1 (control), D2, D3, D4, and D5 refers to 0, 2, 3, 4, and 5 days feed deprivation, respectively.

Parameters	Starvation Period (days)				
	0	2	3	4	5
Crude Protein (%)	16.89	16.61	16.84	16.38	16.15
Crude Lipid (%)	9.24	8.39	6.87	6.46	5.42
Moisture (%)	80.34	80.55	80.68	80.74	81.37

Table 9. Fish muscle biochemical composition of GET Excel tilapia (% mean wet wt. basis) upon termination of experiment. Values are mean ± SD of three replicates.

Parameters	Treatments				
	1	2	3	4	5
Crude Protein (%)	16.51 ±0.43	16.49 ±0.25	16.49 ±0.23	16.39 ±0.13	16.25 ±0.20
Crude Lipid (%)	10.05	12.37	9.48	9.12	9.67
Moisture (%)	79.67	79.03	79.83	80.62	80.48

Note: Mean values obtained are not significantly different (ANOVA, P > 0.05).

Physico-chemical parameters

Water temperature in the aquaria ranged between 23.9 - 26.2°C and 26.0 - 28.6°C before and after water management, respectively and 25.4 - 27.6°C during the afternoon. Dissolved oxygen ranged between 5.78 - 8.42 mg $O.L^{-1}$ and 5.53 - 7.79 mg $O.L^{-1}$ before and after water management, respectively and 6.10 - 7.95 mg $O.L^{-1}$ during the afternoon. Total ammonia nitrogen (TAN) ranged between 0.10 - 0.69 mg $N.L^{-1}$ before and after water management.

DISCUSSION

Results of the study have demonstrated that tilapia juveniles subjected to varying periods of starvation are capable of compensatory growth once refeeding was administered. Both absolute growth and specific growth rates also indicate that growth performance following feed deprivation can even exceed that of the control. This type of growth response is generally referred to as compensatory or 'catch-up' growth (Russell and Wootton, 1992) that leads to the same body size as the controls. Overcompensation, a type of compensatory growth that results in a larger body size than control individuals (Skalski *et al*., 2005), is known to occur in previously starved fish. This phenomenon was observed in T2 and T3 individuals.

Growth performance in T4 and T5 lagged behind the rest of the treatments but like T2 and T3 showed marked improvement in feed conversion efficiency. During the fourth period, feed conversion efficiency of the control (T1) was significantly lower than the rest of the treatments. Although the selected groups had undergone periods of starvation, total feed intake was relatively the same among treatments (i.e., no significant differences from the control). This result illustrates that extra feed was ingested when the juveniles were hyperphagic, and that the additional feed did not provide an extra boost to growth as observed in other studies (Hayward *et al*., 1997).

The length of starvation affected the degree of growth compensation in this study. Fish deprived of feed for longer periods grew more slowly than fish starved for shorter periods. It has been demonstrated that compensatory growth is dependent upon the severity and duration of feed restriction (Jobling *et al*., 1993). Larger fish may require longer periods of feeding restriction before the 'nutritional stress' becomes sufficiently severe to induce a compensatory growth response (Jobling *et al*., 1994). It should be noted, however, that because of non-availability of bigger holding tanks, the fish used in the present study were juveniles (initial average weight of 1.46g). Although juvenile fish might be too small to handle severe starvation, results of this study imply that a short period of starvation is enough to trigger compensatory growth in GET Excel tilapia juveniles.

Partial growth recovery was apparent in T2 and T3 juveniles 12 days after starvation and in T4 and T5 juveniles after 18 days. By the end of the 30-day experiment, full growth recovery in terms of weight gain and SGR was observed in starved GET Excel tilapia, indicating that this fish is capable of a complete compensatory growth response after a short period (5 days) of feed restriction. This result is similar to the work of Kim and Lovell (1995) who found that catfish fed daily to apparent satiation or restrictively fed for 3, 6, or 9 weeks exhibited the same weight, feed conversion, dressing yield, and body composition values in all feeding regimes. Other studies reported observations on overcompensation, such as the work of Hayward *et al*. (1997) on hybrid bluegill which significantly outgrew continuously fed controls after repeated two-day starvation and re-feeding schedule.

