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Is growth retardation present in Nile tilapia *Oreochromis niloticus* cultured in low water exchange recirculating aquaculture systems?

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ABSTRACT

It has been suggested that fish cultured in recirculating aquaculture systems (RAS) grow less as compared with fish cultured in flow-through systems due to the accumulation of substances. In the Netherlands, the commercial culture of Nile tilapia *Oreochromis niloticus* in 300 and 600 MT's systems is done exclusively in RAS operated at water exchange rates as low as 30 L/kg feed/day due to nitrate control by single-sludge denitrification reactors. The use of such nearly closed RAS raises the question whether growth retardation (GR) is present in Nile tilapia. This study is the first to investigate the existence of growth retardation in Nile tilapia by comparing the growth, feeding behaviour and stress response of Nile tilapia cultured in RAS with different levels of substances accumulated. Three RAS, operated at 30 L/kg feed/day (HIGH accumulation), 70 L/kg feed/day (MIDDLE accumulation) and 1500 L/kg feed/day (LOW accumulation) were used. Each RAS contained 24 glass aquaria with individually housed fish. To determine whether GR is size-dependent, per RAS 3 fish size categories were tested in the 57 day experimental period: large (288.7 ± 34.2 g; $N=8$), medium (162.4 ± 23.4 g; $N=8$) and small (81.4 ± 21.0 g; $N=8$). Experimental fish were fed *ad libitum*, twice per day. Feeding behaviour was determined once per week and was measured as the time taken by each fish to eat the first pellet (latency, LAT) and the total time spent feeding (total feeding time, TFT). Temperature, pH, conductivity, alkalinity, dissolved oxygen, dissolved CO_2, nitrogen compounds (TAN, NO_2-N and NO_3-N), chemical oxygen demand and orthophosphate-P were measured over time. At day 57 fish were weighed, blood sampled and returned to their tanks for an extra experimental period of 15 days, and subjected at day 72 to an acute stress followed by blood sampling. Blood was analysed for glucose and cortisol.

Results showed that the water quality parameters measured in the 3 RAS (with the exception of alkalinity) were still within the optimum range for growth of Nile tilapia. Large individuals showed a tendency to grow more in the LOW treatment (2.66 ± 1.35 g/kg$^{0.8}$/day) as compared with the MIDDLE treatment (0.93 ± 1.63 g/kg$^{0.8}$/day). On the contrary, small individuals grew significantly less in the LOW treatment (3.60 ± 1.74 g/kg$^{0.8}$/day) as compared with the HIGH (7.22 ± 1.58 g/kg$^{0.8}$/day) and MIDDLE (6.82 ± 4.54 g/kg$^{0.8}$/day) treatments. Small fish were more motivated to eat (lower latency) in the MIDDLE (4.63 ± 5.24 min) as compared with the LOW treatment (8.94 ± 6.41 min). In the HIGH accumulation treatment higher glucose levels were observed in the small fish, before and after acute stress, as compared with the LOW accumulation treatment. In conclusion, this study showed that the extent to which the accumulation of substances in RAS affects growth depends on fish size: large individuals show a trend towards growth retardation in the highest accumulation RAS while small individuals, on the contrary, seem to grow better in such systems.

1. Introduction

Over the past years, recirculating aquaculture systems (RAS) have seen an increased attention as the most sustainable type of aquaculture production system. Several reasons have contributed to this view, in particular their ability to reduce water and land use and allow an environmentally safe waste management treatment (Timmons and Ebeling, 2007). However, despite their environmental advantages, it has been suggested that fish cultured in RAS grow less compared to fish cultured in flow-through systems e.g., sea bass *Dicentrarchus labrax* (Deviller et al., 2005). The accumulation of substances, whether originating from the biofilter (e.g., bacteria metabolites) and/or the fish (e.g., alarm pheromones, cortisol) and/or the feed (e.g., heavy metals) (Martins et al., 2007, 2009) may be responsible for such growth retardation (GR). Accumulation of substances in RAS depends on feed composition, RAS configuration and the water exchange rate (L/kg feed/day). In the Netherlands conventional commercial RAS systems for eel and African catfish are operated at ±300 and 100 L/kg feed/day respectively. Innovative

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300 and 600 MT/year Nile tilapia *Oreochromis niloticus* RAS are exclusively operated at water exchange rates as low as 30 L/kg feed/day. These low water exchange RAS in Tilapia culture were realised through the introduction of commercial scale single-sludge denitrification reactors in 2005 (personal communication FishionAquaculture, Helmond, The Netherlands) using fish manure as the only carbon source to fuel the process. This development was forced by (1) restrictions in waste volume discharge to the local sewer (2–3 m^3/h), (2) groundwater permits restricting groundwater extraction at one production location to 10 m^3/h or less and (3) the energy costs for heating groundwater to a water temperature of 28 °C.

The use of nearly closed RAS raises the question whether these systems accumulate substances that may compromise Tilapia's growth. A recent study by Martins et al. (2009), compared the effect of substances accumulated in RAS on the development of early life stages of common carp. The authors showed a clear negative effect of water originated from high accumulation RAS on hatching percentage, embryonic development and larvae growth. They suggested that the accumulation of nitrate, orthophosphate-P and the heavy metals, arsenic and copper, in RAS, should be further explored as potential growth inhibiting factors.

