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Accumulation of Microcystins in Nile Tilapia, *Oreochromis niloticus* L., and Effects of a Complex Cyanobacterial Bloom on the Dietetic Quality of Muscles

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Abstract The aim of this study was to investigate the kinetics of accumulation and elimination of microcystins in the tissues of Nile tilapia (*Oreochromis niloticus*) and to evaluate the effect of cyanobacterial exposure on fish muscle quality (levels of total fat and ash, protein, dry matter and the composition of saturated, monounsaturated and polyunsaturated fatty acids). Fish were exposed for 28 days to a natural cyanobacterial bloom with total microcystin concentration around 1,200 μg g^{-1} biomass dry weight. The hepatopancreas accumulated microcystins up to 350 ng g^{-1} fresh weight, but concentrations in muscle were generally below the detection limit (2 ng g^{-1} fresh weight). Following the exposure, fish were moved to the clean water, but only minor microcystin removal from the hepatopancreas was observed during a 4 week depuration period. Exposures of tilapia to the complex cyanobacterial bloom had only minor and temporary impacts on the investigated parameters of dietetic quality.

Keywords Fatty acids · Cyanobacteria · Bioaccumulation · Fish

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Freshwater cyanobacterial blooms represent a serious ecological and health problem worldwide, but the dynamics of their toxicity are still poorly understood (Yang et al. 2006). With respect to human use, understanding the impacts of toxic cyanobacteria on commercial fish and other aquaculture organisms is of special concern. Cyanobacteria produce a range of toxins and the hepatotoxic nonribosomally synthesized microcystin peptides are the most studied group. Nearly 80 structural variants of microcystins were found to be produced by *Microcystis*, *Anabaena*, *Oscillatoria* (*Planktothrix*) or *Nostoc*. Microcystins may form up to 1% of the dry biomass of cyanobacteria, and toxicological investigations showed high toxicity and potential tumor promotion in humans. Consequently, the World Health Organization recommended a tolerable daily intake (TDI) for microcystins of 0.04 (mu)g/kg body weight/day (WHO 1998). The exposure routes for microcystins include food, especially muscle tissue with accumulated toxins (Zhang et al. 2010).

However, under natural conditions, fish are not exposed to isolated toxins but to a complex matrix of toxins in a cyanobacterial bloom, and a narrow focus on toxicity may not be sufficiently comprehensive. For example, cyanobacterial cells may have nutritional value for fish, as some have been shown to be relatively rich in certain fatty acids, and also contain a spectrum of amino acids similar to that of green algae (Ahlgren 1992). A number of studies have focused on isolated toxins, but the impact of a complex

bloom on the dietetic quality parts of fish has not been studied in detail. Several studies investigated effects of toxic blooms on cyprinid fish (such as model zebrafish or commercial species of common and silver carps). However, other fish species are also used in worldwide aquaculture, and cichlid tilapias represent a significant component of this industry (Deblois et al. 2008; Magalhaes et al. 2001; Soares et al. 2004).

The first objective of the present study was to investigate the accumulation and elimination of microcystins in the tissues of Nile tilapia following a 4-week exposure to a complex natural water bloom of cyanobacteria. This provided a more realistic exposure scenario in comparison to most of the previous studies that focused on either isolated toxins or fish experimentally fed with cyanobacteria. Further, we investigated the impacts of a complex bloom on the composition of fish muscles. Potential adverse effects on the dietetic quality of fish fillets might be of concern, especially in less developed countries.

Materials and Methods

Nile tilapia (*Oreochromis niloticus*; length 211 ± 14 mm, average body weight 171 ± 34 g) were obtained from a single artificial stripping (Fishpond Tisová, Czech Republic). Fish were acclimatized for 1 week before the start of the study in a small pond without a cyanobacterial bloom and without microcystins. The study was conducted from August to October 2007 and included a 28-days exposure period followed by a depuration period, when fish were transferred to clean water.

