Reducing Fertilization Rate and Nitrogen Input have no Effect on Nile Tilapia Production in Periphyton-Based Culture with Supplemental Feeding

Wirat Jiwyam*, Ratchaneegorn Mapanao and Nudtha Nithikulworawong

ABSTRACT

Nile tilapia, *Oreochromis niloticus*, with an average individual weight of 87.4 g at a density 3 fish·m^{-2} were used to determine growth and production in periphyton-based tanks. The trial was conducted in 10 m^2 concrete tanks for a 120-day period. A 10 cm deep layer of soil was placed at the bottom of each tank. Bamboo poles with an approximate submerged surface area of 50% of the total tank surface area were inserted vertically into the soil to act as substrate for periphyton. A biweekly fertilizer dose of triple superphosphate (TSP) and cow manure at the rates of 35 g·tank^{-1} and 250 g·tank^{-1}, respectively, were applied to the experimental tanks. In order to test the effect of reducing nitrogen input, four levels of urea were used with TSP and cow manure; 60, 30, 15 or 7.5 g·tank^{-1}. The fish were fed daily with a commercial diet containing 30% crude protein at 50% satiation throughout the experimental period. The results indicated that reduction of fertilization rate and nitrogen input have no effect on water quality or production of Nile tilapia in periphyton-based culture. The system gave an average gross yield of 0.86 kg·m^{-2} with 92% survival.

Keywords: Fertilization, Nitrogen input, Periphyton-based, Supplementary feeding

INTRODUCTION

The assemblage of attached organisms on a submerged surface, including associated non-attached fauna, is referred to as periphyton (van Dam *et al*., 2002). In aquaculture, periphyton has been used to improve water quality and the production of cultured species (Khatoon *et al*., 2007). Adding substrates to enhance periphyton growth in fertilized ponds has been shown to increase fish production. A number of studies have shown higher fish production in ponds with substrates for periphyton development than in ponds without substrates (Hem and Avit, 1994; Wahab *et al*., 1999; Ramesh *et al*., 1999; Keshavanath *et al*., 2001; Azim *et al*., 2001a; Azim *et al*., 2002a). This is due to the fact that filter feeding of planktonic algae is unlikely to fully cover the energy demands of most herbivorous carp and tilapia species (Dempster *et al*., 1995). Therefore, they generally require additional food sources such as benthic algae, algal detritus or plant fodder (Dempster *et al*., 1993; Yakupitiyage, 1993). Periphyton that grows on a substrate in freshwater ponds can serve as one of these additional food sources (Azim and Wahab, 2005). Improving the conversion of nutrients into harvestable products, through the adoption of periphyton-based production in existing pond systems, is one solution worth exploring (Azim *et al*., 2003). Additionally, Nile tilapia can grow better by grazing on periphyton rather than filtering suspended algae from the water column (Huchette *et al*., 2000; Azim *et al*., 2002b).

Program of Fisheries Science, Faculty of Applied Science and Engineering, Nong Khai Campus, Khon Kaen University, Nong Khai Province, Thailand
* Corresponding author. E-mail address: wirjiw@kku.ac.th
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Based on a recommended fertilization scheme (Lin *et al*., 1997), maximum yields of tilapia are attained with nitrogen inputs of 2 to 4 $kg·N^{-1}·ha^{-1}·day^{-1}$, phosphorus input levels sufficient to prevent P limitation, and a N:P ratio of 4:1. However, the current practice of fertilizer- and supplemental feed- applied systems are inefficient; only 5 to 15% of nutrients are retained in the fish biomass (Edwards, 1993). Most of the remainder is lost to the sediments. Improving the efficiency of nutrient use is of major importance in improving the low input systems of aquaculture technologies (Azim *et al*., 2004). Periphyton-based systems have shown higher nutrient utilization efficiency when compared to traditional substrate-free systems (Verdegem *et al*., 2005; Uddin, 2007). An optimum fertilization rate for the traditional substrate-free ponds has been expected to be sufficient for maximizing fish production in periphyton-based systems. However, the optimum fertilization rate for periphyton-based ponds was the bi-weekly (every two weeks) application of cow manure, urea and TSP at rates of 4500, 150 and 150 $kg·ha^{-1}$, respectively (Azim *et al*., 2001c). Feeding at 50% satiation ration, while adding fertilization, could produce growth similar to 100% satiation feeding (Diana, 1997). However, there is currently no data about the effect of reducing fertilization rate and nitrogen input on Nile tilapia production in periphyton-based ponds with supplementary feeding. The reduction of nutrient inputs may be beneficial for reducing production costs and the nutrients retained in pond effluent, which impact the environment. Therefore, the purpose of this study was to evaluate the effect of lowering fertilization rate and nitrogen input on growth and production of Nile tilapia *O. niloticus* in a periphyton-based aquaculture system with supplementary feeding.