To our knowledge, this study is the first to investigate growth retardation in a freshwater species, Nile tilapia, cultured in RAS. The objective of this study was to test whether growth retardation really occurs in Nile tilapia cultured in RAS (and not what the causative factors were). Therefore we decided to compare RAS with a maximal chance of detecting growth retardation. Because growth inhibiting factors (GIF) may originate from the fish, biofilter and/or the feed, it was our intention to operate RAS in which all possible sources were present. Therefore, the growth of Tilapia was compared between RAS different in stocking density, age of the biofilter, feed load and water exchange rate. The water quality, growth performance, feeding behaviour and stress response were compared between these systems in an attempt to understand the consequences for Tilapia of using nearly closed RAS as applied in practice.

2. Material and methods

2.1. Recirculating aquaculture systems

Three RAS were used (Fig. 1) differing in the level of water exchange per kg feed and thus on substances accumulated. As it is not known whether these substances arise from the accumulation of fish and/or system and/or feed related substances, a combination of all these potential sources were assumed to create the differences in the experimental treatments. Therefore, a high accumulation of substances was created in the HIGH accumulation RAS (total system volume was 4.1 m^3) by stocking all-male Nile tilapia (Swansea silver strain, TilAqua, The Netherlands) at high densities, at a high feed load, using an "old" biofilter (in use for more than 5 years) and a low water exchange rate (30 L/kg feed/day). The MEDIUM accumulation RAS (system volume was 4.1 m^3) was identical to HIGH accumulation RAS, except on the water exchange rate used which was 70 L/kg feed/day. Both HIGH and MEDIUM accumulation RAS used a suspended single-sludge denitrifying reactor fueled with fecal carbon waste only. This reactor was an upflow sludge blanket (USB) denitrifying reactor with a volume of 0.48 m^3, and an internal diameter of 0.38 m as previously described by Eding et al. (2003). All the effluent water from the hydrotech filter was used as inlet water in the denitrification reactor. The LOW accumulation RAS (total system volume was 2.9 m^3) functioned as a control system with low accumulation of substances using low stocking densities, low feed loads, "young" biofilter (in use for 1 month) and high water exchange rates (1500 L/kg feed/day, Table 1).

The HIGH and MIDDLE accumulation systems contained 4 tanks of 450 L (flow rate: 23 L/min), with high densities group-housed fish. The group-housed fish were used only as a possible source of fish-related GIF. As it is not known if fish-related substances that accumulate in RAS are produced by fish of a certain size class, 3 size classes were used in the group-housed fish (Table 2). These fish were fed restrictively using automatic feeding belts. The feed used was a commercial Tilapia feed (3 mm floating pellets; 44% crude protein, 10% fat, 25% carbohydrates, 11.5% ash; Skretting, France).

The HIGH and MIDDLE accumulation RAS contained each 24 glass aquaria (32 L, flow rate: 2 L/min) with individually housed Tilapia that shared the same inlet water as the group-housed fish. All fish-related measurements were done in these individually housed experimental animals as will be described later. The LOW accumulation RAS, contained only 24 tanks of 32 L with individually housed Tilapia (experimental fish, see later), thus low stocking density and low feed load.

RAS were kept undisturbed (no sampling procedures) during 57 days of growth period. Daily water exchange was performed according to a standard procedure consisting of calculating the water exchange based on the feed load of the previous day and refreshing 30 L/kg feed/day (HIGH), 70 L/kg feed/day (MIDDLE) or 1500 L/kg feed/day (LOW) from sump 1 (see Fig. 1; conductivity and alkalinity of supply water were 189 μS/cm and 72.5 mg $CaCO_3$/L, respectively). The total volume of water to be replaced was removed every day from the system which had its inlet tap closed. Only after the water removal was completed the new well water was allowed in the system. Alkalinity was never supplied to the systems throughout the experimental period. No water was lost from the systems except for water loss due to evaporation that was topped up before daily water exchange.

2.2. Experimental animals

The Tilapia used in the experiment were kept for 4 weeks in adaptation period in RAS without denitrification reactors operated at water exchange rates of 500 L/kg feed/day. During the experimental period, the inlet water of the group-housed fish tanks was common to the inlet water of 24 tanks of 40 L in which fish were housed individually. Individually housed fish were kept visually isolated from one another by placing a black plastic around the tank, except in the front side to allow daily observations of the fish. As Nile tilapia develops strong social hierarchies (de Oliveira Fernandes and Volpato, 1993), fish were kept isolated to overcome a possible influence of social rank on growth. It should be noted that previous experiments with individually housed Nile tilapia showed that the deprivation of social interaction does not appear to constitute a source of stress (Barcellos et al., 1999a).

Fish were fed *ad libitum* twice a day (08:00 and 16:00) by hand using the same feed used for the culture fish (see earlier). The feeding procedure consisted of providing sequential portions of 5 pellets to each fish, randomly. New pellets were added only after the first portion was completely eaten. The left over pellets were collected and counted after 1 h of feeding. Individual feed intake was recorded daily from the beginning of the experiment till day 57.

As it is not known whether GR is size-dependent, 3 size categories (8 individuals per size category) were used during the experiment. Starting weights for the 3 size categories were: 288.73 ± 34.15 g, 162.39 ± 23.41 g and 81.36 ± 20.87 g for the large, medium and small-size fish. Fish were weighed at the start and after 57 days of growth period.

2.3. Measurements

Individual fish-related measurements consisted of body weight (at the start and end of the 57 day growth period), daily feed intake, feeding behaviour and stress response.

Feeding behaviour was measured as latency (LAT, min) and total feeding time (TFT, min). Both behaviours were measured directly using a stopwatch. Latency was defined as the time that each fish took to eat the first pellet. Total feeding time was defined as the time between the first and the last pellet being consumed (Martins et al., 2005).