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Estimation of ammonia excretion rates during a period of red tilapia, *Oreochromis* sp. culture, considering biomass increase in a water recirculating system

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Abstract: A completely randomized experimental design was conducted to estimate ammonia excretion rate by red tilapia (*Oreochromis sp.*) during different stages of its growth in a recirculating water system units typically designed to meat the experiment purposes. Eight weight groups each containing 75individuals of 20, 30, 40, 60, 80, 120, 180 and 200g average weight were considered as treatments in duplicates. The rate of ammonia excretion by each fish group was measured during a 24-hr period. The data was extended to estimate total ammonia excreted by a group of red tilapia during a 115-day culture period (20-200g). The mean daily ammonia excretion rate was significantly different between treatments ($P<0.05$). These rates were 34.06±1.23, 32.56±3.28, 27.06±0.87, 17.46±2.19, 12.54±1.30, 12.48±1.41, 7.87±1.82 and 5.83±0.19mg kg fish^{-1} h^{-1} for 20, 30, 40, 60, 80, 120, 180 and 200 g fed fish, respectively. It was estimated that on average 39.4% of input

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feed nitrogen excreted as ammonia-N during the culture period. Results of this study well addressed the regime of ammonia production in different stage of red tilapia growth in a recirculating water system.

Keywords: Body weight, Ammonia excretion, Red tilapia, Recirculating water system

Introduction

Water quality problems in recirculating a aquaculture system are related to accumulation of fish metabolites (Fivelstad 1988 ; Colt & Watten, 1998). Nitrous oxide, nitric oxide, nitrate and ammonia/ammonium are soluble forms of nitrogen in the water, causing eutrophication of estuaries and coastal waters (Mandar & Forsberg, 2000). In this respect, ammonia is the major excretory products, originating from protein nitrogen metabolism in teleost fish which is formed in the liver, transported in the blood and excreted across the gills (Ramnarine *et al.*, 1987; Forsberg, 1996 ; Green *et al.*, 2002). Besides ammonia, smaller amounts of urea, tri-methylamine oxide, keratin and uric acid are excreted as well (Forster & Goldstein, 1969 ; Dosdat *et al.*, 1996 ; Iwata *et al.*, 2002). In aquaculture, control of waste is essential to manage high production of fish with the aim of reducing the produced pollutants and establishment of an equilibrium sustainable agriecosystem (Mumpton & Fisherman, 1997). Ammonia excretion is influenced by body weight (Savitz *et al.*, 1977 ; Ramnarine *et al.*, 1987), the composition of natural diets (Elliot, 1976), genetic differences in the utilization of dietary protein (Kaushik *et al.*, 1984 ; Ming, 1985), temperature, salinity, alkalinity and dissolved oxygen levels of water body (Davis & Stickney, 1978 ; Degani & Levanon, 1988 ; Brunty, 1997) and fish species (Brunty, 1997). Scientists have tried to address rates of ammonia excretion in different kind of fish in relation to factors affecting its excretion (Rychly, 1980 ; Kaushik 1981; Kaushik *et al.*, 1984 ; Ming, 1985 ; Fivelstad *et al.*, 1990 ; Kaushik & Cowey, 1991 ; Forsberg, 1996). Kaushik (1980) reported the effects of nutritional status on daily pattern of nitrogen excretion in cyprinids. Diurnal ammonia excretion rate by gilthead sea bream, *Sparus aurata*, (Porter *et al.*, 1987), sea bass, turbot and seabream (Dosdat *et al.*, 1996 ; Robaina *et al.*, 1999) was reported as well. However these data could not be practically used to estimate regime of ammonia excretion by a group of target fish during a period of culture in a culture system. There is few data, regarding ammonia excretion by red tilapia during a period of its culture from fry to marketable size (Lin, 1992 ; Brunty *et al.*, 1997 ; Rafiee, 2005). The influence of system performance associated with an increase on fish biomass has also not been well demonstrated in tilapia. Therefore, the main objective of this study was to evaluate the regime of ammonia excretion by red tilapia, as a factor of biomass increase, in a recirculating system.

