SUPPLEMENTAL FEEDING FOR SEMI-INTENSIVE CULTURE OF RED TILAPIA IN BRACKISHWATER PONDS

Ninth Work Plan, New Aquaculture Systems/New Species Research 5 (9NS5)
Final Report

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ABSTRACT

An experiment was conducted at the Asian Institute of Technology, Thailand, to investigate effects of feeding regimes on growth of sex-reversed Thai red tilapia (*Oreochromis* sp.). There were five different supplemental feeding regimes: 0, 25, 50, 75, and 100% of satiation. Red tilapia fingerlings (33.2 to 33.4 g size) were stocked at 62.5 fish m^{-3} in fifteen 0.8-m^3 net cages suspended in a 200-m^2 earthen pond and cultured for 90 days. The pond was maintained at 10‰ salinity and fertilized weekly at rates of 4 kg N and 1 kg P ha^{-1} d^{-1}. Growth performance of red tilapia was significantly better in the feeding treatments than in the non-feeding treatment. Red tilapia growth and average feeding rate increased, but the Feed Conversion Ratio (FCR) and net economic return decreased with increasing percentages of satiation feeding levels from 25 to 100%. Considering low FCR, good growth and yield performance, high economic return, and potential for growing to greater size, 50% satiation feeding was the most efficient feeding rate.

INTRODUCTION

Many tilapia species are euryhaline and can grow in saline water after proper acclimation (Suresh and Lin, 1992). Varieties of red tilapia have been successfully cultured in saline waters (Watanabe, 1991). However, most of those tilapia culture trials were conducted in intensive systems with pelleted feeds, requiring frequent water exchanges or use of cages. Compared to the voluminous literature available for semi-intensive culture of tilapia in freshwater ponds, information on semi-intensive culture in saline ponds is almost nonexistent. On the other hand, there has been a strong desire to culture tilapia in brackishwater ponds in Southeast Asia as well as in Central and South America during the last few years (Green, 1997). There are a large number of shrimp ponds available in these regions, resulting from either failure in shrimp farming or desires to diversify shrimp culture. These ponds provide a good opportunity for aquaculture, and tilapia appears to be the most appropriate choice for such a culture system because there are few other domesticated finfish species that feed on low-cost natural foods, such as detritus and plankton. This interest in brackishwater culture is particularly strong in Thailand and Vietnam, where shrimp culture is now commonly reduced to one crop per year, leaving the ponds empty for half a year. Tilapia culture is also attractive to shrimp farmers to utilize abundant phytoplankton in shrimp ponds or in pond effluents.

The results from a recent PD/A CRSP experiment conducted in Thailand showed that Thai red tilapia (*Oreochromis* sp.) grew better at 10‰ than at other salinities in ponds fertilized using common CRSP fertilization guidelines (4 kg N and 1 kg P ha^{-1} d^{-1}; Yi et al., 2002). However, under this semi-intensive culture system with fertilizer as the sole nutrient input, growth of red tilapia slowed down after they reached 100 g, resulting in small size at harvest (about 150 g) after five months of culture. Market price in many countries is much higher for larger tilapia. To rear tilapia to a size greater than 500 g to fetch the higher market price, supplemental feeds are usually required. In freshwater ponds the most efficient culture system is to grow tilapia to 100 to 150 g with fertilizer alone, then begin supplemental feeding at 50% satiation feeding level (Diana et al., 1994, 1996). However, such information is not available for red tilapia culture in brackish water.

The purpose of this experiment was to determine the appropriate levels of supplemental feeding in fertilized ponds with 10‰ salinity for maximum growth of Thai red tilapia.