The fish were caged and exposed to the cyanobacterial bloom, which developed naturally in an experimental pond (4 cages, approximate size 1 m^3, 100 fish per cage). In parallel, control fish were kept in an adjacent experimental pond without the occurrence of any apparent cyanobacterial blooms. Parameters of the water in both ponds were as follows (mean ± standard deviation of the experimental and control variant ponds, respectively): water temperature 17.8 ± 1.3, 17.9 ± 1.3°C; dissolved oxygen 93% ± 32%, 114% ± 35%; pH 8.2 ± 0.6, 8.5 ± 0.8; ammonia 0.22 ± 0.06, 0.18 ± 0.12 mg L^{-1} $N\text{-}NH_4$ (APHA 1981).

During the exposure period, the water bloom was assessed every week for concentrations of chlorophyll *a* using the ISO method (ISO 10260 1992). Phytoplankton composition and the numbers of cells were counted microscopically. Concentrations of microcystins in the biomass were determined by reverse phase high performance liquid chromatography with UV/VIS detection using established methods (Blaha and Marsalek 2003).

After the first 28-days of exposure, fish were moved into the 1,000 L tanks (4 tanks, 50 fish per tank) with clean potable water. During this 28 days depuration period, fish were kept at a 12-h light/12-h dark photoperiod with water being changed daily. Parameters of the water were as follows: temperature 26.7 ± 0.2; dissolved oxygen 60% ± 10%; pH 7.3 ± 0.2; ammonia 2.0 ± 1.5 mg L^{-1} $N\text{-}NH_4$.

Each week of the study, 10 individuals were collected from both the control and exposed group, and killed by mechanical stunning and bleeding from a cut through gill vessels. Fish tissues (hepatopancreas and muscle) were dissected and transported on ice to the laboratory for analysis. Sampling of the animals was in compliance with appropriate laws and regulations, and was approved by the Ethical Committee of the University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.

Microcystins from the fish tissues were extracted and analysed by high-performance liquid chromatography/mass spectrometry (HPLC/MS) as described previously (Adamovsky et al. 2007; Kohoutek et al. 2010). Briefly, tissue sample (0.4 g fresh weight) was homogenized with methanol (3 mL), sonicated for 30 min and centrifuged at 4,000 g for 10 min. Supernatant was collected and the pellet re-extracted three times using the same procedure. Obtained methanol fractions were pooled and extracted (three times) with 1 mL of hexane to remove lipids (hexane layers discarded). Extract was then evaporated at 40°C, and the residue dissolved in 50% methanol. Analyses were performed using the Agilent 1200 HPLC system coupled to 6410 Triple-Quad MS (Agilent, USA). Separation was achieved on a Supelcosil ABZ + Plus column, 150 × 4.6 mm, 5 mm (Supelco, Bellefonte, PA, USA) with the flow rate 1 mL/min and linear gradient of water and methanol. MS ion source was in positive ESI mode using nitrogen as drying gas (gas temperature 350°C), gas flow 11 L/min, nebulizer pressure 50 psi, capillary voltage 5,500 V. Spectra were collected in the SIM mode and the masses of microcystin-RR, -YR and -LR were scanned. Toxins were quantified using external calibration (Kohoutek et al. 2010). The method detection limit (MDL; per gram of tissue, fresh weight) was 3.0 ng/g (SD = 5%) for MC-RR and 27.0 ng/g (SD = 7%) for MC-YR and -LR, respectively.

Standard parameters for the composition of muscle tissue included dry matter, and content of proteins, fats and ash. Total lipids were determined by a 12-h Soxhlet extraction with diethylether. Dry matter content was determined by drying the sample to a constant weight at 105°C (for 24 h). Ash level was determined using a gravimetric method of incineration in an electric oven at 550°C. The content of nitrogenous substances was measured by the Kjeldahl method using a Kjeltec 23 apparatus (Tecator, Sweden). The net nitrogen content was then recalculated for nitrogenous substances using the equation N × 6.25. Besides the basic parameters, the composition

of fatty acids (saturated, monounsaturated and polyunsaturated) was determined by gas chromatography. Tissues were extracted with a mixture of methanol and chloroform (Folsch et al. 1957), and analyzed using a HP 4890D chromatograph (Hewlett Packard, USA).