Materials and Methods

This study was carried out at the Laboratory of Aqua-biotechnology Station, Faculty of Agriculture, University Putra Malaysia, in 2002. The total ammonia excretion (TAN = NH_3-N + NH_4^+-N) was evaluated in red tilapia in different weight classes. Eight weight groups of red tilapia with individual body weight of 20±1.25, 30±1.74, 40±1.17, 60±3.49, 80±4.67, 120±4.23, 180±6.46 and 200±7.12g (Mean ± SD) were used *in duplicate*. It was estimated that the mean individual fish weight in each group would reach the next mean individual weight group after a 21 day culture period (Rafiee *et al.*, 2002). Following recorded componential regression formula (A) of biomass increase [(Y= biomass of fish X= time (days)] was used in this research (Rafiee *et al.*, 2002).

$$\text{A)} \quad Y = 0.4244\,X^2 + 83.729\,X + 1318.7 \qquad R^2 = 0.99$$

Each experimental unit consisted of a rectangular fiberglass rearing tank (110 x 84 x 100cm) equipped with three raft hydroponic troughs (110 x 30 x 5cm) to limit penetration of light into the fish tank and reduce algal growth (Fig. 1). The troughs were fixed 20cm above the fish tank. Each tank was filled with 640 L of dichlorinated tap water and stocked with 75 fish. Therefore, the biomass of fish was equal to the number of fish × average weight of fish; i.e., 1.5, 2.25, 3, 4.5, 6, 9, 13.5 and 15Kg for the respective fish groups. A gentle aeration (3.1 L min^{-1}) was provided for each tank. Red tilapia groups were acclimatized to fish rearing tanks for one week. After acclimation process, each fish group netted, weighed and randomly transferred into the new similarly prepared rearing tank and cultured for a 24 hrs. period.

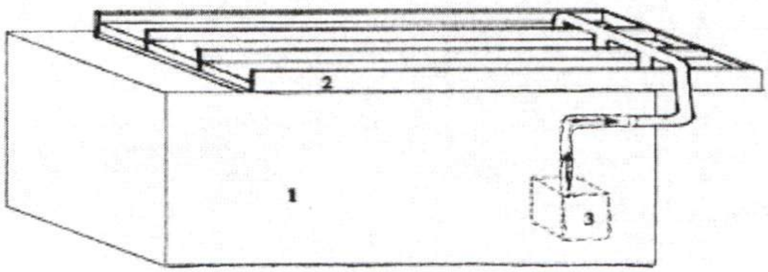


Figure 1: Schematic feature of the system; (1) the fish tank, (2) the hydroponics' troughs and (3) the water pump

The fish were fed twice a day *ad libitum* at 09.00 and 13.00 hrs. The feed was a commercial floating pellet (Car-gill Company), with 24% protein, 6% fat, 6% fiber and 11% moisture. The amount of feed consumed by fish was different among the different weight groups (Table 1).

Table 1: The amount of feed supplied (±SD) for feeding different weight groups of red tilapia during the experiment

Fish groups (g)	20	30	40	60	80	120	180	200
Feed (g)	70.54± 2.54	64.34± 9.25	136.15± 4.35	138.02± 17.22	115.16± 11.96	141.23± 16.00	109.08± 14.62	90.02± 3.06

Dissolved oxygen (DO), temperature (T) and pH, in fish rearing tanks were measured at the initiation of the experiment and 4, 8, 12, and 24 hrs. later. DO and T in fish rearing tanks were measured, using YSI instrument, Model 57. The pH of water was determined by Orion model 410A. pH, water temperature and dissolved oxygen ranged between 7.23-7.27, 26-29°C and 5.73-6.23 mg/L, respectively, throughout the course of experiment.

The concentration of total ammonia (NH_3-N + NH_4^+-N) was measured by taking three samples of water (100ml) from each fish tank. The samples were kept in a freezer (-18°C) for less than 4 hrs. until ammonia analysis process. Water sampling for total ammonia measurement was conducted at the same intervals as other parameters. The concentration of total ammonia-N was measured, using phenate method (APHA, 1984).