METHODS AND MATERIALS

This experiment was carried out at the Asian Institute of Technology (AIT), Thailand, from March through June 2001. The experiment was conducted using Thai red tilapia in a randomized complete block design in 15 cages (1 × 1 × 1.2 m) suspended in a 200-m^2 fertilized earthen pond at 10‰ salinity. Five treatments were used to test effects of different supplemental feeding regimes: 1) 0% (no feeding); 2) 25% satiation feeding; 3) 50% satiation feeding; 4) 75% satiation feeding; and 5) 100% satiation feeding. Satiation rations in treatments 2 through 5 were determined by estimating the total amount of floating pelleted feed (crude protein 30%, Charoen Pokphand

Co., Ltd., Bangkok, Thailand) consumed by red tilapia from 0800 to 0900 h and 1400 to 1500 h every Saturday. The tested percentages of satiation feeding were used to calculate the amount of feed to apply for respective treatments from Sunday through Friday.

Sex-reversed fingerlings of Thai red tilapia (33.2 to 33.4 g size) were purchased from a local farm and acclimated to 10‰ salinity in acclimation tanks by raising salinity level 5‰ every two days. The fingerlings were then stocked at 62.5 fish m^{-3} in all cages on 30 March 2001. During the experiment average weights of red tilapia were determined biweekly by bulk weighing 50% of the initial stock in each cage. Fish were randomly sampled by dip net. Red tilapia in every cage were harvested, counted, and bulk weighed on 28 June 2001 after 90 days of culture. Daily weight gain (g $fish^{-1}$ d^{-1}), yield (kg m^{-3}), and extrapolated yield (kg m^{-3} yr^{-1}) were calculated.

The pond was fertilized weekly with urea and triple superphosphate (TSP) at 4 kg N and 1 kg P ha^{-1} d^{-1}. Initial pond fertilization took place two weeks prior to stocking fish. Salinity was initially regulated by trucking hypersaline water (150‰) to AIT and diluting it to 10‰. Salinity was maintained at 10‰ and monitored weekly. Water depths in the pond and cages were maintained at 1.0 and 0.8 m, respectively, throughout the experiment by adding water at about 10‰ salinity weekly to replace evaporation and seepage losses. All cages were aerated for six hours daily from 0200 to 0800 h using one airstone in each cage.

Integrated water samples were collected from the entire water column near the two ends and the center of the pond and also from each cage biweekly at approximately 0900 h. Analyses were done for pH, alkalinity, total ammonium nitrogen (TAN), nitrite-nitrogen (nitrite-N), nitrate-nitrogen (nitrate-N), total Kjeldahl nitrogen (TKN), soluble reactive phosphorus (SRP), total phosphorus (TP), chlorophyll *a*, total suspended solids (TSS), and total volatile solids (TVS) using standard methods (Parsons et al., 1984; APHA et al., 1985; Egna et al., 1987). At the time of collecting water samples, Secchi disk visibility was measured using a Secchi disk, while temperature and dissolved oxygen (DO) were measured with a YSI model 54 oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Diel measurements for temperature, DO, and pH were conducted in each pond at 0600, 0900, 1600, 1800, 2200, and 0600 h once a month.

Data were analyzed statistically using analysis of variance, paired-sample t-test, and linear regression (Steele and Torrie, 1980) with SPSS (version 7.0) statistical software package (SPSS Inc., Chicago, Illinois, USA). Differences were considered significant at an alpha of 0.05. Statistical analyses for survival rates (%) were performed on data after arcsine transformation. Mean values of survival rates in this text are listed in normal scale followed by their confidence limits. All other means are given with ± 1 standard error (SE).

A partial budget analysis was conducted to determine economic returns of red tilapia in the different treatments (Shang, 1990). The analysis was based on farm-gate prices in Thailand for harvested red tilapia and current local market prices for all other items expressed in US dollars (US$1 = 40 baht). Farm-gate prices of red tilapia were $0.50 and $0.75 kg^{-1} for the sizes 100 to 200 g and 200 to 300 g, respectively. Market prices of sex-reversed red tilapia fingerlings ($0.0125 $piece^{-1}$), electricity ($0.05 kWh^{-1}), pelleted floating feed ($0.4875 kg^{-1}), urea ($0.1875 kg^{-1}), and TSP ($0.3125 kg^{-1}) were applied to the analysis. The calculation for cost of working capital was based on an annual interest rate of 8%.