For statistical analyses, Student's t-test and analysis of variance followed by the Tukey's test were used. For all statistics, *p*-values less than 0.05 were considered statistically significant. Calculations were performed using Statistica 8.0 software (StatSoft Inc., Tulsa, OK, USA).

Results and Discussion

In the experimental pond, the cyanobacterial biomass was dominated by the coccal cyanobacteria *Microcystis aeruginosa* and *M. ichthyoblabe*, with chlorophyll *a* concentrations ranging from 65 to 206 μg L^{-1} and cell densities from 169 to 971 × 10^3 cells/mL. The control pond contained mostly chlorococcal green algae with chlorophyll *a* concentrations of 5–203 μg L^{-1} and cell densities of 1.5–107 × 10^3/mL. During the exposure period, concentrations of total microcystins in the cyanobacterial biomass ranged 1,187–1,211 μg g^{-1} dry weight, which corresponded to 17.4–25.4 μg of total microcystin/L of water. Three dominant microcystin variants were present in cyanobacteria with the following average concentrations: microcystin-RR 537, microcystin-YR 49 and microcystin-LR 560 μg g^{-1} dry weight. The concentrations were comparable or higher than previously reported in the Czech Republic (Blahova et al. 2007).

Microcystin concentrations in the tissues were found to increase with exposure time (Fig. 1). No detectable microcystins were found in the control fish. The highest total concentrations were found in the hepatopancreas, and the maximum concentration was 350 ng × g^{-1} fresh weight after 4-weeks of exposure (Fig. 1b). The dominant variant was microcystin-LR (Fig. 1a). Concentrations of microcystins in the muscle of investigated fish were below the detection limit in most of the samples. Occasional positive samples were found with maxima around 15 ng × g^{-1} fresh weight (detailed results not presented). Our results confirm the accumulation of microcystins in tilapia tissues as reported previously (Deblois et al. 2008; Magalhaes et al. 2001), but the concentrations seem to be lower than previously reported in *Tilapia rendalli* experimentally fed with toxic *Microcystis aeruginosa* (Soares et al. 2004). Low concentrations of microcystins in the edible parts of tilapia observed in the present study thus indicate rather minor health risks from possible consumption of contaminated fish.

After the exposure, fish were moved into clean dechlorinated potable water for 4 weeks. Interestingly, only a minor decrease in total microcystin concentrations was

