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Microcystin Accumulation in Nile Tilapia, *Oreochromis niloticus* and Giant Freshwater Prawns, *Macrobrachium rosenbergii* in Green Water System Cultivation

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ABSTRACT

Phytoplankton including blue-green algal or cyanobacterial blooms frequently occurred in aquaculture ponds. Some cyanobacteria produced cyanotoxins that may accumulate in the food web and eventually in the aquaculture products. In this study, accumulatation of microcystins in Nile tilapia (*Oreochromis niloticus*) and giant freshwater prawn (*Macrobrachium rosenbergii*) cultured in green water system was investigated. Nile tilapia was cultured in green water system and fish food; green water system with *Microcystis aeruginosa* Kützing and fish food and green water system with *M. aeruginosa*. Giant freshwater prawn was cultured: in green water systems with and without toxic *M. aeruginosa*. Microcystins of 8.32 ± 0.76 and 9.35 ± 1.45 $\mu g \cdot kg^{-1}$ d.w. were detected in fish cultured in green water system with *M. aeruginosa* and fish food and in green water system with *M. aeruginosa*, respectively. Microcystins of 14.42 ± 1.63 $\mu g \cdot kg^{-1}$ was found in prawn samples. It implied that aquaculture products were likely to be contaminated with microcystins. This finding is useful for aquaculture in terms of food safety.

Keywords: *Microcystis aeruginosa* Kützing; Microcystins; Aquaculture; Green Water System

1. Introduction

Thailand is the fourth ranking Nile tilapia (*Oreochromis niloticus*) producer in the world since 2000. Its production has increased almost exponentially [1]. It is consumed and exported to other countries. Nile tilapia is mostly raised in earthen ponds using manures and other recyclable wastes, as low cost commercial pellet feeds are not necessary for growing tilapia and the traditional cultivation, nutrient-enriched water, "green water", produced by the addition of animal manure or fertilizer is sufficient to achieve a marketable fish [2] as well as the prawns [3]. There are many aquaculture ponds throughout Thailand where giant freshwater prawns, *Macrobrachium rosenbergii*, are cultured. In fact, the prawns can be grown in all freshwater bodies [3]. They are commercially important because they are widely used for human consumption. Domestic consumption was 70 % of total production [1]. Green water systems can cause eutrophication of surface water, resulting in increased occurrence of toxic cyanobacterial bloom, especially *Microcystis* [3]. The occurrence can create a significant water quality problem, including their ability to produce toxins, namely microcystins (MCs). The toxins accumulate in aquatic organisms and are transferred to higher trophic levels. It involves the risk for human exposure through the consumption of contaminated aquatic organisms [4-6]. In Thailand, cyanobacterial genera with known toxin- producing taxa occurred in many reservoirs in all regions. *Microcystis aeruginosa* is the most frequently blooms [7-9]. Ruangrit *et al.* [10] found high amount of *M. aeruginosa* in prawn pond and microcystins were detected in prawn. Therefore, it is needed to clarify whether MCs are able to accumulate in aquatic organism cultured with traditional method. The data would be useful for food safety aspect and public health to avoid the damaging effect of cyanobacteria and their toxins.

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2. Materials and Methods

2.1. Culturing of Nile Tilapia and Giant Freshwater Prawn

Nile tilapia about 5 cm in size and giant freshwater prawn about 5 - 7 cm in size were obtained from the Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai, Thailand. The fish were cultured in 3 cement ponds, 1.5 m × 1.5 m and the depth of 0.50 m containing green water 0.30 m deep, 30 fish in

each pond. Feeding treatments were; 1) Green water system (Tr. 1). 2) Green water system with 18 - 30 × 10^6 cells·L^{-1} *M. aeruginosa* from natural pond and combined with commercial pellet feed (Tr. 2). 3) Green water system with 18 – 30 × 10^6 cells·L^{-1} *M. aeruginosa* (Tr. 3).

The prawns were cultured in two cement ponds of similar size, 30 prawns were in the pen (0.45 m × 0.45 m with water depth of 0.30 m) made of blue net (mezh size 2 mm) attached to the pond, 30 prawns were outside the pen. Feeding treatments were: Green water system (Tr. 1) and green water system with *M. aeruginosa* combined with commercial pellet feed (Tr. 2).