The TAN excretion rate by fish between two time intervals was calculated using the following formula:

$$A = V_2 (N_1 - N_2) / B / T_{2\text{-}1}$$

Where:

A = TAN excretion rate (mg kg $fish^{-1}$ h^{-1})

V_2 = volume of the water at the time 2 (L)

N_1 = TAN concentration at the time 1(mg L^{-1})

N_2 = TAN concentration at the time 2 (mg L^{-1})

B = total wet weight of the fish (kg)

$T_{2\text{-}1}$ = time interval between sampling 1 and 2 (h)

Total ammonia excretion within 24 hrs. (DM) was calculated based on the following experimental formula;

$$DM\ (\text{mg kg fish}^{-1}\ h^{-1}) = V[(N_2 - N_1)\times T_{2\text{-}1} + (N_3 - N_2)\times T_{3\text{-}2} + (N_4 - N_3)\times T_{4\text{-}3} + (N_5 - N_4)\times T_{5\text{-}4}] / 24 + B$$

Where:

DM = mean Daily total ammonia excretion (mg kg $fish^{-1}$ h^{-1})

The percentage of feed nitrogen excreted as ammonia-N was calculated based on data of protein-nitrogen (%) in diet and rate of daily consumed feed and daily total ammonia-N excreted by fish in each fish group and biomass of fish (APHA, 1984).

Data on ammonia excretion rates were subjected to One-way ANOVA and differences between the means were tested with Duncan's New Multiple Range Test, using SPSS, version 10.01 (Zar, 1999).

Results

Percentage of the input feed nitrogen excreted as ammonia-N for 20, 30, 40, 60 and 80g fish was lower compared to the other fish groups (Fig. 2). The highest and lowest feed nitrogen excretion rates of 54.2±8.4% and 30.49±5.33% were observed in groups of 180 and 60g fish, respectively.

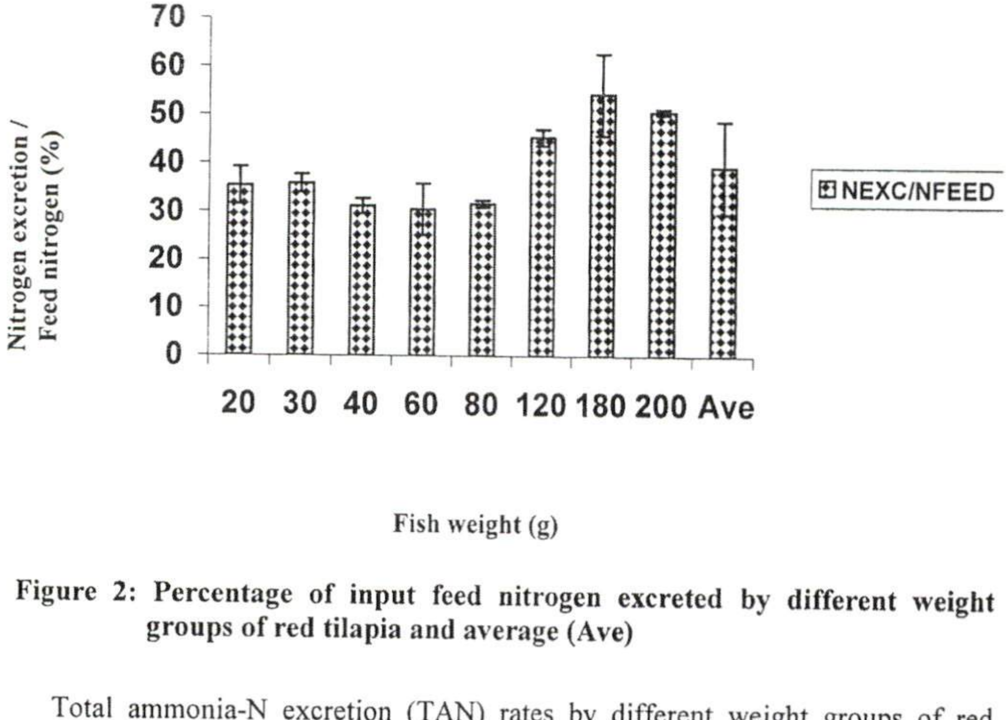


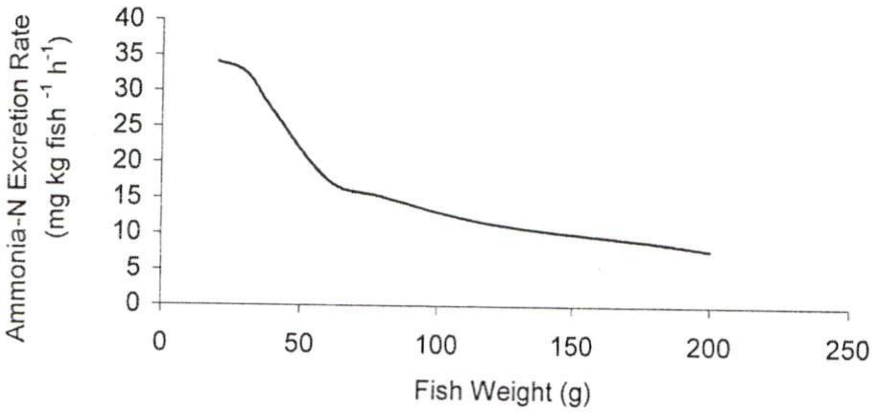
Figure 2: Percentage of input feed nitrogen excreted by different weight groups of red tilapia and average (Ave)