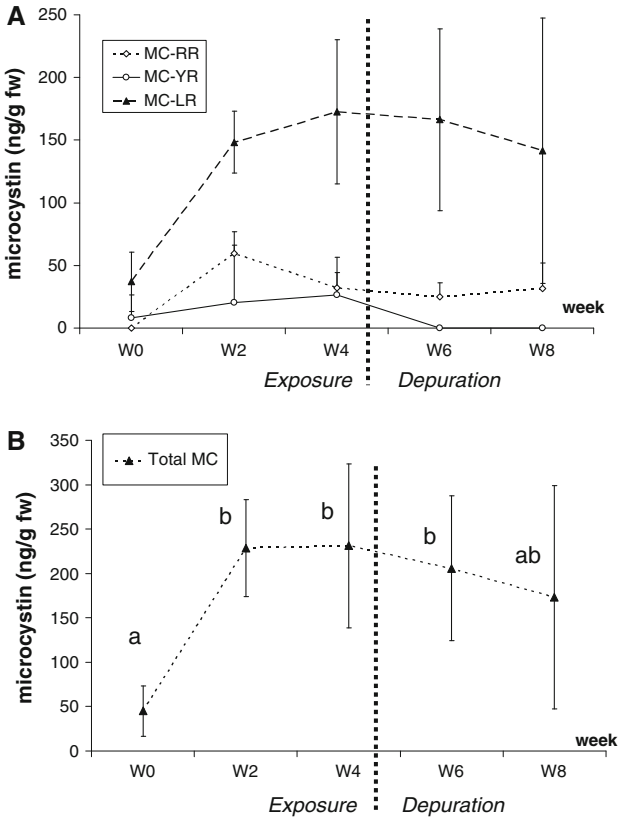

Fig. 1 Microcystin concentrations in the hepatopancreas of Nile tilapia exposed to cyanobacteria. **a** concentration of individual congeners, **b** total microcystin concentration (ng/g fresh weight, mean ± SD, n = 10 fish per group). Fish were exposed for 28 days (4 weeks—W0–W4) followed by a 28 days depuration period (W6–W8); both periods are separated by a *vertical dashed line*. Results of the statistical analysis (ANOVA + Tukey's test) are shown for panel **b** (groups marked with the *same letter* "*a/b*" are not significantly different)

observed during the period (Fig. 1, weeks 6–8). Microcystin-YR was the only variant which was not detectable after prolonged exposures (Fig. 1a). These data seem to indicate high species-specificity in the metabolism, because previous studies with cyprinid species showed relatively fast microcystin depuration in common carp and silver carp (Adamovsky et al. 2007), as well as in goldfish after intraperitoneal injection of microcystin-LR (Malbrouck et al. 2003).

In additional to the accumulation and elimination of microcystins, we also investigated the effects of a cyanobacterial bloom on the composition of fish muscle tissue. Basic parameters of the dietetic quality (i.e. dry matter, ash, content of proteins and fats, Table 1) were not substantially affected by the exposure to cyanobacteria. The only significant difference was observed during week 2, when higher levels of dry matter were found in the exposed fish in comparison with the control (highlighted by italics; * asterisk indicates statistically significant difference,

Table 1 Basic muscle composition of Nile tilapia in experimental ponds during exposure to a cyanobacterial bloom and a subsequent depuration period

Week No	Total fat (% dw)		Total ash (% dw)		N-substances (% dw)		Dry matter (% fw)	
	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
Exposure								
1	12.3 ± 2.7	9.3 ± 2.1	5.9 ± 0.2	6.0 ± 0.5	83.3 ± 2.5	83.4 ± 0.9	21.3 ± 1.8	21.8 ± 1.0
2	10.9 ± 4.0	12.5 ± 3.1	6.0 ± 0.5	5.9 ± 0.2	85.2 ± 1.7	83.0 ± 3.1	*22.5 ± 0.2*	*23.9 ± 0.5**
3	10.0 ± 4.6	9.8 ± 3.8	6.0 ± 0.5	6.2 ± 0.4	84.4 ± 1.1	85.8 ± 0.7	21.6 ± 0.9	21.7 ± 0.8
4	11.7 ± 4.9	9.3 ± 2.7	5.9 ± 0.1	6.0 ± 0.1	84.2 ± 1.5	83.7 ± 0.6	22.4 ± 0.9	21.4 ± 0.7
Depuration								
5	9.9 ± 0.8	11.9 ± 3.2	5.9 ± 0.2	6.2 ± 0.4	84.5 ± 1.7	83.8 ± 1.4	23.0 ± 0.2	22.5 ± 0.4
6	13.1 ± 2.0	9.3 ± 0.6	5.9 ± 0.5	6.2 ± 0.3	85.6 ± 2.1	87.6 ± 0.1	23.6 ± 0.9	22.2 ± 0.3
7	7.7 ± 3.2	12.6 ± 3.7	5.8 ± 0.1	5.9 ± 0.2	88.6 ± 0.9	89.6 ± 1.1	21.9 ± 0.8	21.6 ± 0.5
8	11.7 ± 2.6	9.1 ± 2.3	5.8 ± 0.3	6.1 ± 0.4	85.0 ± 1.3	87.2 ± 0.7	22.2 ± 1.3	22.2 ± 0.7