MATERIALS AND METHODS

The experiment was conducted at Khon Kaen University's Nong Khai Campus, Thailand, from August to October 2010. The trial was conducted in twelve concrete tanks 2.75x3.75x1.2 m), each which had a 10-cm layer of sun-dried bottom soil from an earthen fishpond placed on the bottom. All experimental tanks were filled with well water, and the water depth was maintained at 80±2 cm above the soil surface throughout the trial. Bamboo poles (average diameter 5.5 cm, length 150 cm) were placed vertically into the tank bottom, at a total density of 35 poles per tank (3.5 $poles·m^{-2}$), giving an additional submerged surface area approximately equal to 50% of the total tank surface area.

Based on recommended of fertilization rates for Nile tilapia in Thailand (Lin *et al*. 1997), all tanks were fertilized with urea, triple super phosphate (TSP) and dried cow manure at two weeks intervals (Table 1). Before the fertilizers were applied, the urea was dissolved in water, and the TSP was soaked in water overnight. After the first fertilization and bamboo pole installation, the tanks were left for 10 days to allow plankton development in the water column and for periphyton growth on the bamboo substrates, and then stocked with fish. Thereafter, fertilization continued at a bi-weekly intervals throughout the experimental period.

Table 1. Fertilization regimes ($g·tank^{-1}·2\text{-}week^{-1}$ interval) for Nile tilapia raised in experimental concrete tanks.

Treatment	Fertilization regime	N (g)	P (g)	N:P
T1	60 g. Urea + 35 g. TSP + 250 g. cow manure	32.75	8.4	3.90: 1
T2	30 g. Urea + 35 g. TSP + 250 g. cow manure	18.75	8.4	2.23: 1
T3	15 g. Urea + 35 g. TSP + 250 g. cow manure	11.75	8.4	1.40: 1
T4	7.5 g. Urea + 35 g. TSP + 250 g. cow manure	8.25	8.4	0.98: 1

Note: N in cow manure = 1.9 %; P in cow manure = 0.56%

A population of sex-reversed juveniles of Nile tilapia with an average weight of 87.39± 14.10 g were stocked in the tanks in triplicate at a density of 30 fish·tank^{-1}. During the experiment the fish were fed with a commercial pellet containing 30% crude protein at 50% satiation level daily. At the first feeding, fish were hand-fed twice a day at 8:00 and 17:00 to apparent satiation (100% satiation), and daily feed intake was recorded. After that, the 50% satiation level was determined based on the average feed consumption of fish in the first feeding. The amount of feed was re-adjusted every two weeks. All fish were weighed at the beginning and at the end of the experiment.

Fish from all treatments were harvested after 120 days, and the final weight, weight gain, specific growth rate, yield and survival rate were calculated as follows: final mean weight (g) = Tw/Nf, where Tw = sum of individual weights of harvested fish and Nf = number of fish; mean weight gain (g·fish^{-1}) = (Wf – Wi), where Wi = initial mean weight and Wf = final mean weight; specific growth rate (%) = 100 [(lnWf – lnWi)]/culture period (days), gross yield (g·m^{-2}) = Tw/10 m^2; net yield (g·m^{-2}) = (Tw – Ti)/10 m^2, where Ti = sum of individual weights of stocked fish; survival rate (%) = 100 Nf/Ni, where Nf and Ni = final and initial number of fish, respectively. At the end of the experiment, surviving fish were randomly sampled from each tank and measured for body weight and total length.