RESULTS

Survival of red tilapia in cages ranged from 96.7 to 99.8% and did not differ significantly among treatments ($P > 0.05$). Significant difference in caged red tilapia growth between the non-feeding treatment (0% satiation feeding) and the feeding treatments (25 through 100% satiation feeding) was shown at the first fish sampling (15 d after stocking, $P < 0.05$), while

Table 1. Growth performance of all-male Nile tilapia fed at 0, 25, 50, 75, and 100% of satiation in 0.8-m^3 cages. Mean values with different superscript letters in the same row were significantly different among treatments ($P < 0.05$).

Performance Measures	Treatments				
	0%	*25%*	*50%*	*75%*	*100%*
STOCKING					
Total Weight (kg $cage^{-1}$)	1.7 ± 0.0	1.7 ± 0.0	1.7 ± 0.0	1.7 ± 0.0	1.7 ± 0.0
Mean Weight (g $fish^{-1}$)	33.3 ± 0.0	33.3 ± 0.1	33.3 ± 0.0	33.3 ± 0.0	33.3 ± 0.0
HARVEST					
Total Weight (kg $cage^{-1}$)	6.9 ± 0.1^a	9.8 ± 0.1^b	10.8 ± 0.4^c	11.8 ± 0.1^d	12.4 ± 0.3^d
Mean Weight (g $fish^{-1}$)	138.4 ± 2.2^a	203.2 ± 2.5^b	221.5 ± 5.0^c	241.9 ± 5.1^d	253.9 ± 2.8^d
Mean Weight Gain (g $fish^{-1}$)	105.1 ± 2.2^a	169.9 ± 2.5^b	188.2 ± 5.0^c	208.6 ± 5.1^d	220.6 ± 2.8^d
Daily Weight Gain (g $fish^{-1}$ d^{-1})	1.17 ± 0.10^a	1.92 ± 0.14^b	2.11 ± 0.18^c	2.33 ± 0.15^d	2.47 ± 0.19^e
Net Yield (kg m^{-3} $crop^{-1}$)	6.5 ± 0.1^a	10.2 ± 0.1^b	11.4 ± 0.5^c	12.7 ± 0.2^d	13.4 ± 0.3^d
(kg m^{-3} yr^{-1})	26.4 ± 0.4^a	41.3 ± 0.3^b	46.3 ± 2.2^c	51.6 ± 0.6^d	54.2 ± 1.4^d
Gross Yield (kg m^{-3} $crop^{-1}$)	8.6 ± 0.1^a	12.3 ± 0.1^b	13.5 ± 0.5^c	14.8 ± 0.2^d	54.2 ± 1.4^d
(kg m^{-3} yr^{-1})	34.8 ± 0.4^a	49.8 ± 0.3^b	54.7 ± 2.2^c	60.0 ± 0.6^d	62.6 ± 1.4^d
FCR	----	0.67 ± 0.01^a	0.93 ± 0.04^b	1.15 ± 0.01^c	1.28 ± 0.03^d
Survival (%)	99.8	96.7	98.3	99.3	98.3
	(93.8–100.0)	(93.0–99.1)	(82.0–100.0)	(82.1–100.0)	(82.0–100.0)