Total ammonia-N excretion (TAN) rates by different weight groups of red tilapia over the first, second, third and fourth time intervals and mean daily time periods is shown in Table 2. The mean of daily TAN excretion rates decreased with an increase in the fish biomass. These rates in 20, 30, 40, 60, 80, 120, 180 and 200g fish groups were 34.1, 32.6, 27.1, 17.5, 16.2, 12.5, 12.5, 8.9 and 5.9mg kg $fish^{-1}$ h^{-1}, respectively (Table 2). Maximum and minimum daily TAN excretion was recorded in groups with 20 and 200g fish, respectively. The results showed a significant correlation between the weight groups of the red tilapia and total ammonia excretion (r^2 = 0.947, P<0.01), (Fig. 3). The TAN excretion rate increased after feeding the fish in the morning and afternoon; this rate was higher in the morning compared to feeding time in afternoon. The maximum TAN excretion was recorded 8 hrs. after feeding the fish. Mean daily excretion of TAN for 20, 30, 40, 60, 80, 120, 180 and 200g fish groups is shown in Fig. 4. These results showed that the TAN excretion increased with an increase in the red tilapia biomass. There were also a significant correlation between biomass of fish and TAN excretion (r^2 = 0.988, P<0.01), (Fig. 4).

Table 2: The mean (±SD) total ammonia-N (mg kgfish^{-1} h^{-1}) excreted by the different weight classes of red tilapia within a 24 hrs. experimental period

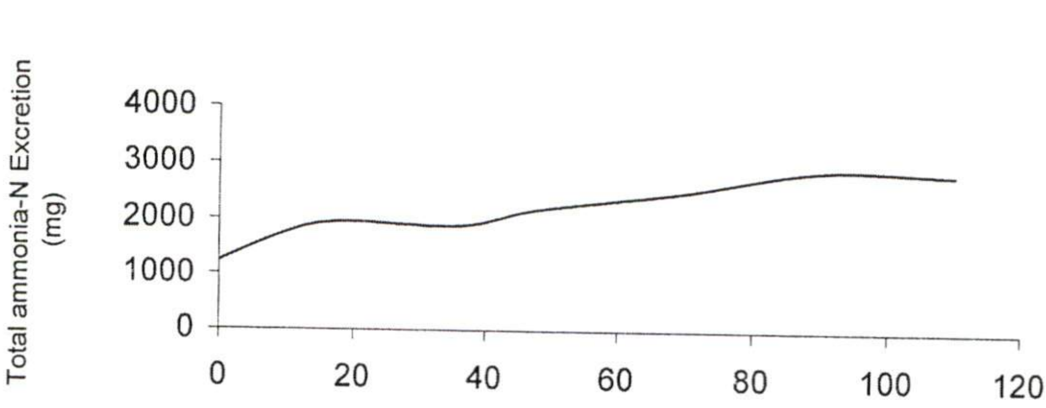
	Time Intervals				
Fish groups (g)	**9.00-13.00**	**13.00-17.00**	**17.00-21.00**	**21.00-9.00**	**Daily Mean**
20	26.45±13.27^b	73.39±14.48^c	31.57±4.82^c	24.32±1.41^d	34.06±1.23^d
30	37.55±0.44^c	67.54±4.46^c	39.45±11.15^c	16.94±4.07^c	32.56±3.28^d
40	24.89±2.34^b	60.68±5.36^c	35.79±3.02^c	13.67±0.10^c	27.06±0.87^c
60	13.11±4.44^a	33.44±4.82^b	34.37±3.77^c	7.95±2.27^b	17.46±2.19^b
80	16.66±5.33^a	34.68±8.51^b	16.05±1.98^b	6.61±1.54^b	12.54±1.30^b
120	19.25±1.44^a	26.47±4.53^b	13.07±2.23^b	5.52±0.00^b	12.48±1.41^b
180	12.15±1.23^a	12.43±1.54^a	7.88±1.30^a	4.91±1.47^a	7.87±1.82^a
200	11.58±1.32^a	6.57±0.59^a	8.89±0.20^a	2.54±1.06^a	5.83±0.19^a

Numbers followed by the same superscript letters in a column are not significantly different at the 0.05 % level.