Water quality in the experimental tanks was monitored weekly between 09:00 and 10:00 AM each sampling day at a depth of 25 cm. The tanks were monitored for water temperature (YSI model 52), dissolved oxygen (APHA, 1989), and pH and conductivity (Hach sensION 5). Composite column water samples were collected every two weeks, and analyzed for chlorophyll *a* (acetone extraction), total ammonia nitrogen (phenate method), nitrite-N (diazotization), nitrate (cadmium reduction and diazotization), soluble reactive phosphorus (ascorbic acid method), total phosphorous (persulfate digestion and ascorbic acid finish), total dissolved solids, total suspended solids, total alkalinity and total hardness following the Standard Methods for the Examination of Water and Wastewater (APHA, 1989). Total nitrogen was analyzed following Stirling (1985). Periphyton samples was collected 10 days after the substrate installation, and were taken at two-week intervals thereafter. At each sampling time, one pole from each tank was randomly selected and a 10x10 cm -band of periphyton was sampled at the mid- depth for dry matter (DM) and ash-free dry matter (AFDM) content analyses. Dry matter was determined by drying the samples overnight at 105 °C to a constant weight, from which the ash content was determined using a muffle furnace (4 h at 540 °C). The final weight of the crucible along with the remains was taken in order to calculate the amount of ash and ash-free dry matter.

Plankton samples were collected by passing 5 L of water taken from each pond through a plankton net (mesh size 45 μm) and made up to a standard volume of 100 mL with distilled water. Samples were preserved with 5% buffered formalin. Plankton numbers were counted using a Sedgewick–Rafter counting cell (S-R cell). One millilitre of sample was placed in to the counting chamber of the S-R cell and was left to stand for 15 min to allow plankton to settle. The plankton on 10 randomly selected fields of the chamber were counted under a microscope. Plankton density was calculated using the following formula:

$$N = (P \times C) / L$$

where N = the number of plankton cells or units per litre of original water, P = the number of plankton counted in 10 fields, C = the volume of final concentrate of the sample (mL) and L = the volume (L) of the pond water sample.

Identification of plankton to genus level was carried out using the keys from Ward and Whipple (1959), Prescott (1962) and Bellinger (1992).

The data on final mean weight, mean weight gain, specific growth rate, survival rate and yield were compared using analysis of variances (ANOVA) and the Tukey-HSD test. The assumptions of normal distribution and homogeneity of the variances were met. The differences were considered statistically significant at an alpha level below 0.05. Treatment means are presented as mean ± standard error (SE).

RESULTS

Table 2 shows the means of water quality parameters during the experimental period. None of the means were significantly different among treatments (P > 0.05). The mean values for total ammonia ranged from 0.15-0.17 $mg \cdot l^{-1}$. The mean values of pH and dissolved oxygen were 7.88-8.08 and 3.06-4.13 $mg \cdot l^{-1}$, respectively. The mean values of Secchi disc visibility and chlorophyll *a* were 22.3-26.8 cm and 299-453 $\mu g \cdot l^{-1}$, respectively. Table 3 shows the mean values of growth and yield

Table 2. Average values (mean ± SE) for water quality parameters in experimental tanks during 120-days rearing Nile tilapia.

Parameters	Treatments			
	1	2	3	4
pH	8.08±0.07	7.92±0.08	7.88±0.07	7.93±0.05
Temperature (ºC)	27.59±0.52	27.62±0.54	27.66±0.53	27.67±0.53
Secchi disc visibility (cm)	22.29±1.3	25.21±2.41	23.60±1.80	26.75±2.46
$NH_3 + NH_4^+$- N (($mg \cdot l^{-1}$)	0.16±0.03	0.15±0.02	0.16±0.02	0.17±0.03
NO_2- -N ($mg \cdot l^{-1}$)	0.03±0.02	0.03±0.02	0.02±0.00	0.02±0.00
NO_3- -N ($mg \cdot l^{-1}$)	0.24±0.03	0.26±0.04	0.27±0.02	0.24±0.02
Total nitrogen ($mg \cdot l^{-1}$)	1.15±0.17	1.05±0.14	1.05±0.14	1.03±0.13
PO_4^{3-} -P ($mg \cdot l^{-1}$)	0.32±0.07	0.33±0.07	0.36±0.08	0.34±0.07
Total phosphorus ($mg \cdot l^{-1}$)	0.78±0.14	0.81±0.13	0.85±0.16	081±0.12
Chlorophyll *a* ($\mu g \cdot l^{-1}$)	453.5±85.8	384.0±74.9	406.7±78.8	298.9±59.3
Dissolved Oxygen ($mg \cdot l^{-1}$)	3.97±0.63	3.82±0.49	4.13±0.43	3.06±0.41
Total alkalinity (mg $CaCO_3 \cdot l^{-1}$)	340.1±9.6	346.5±12.2	354.80±9.32	347.33±16.31
Total hardness ($mg \cdot l^{-1}$)	209.4±7.4	209.7±9.2	219.47±8.68	225.63±11.24