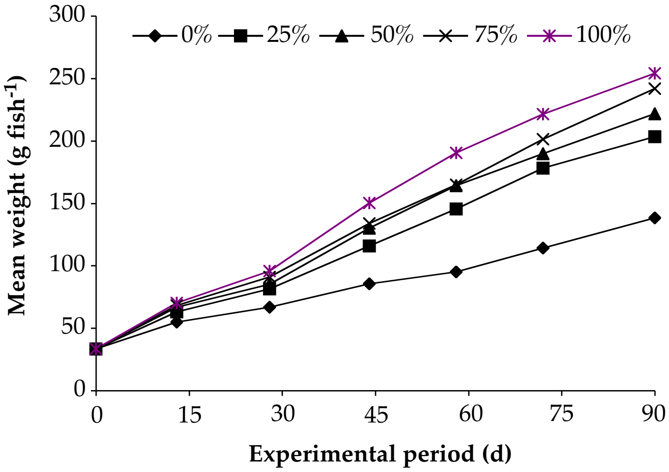

Figure 1. Growth of Thai red tilapia fed at 0, 25, 50, 75, and 100% satiation feeding levels over the 90-day experimental period.

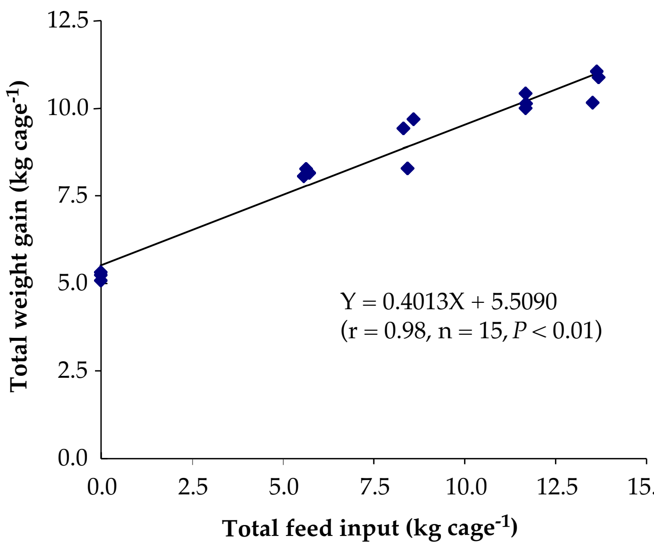

Figure 2. Relationship between total feed input to cages and total weight gain of caged red tilapia.

significant differences among feeding treatments were detected starting from the second fish sampling (30 d after stocking, $P < 0.05$, Figure 1). Growth and yield of caged red tilapia increased significantly with increased feeding rates from 0 to 75% satiation ($P < 0.05$), while there were no significant differences between the 75 and 100% satiation feeding treatments ($P > 0.05$). Mean daily weight gains were significantly different among treatments, with the highest value in the 100% satiation feeding treatment ($P < 0.05$, Table 1).

The feed conversion ratio was significantly better in treatments with lower percentages of satiation feeding ($P < 0.05$, Table 1). Total weight gain of caged red tilapia was linearly and positively correlated ($r = 0.98$, $n = 15$, $P < 0.01$) with total feed input to cages (Figure 2). Compared to weight gain in the non-feeding treatment, addition of feed at 25, 50, 75, and 100% satiation feeding resulted in 57, 75, 95, and 105% greater weight gain, respectively (Figure 3). By subtracting weight gain in the non-feeding treatment from that in the feeding treatments, natural food appears to contribute to 64, 57, 51, and 49% of the energy for weight gain in treatments with 25, 50, 75, and 100% satiation feeding levels, respectively. Feeding rates