The apparent slowing down of the growth toward the end of the 30-day culture can be attributed to the carrying capacity of the culture tank, i.e., the tank is too small for the bigger-sized juveniles undergoing compensatory growth. Carrying capacity of the culture tank would limit growth rate of fish within optimal levels dictated by the culture environment. Under optimal conditions, the juveniles would still be growing at an exponential phase in a sigmoidal pattern, where growth rate eventually slows down upon nearing the asymptotic weight (W_∞). Declining trend in SGR after only 30 DOC points to two important management options: the need to increase size of the culture vessel after 24 DOC, or reduction in stocking density of tilapia juveniles.

Higher daily feed intakes in starved groups than in the control upon resumption of feeding is a behavioral characteristic described as hyperphagia, where most starved animals displayed excessive consumption (beyond the normal rate) upon refeeding (Jobling, 1994). Hyperphagia, however, was observed to be short-term (about two to three days) in this study. Feeding rates in T2 ($7.19\%.BW.d^{-1}$) and T3 ($8.05\%.BW.d^{-1}$) were higher than in the control ($5.95\%.BW.d^{-1}$). This means that a fish starved for two or three days must be given one or two percent higher feed ration than normally given to enable the fish to catch up with the growth of continuously fed fish.

Hyperphagia is often observed during the period of compensation (Saether and Jobling, 1999) and is accompanied by rapid growth and improved feed conversion. In some cases, starved animals have been reported to show improved feed conversion without becoming hyperphagic (Jobling, 1994). Food conversion is usually reported in terms of weight gain per unit weight of feed consumed, so it is possible that the improvements in conversion observed in restricted-refed animals could be related to differences in the composition of the tissues deposited during recovery and normal growth. The differences in feed conversion could indicate differences in metabolic expenditure between restricted-refed and continuously fed animals.

Low mortalities among fish in all treatments (including the control) may be attributed to diverse physiological conditions of the juveniles rather than as a result of starvation. The juveniles came from 30 breeders and were randomly selected from the pooled samples, hence, may have variable tolerance to environmental stress. Mortality could have also been caused by stress from frequent physical handling during periodic weighing of the fish.

The decrease in muscle tissue protein among starved fish at the end of the experiment was also observed by Satoh *et al.* (1984) in adult Nile tilapia (*Oreochromis niloticus*) fasted for 60 days. Like protein, lipid content fish tissue also decreased. This result conforms to the work of Quinton and Blake (1990) in which carcass lipid of the rainbow trout decreased after three weeks of starvation. Researchers had suggested that in short-time starvation visceral fats and muscle fats are utilized as the energy source.

SUMMARY AND CONCLUSION

This study has demonstrated that GET Excel tilapia juveniles exhibited a positive compensatory growth after being subjected to varying periods of starvation. Juveniles starved for shorter periods (2-3 days) recovered faster (<18 days) and showed accelerated growth earlier than those starved for a longer period (4-5 days). Catch-up growth occurred early and at a faster rate when the fish was subjected to shorter starvation time compared to longer period of food deprivation. Differences among treatments, however, were not significant, except during the second and third sampling periods when juveniles starved for shorter periods (T2 and T3) obtained significantly faster growth than juveniles starved longer (T4 and T5).

Survival of starved juveniles was high, indicating that starvation of up to 5 days does not harm the fish nor retard their growth. Starved tilapia also obtained high feed conversion efficiency (FCE) once feeding was reestablished, although a short period (2-3 days) of hyperphagic condition or accelerated feeding was observed.

Crude protein content of muscle tissues from tilapia juveniles apparently decreased with increasing period of starvation, however, final values obtained from all treatments at the end of the experiment did not differ significantly. Crude fat or lipid content likewise decreased with the length of starvation period. Moisture content slightly increased as the period of starvation lengthened.

Results of this study have an important implication on the production of GET Excel tilapia particularly on its early stages. Juveniles can easily recover from short-term starvation without reducing weight gain, SGR, FCE, and protein content. The ability of tilapia juveniles for compensatory growth following feed deprivation can prove to be advantageous and economical for aquaculture during unforeseen situations (e.g. severe weather conditions and shortage of feed supply) when feeding the juveniles for short periods is not possible.

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