Completely randomize design (CRD) with duplicate treatments were carried out. Both fish and prawn were cultured for 2 months. Water samples were collected every two weeks to determine the amount of *M. aeruginosa*, phytoplankton and microcystins.

2.2. Identification and Enumeration of *M. aeruginosa* and Phytoplankton

Morphological classification of *Microcystis* spp. and phytoplankton were done under compound microscope (Olympus model CH30RF200) using related texts such as Komárek and Komáková-Legnerová [12] and Hindak [13]. Cells of *M. aeruginosa* were counted on a haemacytometer.

2.3. Analysis of Microcystins

2.3.1. Extraction of Microcystins

Microcystins were extracted after Kankaanpää *et al.* [4] with modification. Fish and prawn tissues were dissected and freeze-dried at −20˚C for 24 - 72 hours before extraction and ELISA analysis. One mL of 100% methanol was added into 2 g fish and prawn tissues for extraction overnight. The extracts were centrifuged at 12,000 rpm for 30 min and the supernatants were concentrated to 150 µl with a heat block at 50˚C, overnight and centrifuged at 12,000 rpm for 30 min before ELISA analysis.

2.3.2. Microcystin Analysis by ELISA Assay

ELISA Microcystin Plate Kit (Catalog No. EP022), ENVIROLOGIX INC© was used and performed in accordance with the manufacturer's instructions. A standard curve was constructed using three calibrations 0.16, 0.5 and 2.5 µg·L^{-1} supplied with the kit. The absorbance at 450 nm was measured with a microplate reader (Spectra MR, DYNEX Technologies). The microcystin concentration in each extract was expressed as MC-LR equivalent.

3. Results and Discussion

3.1. Identification and Enumeration of *M. aeruginosa* and Phytoplankton in Fish Ponds

Dominant species of phytoplankton excluding *M. aeruginosa* were found to belong to 5 divisions *i.e*. Divisions Chlorophyta, Cyanophyta, Euglenophyta, Bacillariophyta and Pyrrhophyta. Dominant species were Chlorophyta (green algae) such as *Scencedesmus* spp. and *Pediastrum* spp. The species composition was similar in each treatment except Tr. 2, *Microcystis wesenbergii* was found as dominant species.

Tr. 1 had lowest amounts of phytoplankton. Whereas the highest amounts of phytoplankton were found in Tr. 3 and Tr. 2, respectively (**Figures 1** and **2)**.

3.2. Microcystin Contents in Fish Samples

High microcystin contents in fish samples were detected. Microcystin contents of 8.32 ± 0.76 and 9.35 ± 1.45 µg·kg^{-1} d.w. were found in the fish of Tr. 2 and Tr. 3, respectively. Whereas microcystins in the corresponding Tr. 2 and Tr. 3 ponds were 20.08 ± 0.24 and 19.52 ± 0.49 µg·L^{-1}, respectively.

Microcystins were released from dead *Microcystis* cells causing high content of microcystin in the water. However, microcystin in the fish was obtained from ingestion of *Microcystis* cells by the fish [14,15].

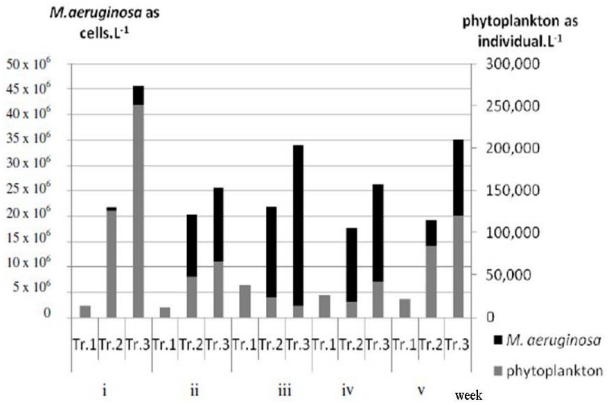

Figure 1. Amounts of phytoplankton and *M. aeruginosa* in each treatment (Replicate I fish pond).