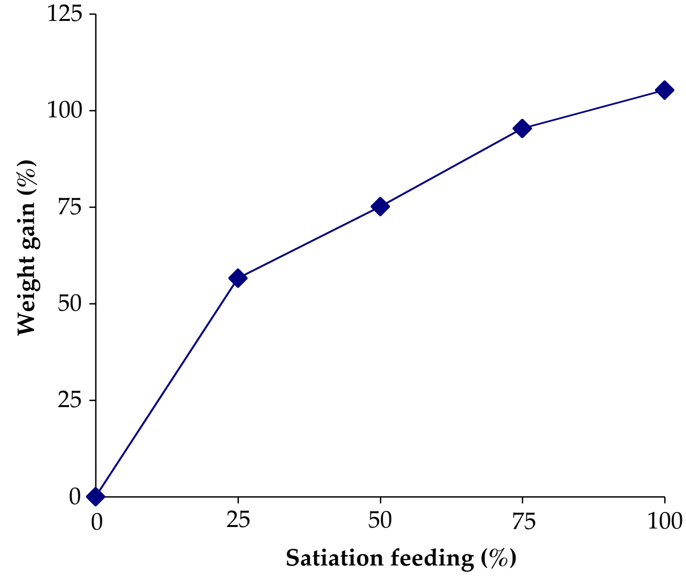

Figure 3. Percentages of additional total weight gains for each feeding treatment compared to the total weight gain of non-feeding treatment.

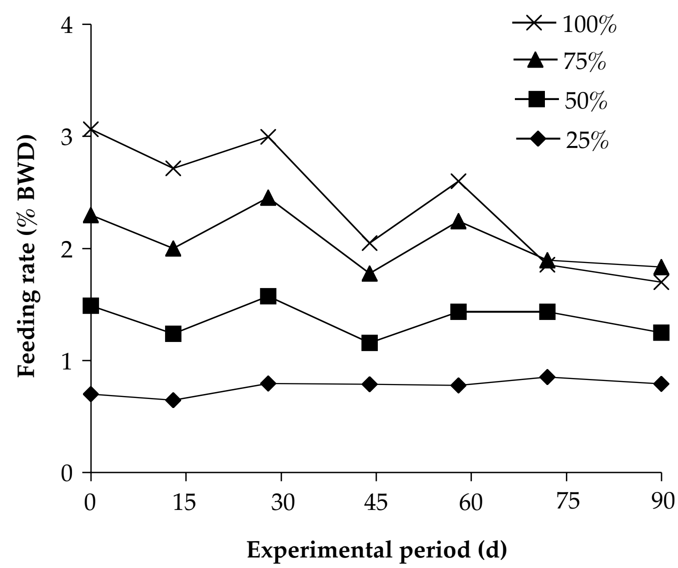

Figure 4. Mean feeding rates (% BWD) for red tilapia at 25, 50, 75, and 100% satiation feeding levels over the 90-day experimental period.

varied from 0.64 to 3.06% body weight per day (BWD) in the feeding treatments and increased with increasing percentage of satiation feeding levels (Figure 4). The average feeding rates were 0.76, 1.37, 2.07, and 2.42% BWD in the feeding treatments with 25, 50, 75, and 100% satiation feeding. Feeding rates also appeared to decline over time in the highest feeding treatments.

All water quality parameters except Secchi disk visibility were measured in cages and open water, and no significant differences were found among cages or between cages and open water ($P > 0.05$). The mean values of water quality parameters measured in cages and ponds at each sampling are summarized in Table 2. Mean temperature ranged from 29.9 to 32.2°C. Values of pH at the end of the experiment were significantly higher that those at the beginning ($P < 0.05$). Concentrations of

Table 2. Mean (± SE) values of water quality parameters measured in cages and open water at 0900 h (except DO, which was measured at dawn) at various points during the experimental period. Parameters (excluding Secchi disk visibility) marked with an asterisk had significant differences among the time periods.