Table 3. Growth parameters (mean ± SE) of Nile tilapia in periphyton-based tanks, fertilized with various N: P ratios, and fed at 50% satiation for a 120-day culture period.

Growth parameters	Treatments			
	1	2	3	4
Initial mean weight (g)	88.81±1.69	87.38±1.40	87.25±1.44	86.13±1.41
Initial mean length (cm)	17.12±0.08	17.04±0.09	17.05±0.09	17.09±0.05
Final mean length (cm)	25.6±0.2	26.2±0.1	26.8±0.1	28.6±2.4
Final mean weight (g)	290.9±7.3^a	315.0±5.8^b	330.3±5.6^b	317.6±5.4^b
Mean weight gain (g)	199.5±43.8	226.6±4.5	242.5±21.4	231.8±13.8
Specific growth rate ((%$\cdot day^{-2}$)	1.04±0.14	1.14±0.03	1.18±0.06	1.16±0.04
Survival rate (%)	93.3±6.7	78.3±21.7	97.8±2.2	98.9±1.1
Feed conversion ratio	0.92±0.26	1.32±0.64	0.73±0.08	0.69±0.04
Gross yield ($kg \cdot m^{-2}$)	0.82±0.18	0.74±0.21	0.97±0.07	0.94±0.03
Net yield ($kg \cdot m^{-2}$)	0.55±0.18	0.48±0.22	0.71±0.08	0.69±0.03

Means in the same row with different superscripts are significantly different ($p < 0.05$).

parameters of the experimental fish. The mean initial weights of the experimental fish were not significantly different ($P > 0.05$). Final mean weight of the fish at the highest nitrogen fertilization level (T1) was significantly lower than for fish at the lower nitrogen fertilization levels ($p < 0.05$). Meanwhile, no significant difference in mean weight gain, specific growth rate, survival rate, feed conversion ratio or yield was found, among all nitrogen fertilization levels. Specific growth rates from all fertilization levels ranged from 1.0-1.2 %·day^{-1}. Survival rates of fish from all treatments were higher than 78%, and were not significantly different ($P > 0.05$). Gross yields were also not significantly different among treatments ($P > 0.05$).

Periphyton biomass was not significantly different among treatments (Table 4), and the mean dry matter ranged from 2.2 – 2.8 mg·cm^{-2}. The groups of plankton found in experimental tanks and their abundance during the experimental period are presented in Table 5.

Table 4. Average periphyton biomass on bamboo poles in experimental tanks during a 120-day culture period of Nile tilapia.

Biomass (mg·cm^{-1})	Treatment			
	1	2	3	4
Dry matter	2.82±0.44	2.15±0.33	2.21±0.20	2.61±0.31
Ash	1.11±0.12	0.91±0.13	0.87±0.06	0.89±0.10
Ash-free dry matter	1.72±0.33	1.24±0.22	1.33±0.14	1.73±0.23

Table 5. Abundance of plankton (cells or colonies·l^{-1}) found in periphyton-based tanks during a 120-day culture period of Nile tilapia.