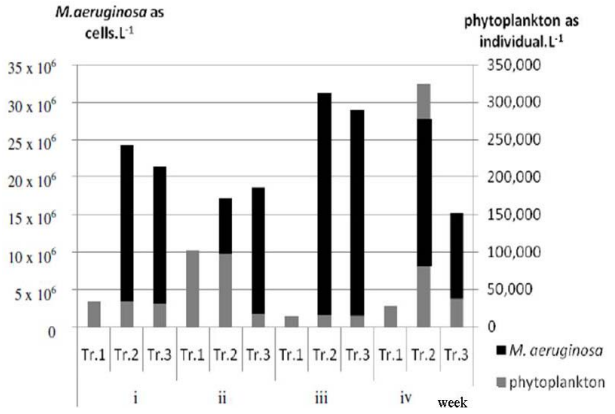

Figure 2. Amounts of phytoplankton and *M. aeruginosa* in each treatment (Replicate II fish pond).

3.3. Identification and Enumeration of *M. aeruginosa* and Phytoplankton in Prawn Ponds

Dominant phytoplankton species excluding *M. aeruginosa* in the prawn pond were diatoms such as *Cyclotella* spp. and *Acthanthidium* spp. The amounts of *M. aeruginosa* and other phytoplankton are shown in **Figure 3**.

3.4. Microcystin Contents in Prawn Samples

Microcystin detected in the second pond (at the end of cultivation) was 21.19 ± 0.31 $\mu g \cdot L^{-1}$ and in the prawn tissue was 14.42 ± 1.63 $\mu g \cdot kg^{-1}$ d.w.

This study was conducted in a short period. Dominant species of phytoplankton excluding *M. aeruginosa* were similar in each treatment because similar green water was used. Both fish and prawn cultivation in Tr. 1 had lowest amounts of phytoplankton. Tr. 2 and Tr. 3 had higher amounts because not only biomass of *M. aeruginosa* was added but also phytoplankton associated with *M. aeruginosa*. Fish samples in Tr. 2 and Tr. 3 which contained high amounts of *M. aeruginosa* also contained high amounts of MCs.

Effect of giant freshwater prawn consumption behavior on the microcystin accumulation in two types of cultivation *i.e.* inside and outside the pens, in the same pond was not different. Microcystins can interact with humic and fulvic substances, suspended particulate matter or sediments. Prawns are bottom dweller and consume feed which falls to the bottom of the pond. If prawns are forced to avoid feeding at the bottom by pen, toxin accumulation could be lower. Unfortunately, the experiment had to conduct in cement pond which had no sediment. It was shown that MC contents from both types of cultivation were comparable.

MC content in the prawn tissue was higher than that in the fish tissue. It might be possible that prawns came into contact with microcysin in sediment which were released by dead *Microcystis* cells at the bottom of the pond. Moreover, a number of studies have demonstrated that MCs can be excreted quickly by fish. Soares [16] showed that 48% of the total MCs ingested by *Tilapia rendalli* were eliminated with feces during a 30-day experiment.

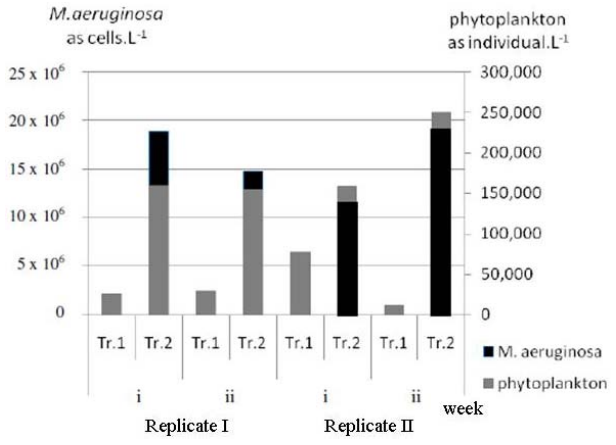

Figure 3. Amounts of phytoplankton and *M. aeruginosa* in each treatment (prawn pond).

4. Conclusion

Tilapia and prawn aquaculture prefer nutrient-enriched water (green water), produced by the addition of animal manure or fertilizer, for supporting the growth of tilapia and prawn. Pond management is essential for a productive aquaculture farm. In this sense, adequate nutrient levels will allow the right biomass and structure of phytoplankton. An excessive supply of nutrients will result in an over-enrichment that eventually will promote algal blooms. Additionally, nutrients in excess will alter phytoplankton composition with a resulting change of dominant species; such changes imply the substitution of larger species for smaller ones, particularly cyanobacteria.

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