Parameters	Day 6	Day 19	Day 34	Day 47	Day 60	Day 74	Day 88
*DO at Dawn (mg l^{-1})	4.61 ± 0.00	----	2.33 ± 0.02	----	1.68 ± 0.01	----	0.30 ± 0.00
Temperature (°C)	31.8 ± 0.0	32.2 ± 0.0	30.0 ± 0.0	32.9 ± 0.0	31.0 ± 0.0	29.9 ± 0.0	30.3 ± 0.0
*pH	7.9 ± 0.0	6.5 ± 0.0	6.2 ± 0.0	6.7 ± 0.0	6.5 ± 0.0	5.2 ± 0.0	5.9 ± 0.0
*Alkalinity (mg l^{-1} as $CaCO_3$)	78 ± 1	42 ± 1	42 ± 1	37 ± 0	15 ± 1	10 ± 0	13 ± 1
*TKN (mg l^{-1})	2.61 ± 0.08	3.29 ± 0.20	6.11 ± 0.13	5.79 ± 0.17	2.41 ± 0.18	3.13 ± 0.08	5.70 ± 0.24
*TAN (mg l^{-1})	0.56 ± 0.03	2.30 ± 0.02	4.59 ± 0.04	3.62 ± 0.15	0.89 ± 0.09	2.21 ± 0.02	2.84 ± 0.03
*Nitrite-N (mg l^{-1})	0.05 ± 0.00	0.24 ± 0.00	0.24 ± 0.00	0.80 ± 0.00	0.28 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
*Nitrate-N (mg l^{-1})	0.23 ± 0.00	0.19 ± 0.00	0.06 ± 0.00	0.58 ± 0.02	0.09 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
*TP (mg l^{-1})	0.16 ± 0.00	0.10 ± 0.00	0.43 ± 0.00	0.17 ± 0.00	0.22 ± 0.00	0.91 ± 0.01	0.44 ± 0.01
*SRP (mg l^{-1})	0.01 ± 0.00	0.03 ± 0.00	0.14 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.43 ± 0.01	0.07 ± 0.00
*Chlorophyll *a* (mg m^{-3})	61 ± 3	14 ± 1	33 ± 1	39 ± 4	49 ± 1	52 ± 4	100 ± 3
*TSS (mg l^{-1})	76 ± 2	79 ± 1	54 ± 1	55 ± 1	96 ± 4	61 ± 1	69 ± 1
TVS (mg l^{-1})	29 ± 2	48 ± 1	19 ± 1	24 ± 1	48 ± 3	27 ± 1	33 ± 1
Secchi Disk Visibility (cm)	55	100	64	64	62	61	41

Table 3. Partial budget analysis (in US$) for red tilapia in each experimental treatment.

Parameters	Feeding Treatments				
	0%	*25%*	*50%*	*75%*	*100%*
GROSS REVENUE					
Red Tilapia	3.45	7.35	8.10	8.85	9.30
Total	3.45	7.35	8.10	8.85	9.30
VARIABLE COST					
Red Tilapia Fingerlings	0.63	0.63	0.63	0.63	0.63
Urea	0.21	0.21	0.21	0.21	0.21
TSP	0.21	0.21	0.21	0.21	0.21
Pelleted Feed	0.00	2.75	4.12	5.69	6.64
Electricity	1.35	1.35	1.35	1.35	1.35
Cost of Working Capital	0.19	0.41	0.52	0.65	0.72
Total	2.59	5.56	7.04	8.74	9.76
NET RETURN	0.86	1.79	1.06	0.11	–0.46

DO at dawn and alkalinity decreased significantly over time ($P < 0.05$). Concentrations of nutrient parameters fluctuated throughout the experimental period, with TKN, TAN, TP, and SRP increasing significantly but nitrite-N and nitrate-N decreasing significantly through the experiment ($P < 0.05$). Concentrations of chlorophyll *a* and TSS were significantly higher at the end than at the beginning of the experiment ($P < 0.05$), while TVS concentrations were not significantly different ($P > 0.05$).

The partial budget analysis for our data indicated that red tilapia culture with supplemental feeding at 25 to 75% satiation feeding levels was profitable (Table 3). Net return in the feeding treatments decreased with increased feeding levels, and the 100% satiation feeding resulted in a negative net return. The net return in the non-feeding treatment was lower than that in both the 25 and 50% satiation feedings but greater than that in the 75 and 100% satiation feedings.