Group/Genus	Treatments			
	1	2	3	4
Phytoplankton				
Bacillariophyta				
Gyrosigma	0-9,000	0-4,670	0-13,330	0-18,000
Nitzschia	0-1,670	0-0	0-330	0-330
Pleurosigma	0-330	0-1,670	0-31,000	0-1,330
Chlorophyta				
Chlorella	-	0-20,670	-	-
Oocystis	0-1,000	0-1,000	0-670	0-4,670
Scenedesmus	-	0-330		0-1,000
Spirogyra	0-10,000	0-330	0-10,000	0-500
Volvox	-	-	0-670	-
Cyanophyta				
Microcystis	0-8,330	0-8,330	330-5,000	0-7,670
Euglenophyceae	1,670-44,670	670-16,330	3,330-32,000	3,000-26,330
Zooplankton				
Rotifera	0-1,000	0-330	-	0-330
Ameoba	0-2,000	0-5,000	0-2,670	-
Other				
Clostridium	0-3,330	0-330	-	-

DISCUSSION

Values for ash-free dry matter of periphyton during the rearing period ranged from 1.2-1.7 mg·cm^{-2}. These values are relatively higher than those reported in several previous studies; 0.73-1.40 mg·cm^{-2} (Azim *et al*., 2001b), 0.04-0.79 mg·cm^{-2} (Azim *et al*., 2002a) and 0.10-0.28 mg·cm^{-2} (Keshavanath *et al*., 2004); however, it was similar to results reported by Azim *et al.* (2001c), of 2.06 mg·cm^{-2}. High gross yield produced in the present study might indicate that optimizing both supplementary feeding and fertilization regimes in periphyton-based systems can further improve Nile tilapia yield. There was no significant difference among treatments in any of the water quality parameters. The levels of total ammonia in all treatments did not reach the level to induce stress in tilapia in pond culture (Lin *et al*., 1997). Although, there were no significant differences in water quality parameters or survival rate among treatments, the results showed that the tanks receiving higher nitrogen fertilizer inputs may lead to higher total nitrogen concentration in the water and lower survival rate of fish. High concentrations of un-ionized ammonia may occur occasionally at high nitrogen fertilization levels, and may affect the survival of the fish (Keshavanath *et al*. 2004). Additionally, a higher survival rate in treatment 4 may have led to higher individual growth of the fish, as indicated by the higher final mean weight. This suggests that lowering the nitrogen fertilizer level may not negatively affect fish growth in periphyton-based tanks, but may, in fact, improve fish survival.

In addition, the final mean weight of the fish in treatment 2 was not significantly different from treatment 3, which had a higher survival rates. This indicates that nitrogen fertilizer can be reduced to a ratio (N:P) of 0.98–1.4 in periphyton-based tanks with supplementary feeding, without having an effect on growth or production of Nile tilapia. However, investigative studies of nutrient limitation show mixed results. In most freshwater studies, phosphorous was identified as the limiting nutrient (Ghosh and Gaur, 1994; Vymazal *et al*., 1994), but nitrogen (Barnese and Schelske, 1994), carbon (Sherman and Fairchild, 1989) and silica can also be limiting, depending on the algal species and on other environmental factors such as hardness and acidity (van Dam *et al*., 2002). High Si:N or Si:P ratios favored diatoms, low N:P ratios favored cyanophytes and high N:P ratios favored chlorophytes in periphyton of the Baltic Sea (Sommer, 1996). Similarly, high Si:P and N:P ratios favored diatoms, and low N:P and Si:P ratios favored cyanophytes in a reservoir in Patagonia (Baffico and Pedroso, 1996). The plankton population indicates the productive status of a pond, representing both direct and indirect sources of food for the fish. Phytoplankton composition in this study was representative of that found in Azim *et al*. (2001a) and Azim *et al*. (2001c). It is well established that phytoplankton productivity is positively correlated with nutrient concentrations (Boyd, 1990). The mean values of secchi disc depth and chlorophyll *a* concentration indicate that phytoplankton biomass may have reached the carrying capacity of the system. Phytoplankton can also affect production of algal periphyton in the system impeding sunlight penetration into the water column and thus hampering periphyton growth. Periphyton and phytoplankton respond differently to increasing amounts of available nutrients. In another study, phytoplankton concentration increased linearly with increasing nutrient concentration, while periphyton showed an optimum above which its density decreased, probably due to shading by phytoplankton and competition for nutrients (Azim *et al*., 2001c). In this study, pond with high secchi disc visibility and low chlorophyll *a* resulted in high periphyton biomass on bamboo poles, although there are no significantly different among treatment. The importance of self-shading in mature perihyton assemblages has already been mentioned and illustrated the importance of light for the productivity of periphyton (van Dam *et al*., 2002). Periphyton standing stock is reduced at greater depths because of reduced light incidence (Konan-Brou and Guiral, 1994; Azim *et al*., 2002a). Light is less important for the non-algal components of the periphyton, but non-algal periphyton probably benefits from organic exudates produced by the algal components (Kuehl *et al*., 1996; Romaní and Sabater, 2000).