A) $Y = 0.0014 X^2 - 0.4384 X + 42.303$ $R^2 = 0.97$

Figure 3: The rate of ammonia-N excretion by red tilapia during a period of culture as a factor of weight where Y= ammonia-N excretion and X= weight



A) $Y = - 0.0654 X^2 + 20.788 X + 1397.5$ $R^2 = 0.9307$

Figure 4: The total ammonia-N excretion by red tilapia as a factor of time in the culture system where Y= ammonia-N excretion by fish biomass and X= time (day)

Discussion

The results of present study indicated that daily mean excretion of ammonia (mg kg fish^{-1} h^{-1}) by red tilapia decrease with an increase in weight, considering the rate of feed consumption by fish (kg feed/kg fish), the input feed nitrogen excretion was different among the different weight groups of red tilapia. The lowest rate was recorded in 60g weight group (30%). According to the literature, the feed nitrogen excretion rates of 35-37%, 32%, 35%, 30-40%, 33-35% and 35% recorded in *Sparus aurata* (Porter *et al.*, 1987), juvenile of red tilapia (Shiau & Cheng, 1990), *Oncorhynchus mykiss* (Davis & Stickney, 1978 ; Bretta & Zalla, 1975 ; Mead, 1985), *Samo salar* (Kaushik, 1980,1981), *O. nreka* (Cai & Robert, 1992) and post-smolt of *S. salar* (Forsberg, 1996), respectively. The represented data confirm the same rate of input feed nitrogen, excreted as ammonia by red tilapia compared to the other teleost fish. Gerking (1971) indicated that the feed nitrogen excreted by the different sizes of bluegill sunfish (*Lepomis macrochirus*) as ammonia-N was different. A diurnal variation in ammonia excretion rate for 30g fingerlings of *Oncorhynchus nerka* was reported by Brett and Zalla (1975). Porter *et al.*, (1987) reported that rate of ammonia excretion by 30, 40, and 90g fish was 70.0, 36.4, and 25.2 mg kg^{-1} h^{-1}, respectively. This study showed that total ammonia excretion by red tilapia decreased with an increase in body weight. Significant correlation between ammonia excretion and weight of red tilapia suggest for possible prediction of ammonia excretion rate by fish in different stages of its growth (r^2 = 0.97, $P<0.01$).

It was also resulted that light or feeding time affecting ammonia excretion rates. In dark, period (at night), mean total ammonia excretion rates decreased in different weight groups of fed red tilapia. However, higher excretion of ammonia could be likely related to utilization of protein as a source of energy or more catabolism of protein within the dark period or a decrease in the environmental temperature (Kaushik, 1980 ; Kaushik & Cowey, 1991 ; Forsberg, 1996). If the assimilation rate of nitrogen by fish and bacteria were measured, the system performance and nitrogen dynamic was predictable and possible. Knowledge about the peaks of ammonia excretion associated with increasing the fish biomass and efficiency of designed system are necessary to balance the ratio between biological filter dimensions and removal of ammonia-N excreted by red tilapia, in this respect escaped ammonia–N from the culture system due to water recirculation, removal of ammonia by natural flora of bacteria in the system must be also determined, which can be considered for further studies. Thus the present data would be usable if incorporated with other data for prediction of ammonia excretion by red tilapia in any red tilapia culture system.

The results of this experiment indicated that an increase in fish biomass (fish growth) significantly affected ammonia excretion in the culture system. The mean of daily TAN excretion rates decreased while fish weight increased. It was also demonstrated that ammonia excretion numerically increased after feeding the fish. The input feed nitrogen excreted as ammonia varied in different weight groups of red tilapia, considering the same biomass/unit. On average, 39.54% of the input feed nitrogen excreted as ammonia-N by red tilapia in the culture system. The regime of ammonia production as a factor of culture time and fish biomass increase due to growth of fish during a period of red tilapia culture, was estimated as well (Fig. 4) and came into formulation follow as:

Total ammonia excretion (mg kg fish^{-1} h^{-1}) = 0.0014 (Weight)^2 - 0.4384 (Weight) + 42.303

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