DISCUSSION

Physical, chemical, and biological parameters did not differ among cages or between cages and open water in the present experiment, indicating that all red tilapia had a similar culture environment and equal access to natural foods. Compared to non-feeding cages, the addition of supplemental feed resulted in significantly higher yields and growth rates. The growth differential between non-fed and fed red tilapia started during the first two weeks of culture, similar to results for Nile tilapia (*Oreochromis niloticus*) cultured in freshwater ponds (Diana et al., 1994), while the growth differential among different levels of food input began after the first two weeks of culture and continued to differentiate throughout the experiment. Moav et al. (1977) reported insignificantly different production between fish with and without supplemental feeding in fertilized freshwater polyculture ponds, which was likely due to lower densities of stocked fish (Diana et al., 1994).

Hepher (1978) developed the concept of critical standing crop (CSC), which is the biomass of fish in any aquaculture system that results in growth reductions for each individual. In the present experiment, treatments differed significantly in growth of red tilapia from the first biweekly fish sampling, and growth continued linearly until harvest, indicating that CSC had to occur at a size less than 50 g. The results support findings in Nile tilapia grown in freshwater ponds (Diana et al., 1994).

The good growth (1.17 g d^{-1}) of red tilapia at high density in non-feeding cages indicates that natural foods were abundant and important to growth in this experiment. This growth rate was comparable to or higher than that in other studies (Green, 1992; Diana et al., 1994, 1996). The good growth of non-fed red tilapia also indicate the abundant natural foods in the experimental pond in the present experiment, which was further implied by declining alkalinity throughout the experiment due to carbon utilization for photosynthesis (Knud-Hansen et al., 1991). Tilapias under culture conditions prefer artificial to natural foods (Schroeder, 1978), and thus, the contribution of natural foods to red tilapia may decrease with increasing availability of artificial feed, giving higher FCR in the treatments with higher percentages of satiation feeding. In other words, supplemental feed was more effective for incremental fish growth at lower percentages of satiation feeding.

Combined fertilizer and feed application resulted in efficient use of nutrients (Green, 1992; Diana et al., 1994, 1996). Applying feed at a lower percentage of satiation makes tilapia more efficient at utilizing natural food. In a previous study, Diana et al. (1994) determined 50 to 75% satiation feeding to be the most efficient feeding rates for Nile tilapia culture in freshwater ponds. The partial budget analysis in the present experiment showed that 25% satiation feeding was most profitable, followed by 50% satiation feeding. However, growth started to slow at the end of the culture period in the 25% satiation feeding (Figure 1). It appears that a 25% ration was not sufficient to support further growth to a size (> 500 g) that would fetch higher market prices. Considering low FCR, good growth and yield performance, high economic return, and potential for growing to greater size, 50% satiation feeding is the most efficient feeding rate in the present experiment.

ANTICIPATED BENEFITS

This experiment showed that Thai red tilapia culture is profitable in cages without feeding or with 25 to 75% satiation feeding in fertilized brackishwater ponds. It also demonstrated that 50% satiation feeding is the most efficient rate. These data provide farmers with a cost-efficient alternative to culture red tilapia in semi-intensive fertilization systems supplemented with pelleted feed using underutilized or abandoned shrimp ponds in coastal zones. Culture of red tilapia in low-salinity brackish water over time may help reclaim these pond areas for agriculture or freshwater aquaculture.

ACKNOWLEDGMENTS

The authors wish to acknowledge the Asian Institute of Technology, Thailand, for providing the research, field, and laboratory facilities. Mr. Manoj Y., Mr. Supat P., Mr. Nhan D.T, and Mr. Tuan H.M. are greatly appreciated for their field and laboratory assistance.

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Cite as: [Author(s), 2002. Title.] In: K. McElwee, K. Lewis, M. Nidiffer, and P. Buitrago (Editors), Nineteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, [pp. ___.]