In the present study, the highest net yield over the 120-day period was 710 $g{\cdot}m^{-1}$ (giving an estimated 21.6 $t{\cdot}ha^{-1}{\cdot}yr^{-1}$). These values gave an extrapolated yield of approximately 59.17 $kg{\cdot}ha^{-1}{\cdot}day^{-1}$. In other periphyton-based systems, reported yields include 5.94 $kg{\cdot}ha^{-1}{\cdot}day^{-1}$ of kalbbaush, *Labeo calbasu* (Wahab *et al*., 1999); 15.83 $kg{\cdot}ha^{-1}{\cdot}day^{-1}$ of rohu, *Labeo rohita* (Azim *et al*., 2001a); 8.37 $kg{\cdot}ha^{-1}{\cdot}day^{-1}$ of rohu and catla, *Catla catla* (Azim *et al*., 2002a). In systems where fish stocking is not limited, production level can be largely extended, as reported from "acadja" enclosure systems (4 to 20 $t{\cdot}ha^{-1}{\cdot}yr^{-1}$) (Welcomme, 1972; Hem and Avit, 1994). Nile tilapia production in ponds fertilized with urea and manure without supplementary feeding was about 0.2 $kg{\cdot}m^{-2}$ per four months (Lin *et al*., 1997), while production from organic tilapia to market size in periphyton-based ponds with reduced feed inputs was 0.5 $kg{\cdot}m^{-2}$ in a 87-day rearing period (Milstein *et al*., 2009) or equivalent to 0.69 $kg{\cdot}m^{-2}$ in a 120-day period, which is lower than the production in the present study. Elsewhere, Nile tilapia production in periphyton-based tanks fertilized with only chemical fertilizer and without supplementary feeding was 0.62 $kg{\cdot}m^{-2}$ for the same rearing period (Jiwyam, 2013). However, in that experiment the fish were stocked as fingerlings. The optimum fertilization rate for periphyton-based ponds was the bi-weekly application of cow manure, urea and TSP at rates of 4500, 150 and 150 $kg{\cdot}ha^{-1}$, respectively. It was estimated that this level of fertilization could support fish production of around 5,000 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$, without recourse to supplementary food (Azim *et al*., 2001c). This was far higher than the fertilization rate used in the present study, which is equivalent to the bi-weekly application of cow manure, urea and TSP at rates of 2500, 7.5-60 and 35 $kg{\cdot}ha^{-1}$, respectively. The estimated annual production from the present study was around 21,600 $kg{\cdot}ha^{-1}$, while the production of Nile tilapia, reared in fertilized ponds with supplementary feeding from another study ranged from 17,861 to 24,777 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ (Diana, 1997). However, the maximum fertilization rate used in the present study was only half of the previously cited study. In that study, feed conversion ratio when Nile tilapia were fed to satiation was 1.42, and reduced to 0.88 when the fish were fed at 50% satiation (Diana, 1997). The average feed conversion ratio from most treatments in the present study was 0.78. However, the higher production from this study when compared to the aforementioned study may suggest that growth and yield is a function of a number of biological and physical factors, including fish life stage, size, sex, social hierarchy and their tolerance to environmental changes, as well as configuration and hydrodynamics of the culture system. The results of this study indicated the benefit of periphyton-based aquaculture in increasing Nile tilapia production with reduced cost and may reduce the nitrogen concentration in pond effluent.

In conclusion, the results from this study indicate that N: P ratio in fertilizer did not affect growth and yield of Nile tilapia in fertilized periphyton-based tanks with supplementary feeding. Reducing nitrogen in fertilizer could reduce nitrogen concentration in the water, which may be retained in pond effluent. The results also indicated very high production of tilapia compared to several previous reports. Therefore, supplementary feeding at 50% satiation is considered optimum in semi-intensive culture using a periphyton-based system.

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