FATE OF METHYLTESTOSTERONE IN THE POND ENVIRONMENT: USE OF MT IN EARTHEN PONDS WITH NO RECORD OF HORMONE USAGE

Ninth Work Plan, Effluents and Pollution Research 2D (9ER2D)
Final Report

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ABSTRACT

The following study examined the persistence of 17α-methyltestosterone (MT) in the environment after its use for masculinizing Nile tilapia in nursery ponds located in the Universidad Juárez Autónoma de Tabasco, Mexico. Fry harvested from spawning ponds were treated with a masculinizing dose of MT (60 mg kg^{-1}) for four weeks. Concentrations of MT were determined by radioimmunoassay. MT was not detectable in the water at any time. In the sediments, MT was not detectable during the first 10 days of treatment. Afterwards MT was detectable in all sampling points (mean = 146.7 pg g^{-1}; SE = 21.3). MT values varied from not detectable to 368.9 pg g^{-1}. Masculinizing efficiency was low in the first trial (87.4% males) but increased significantly afterwards, reaching 92.6% males in the second trial and 98.7% in the third trial.

Another outcome of this investigation is a manual on tilapia masculinization using synthetic steroids. This manual is intended to reach fry producers, extension agents and technicians; it contains a general description of the biology of the tilapias, traditional culture practices, masculinization methods, and a detailed section on safe handling of steroids.

INTRODUCTION

In tilapia culture the production of all-male populations through treatment of fry with 17α-methyltestosterone (MT)–impregnated food has become the most popular procedure. All-male populations have greater growth potential because no energy is shunted toward reproduction and no competition with younger fish occurs (Green et al., 1997). Contreras-Sánchez (2001) demonstrated that significant "leakage" of MT to water and sediments occurs in small, closed systems, probably from uneaten or unmetabolized food. This leakage poses a risk of unintended exposure of hatchery workers as well as fish or other non-target aquatic organisms to anabolic steroids if MT persists in the environment after treatment of tilapia fry.

Some researchers have warned about unintended effects of steroid administration, such as fish-to-fish transfer of steroids (Budworth and Senger, 1993), biased sex ratios in untargeted organisms (Abucay et al., 1997), and paradoxical feminization (Rinchard et al., 1999; Eding et al., 1999). If MT is being added to the food in amounts that efficiently masculinize fish despite steroid loss to the environment, then determining the fate of MT in semi-closed systems such as ponds will yield important information on both safety and efficacy of MT use for masculinization. To determine if MT is detectable within the pond environment, the following study was undertaken.

METHODS AND MATERIALS

Laboratory of Aquaculture at UJAT

Nile tilapia, *Oreochromis niloticus*, fry were collected daily from a spawning tank. Fry were selected by grading with a 3-mm mesh (Popma and Green, 1990), counted, and randomly assigned to either MT-feeding or ethanol (EtOH; vehicle)–feeding treatments. Fry were housed in 1 × 1 × 0.75 m hapas made of mosquito mesh, and hapas were placed in a 7 × 15 m earthen pond. The MT-treated experimental units were located at one end of the pond and the control units at the other end. Three masculinization trials were established at different dates throughout the experiment. A total of seven hapas were assigned to each treatment: three for trial 1 (density = 3,000 fry $hapa^{-1}$), two for trial 2 (density = 2,000 fry $hapa^{-1}$), and two for trial 3 (density = 5,000 fry $hapa^{-1}$). The number of fry per treatment was established based on fry availability.

MT-impregnated food was made by spraying crushed flaked food with MT dissolved in EtOH; control food was made by spraying crushed flaked food with EtOH. Fry were fed MT (60 mg kg^{-1}) or control diet for four weeks. Feeding rate was at 20% per calculated body weight for the first 23 days of treatment and then 10% per calculated body weight through 28 days of treatment (Popma and Green, 1990). After 28 days of dietary treatment, fry were moved