Values are shown as mean ± SD of n = 10 fish

dw Dry weight, *fw* fresh weight

Table 2 Fatty acid composition (%) of Nile tilapia muscle tissue in experimental ponds during exposure to a cyanobacterial bloom and a subsequent depuration period

Week No.	Sum SFA		Sum MUFA		Sum PUFA		Sum PUFA n − 6		Sum PUFA n − 3	
	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
Exposure										
1	42.0 ± 1.8	41.4 ± 2.2	35.0 ± 3.9	33.4 ± 4.1	23.0 ± 4.1	25.1 ± 4.1	16.7 ± 3.1	15.8 ± 0.8	6.3 ± 1.2	9.4 ± 4.2
2	39.2 ± 2.3	39.6 ± 2.8	33.3 ± 9.0	32.1 ± 0.7	27.5 ± 8.2	28.4 ± 3.2	19.4 ± 4.8	19.8 ± 3.9	8.2 ± 3.5	8.5 ± 1.0
3	37.5 ± 0.6	35.9 ± 1.9	32.3 ± 1.9	32.5 ± 2.2	30.1 ± 1.7	31.5 ± 2.6	19.1 ± 1.9	20.3 ± 2.9	11.0 ± 1.2	11.3 ± 0.5
4	36.0 ± 1.4	37.2 ± 1.6	37.4 ± 5.2	32.9 ± 1.2	26.6 ± 5.2	29.9 ± 0.5	17.0 ± 2.1	17.5 ± 1.8	9.5 ± 3.2	12.4 ± 2.1
Depuration										
5	36.1 ± 0.6	36.8 ± 1.0	30.7 ± 1.7	34.5 ± 2.6	*33.2 ± 1.2*	*28.7 ± 1.8**	18.3 ± 0.7	17.7 ± 1.5	14.9 ± 1.3	10.9 ± 2.3
6	36.8 ± 1.0	37.4 ± 2.2	*35.0 ± 1.3*	*31.0 ± 1.9 **	28.2 ± 2.2	31.7 ± 2.6	*15.9 ± 1.2*	*20.4 ± 1.8 **	12.3 ± 2.3	11.3 ± 2.1
7	35.9 ± 0.6	36.5 ± 0.8	28.6 ± 3.3	26.0 ± 5.3	35.6 ± 3.3	37.6 ± 6.1	22.1 ± 1.3	21.3 ± 3.8	13.5 ± 2.2	16.3 ± 2.8
8	38.5 ± 0.3	37.1 ± 1.7	27.8 ± 1.7	34.1 ± 3.4	33.7 ± 2.0	28.8 ± 2.5	18.3 ± 1.5	17.4 ± 2.5	15.3 ± 3.4	11.4 ± 0.1

Values show mean ± SD of n = 10 fish. An asterisks indicates a statistically significant difference between the exposed and control fish within a given sampling period (Student's t-test, $p < 0.05$)

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

Student's t-test, $p < 0.05$). However, the difference was small, and it was not confirmed during the following samplings (Table 1).

Interestingly, another study with the Nile tilapia indicated a similar trend, i.e. elevated dry matter after feeding with algae (Zhao et al. 2006). The same study of Zhao et al. (2006) also reported elevated protein content, which was not observed in our experiment. On the other hand, studies with other fish species showed a different pattern. For example, exposure to a complex bloom in our previous study decreased dry matter in the muscle of the silver carp and had no significant influence on the common carp (Mares et al. 2009). Also the study of Tricarico et al. (2008) found no alterations in the chemical composition of the edible crayfish fed with algae.

In addition to the basic dietetic parameters, the spectrum of fatty acids in muscle tissue was investigated (Table 2). Minor but significant differences between the control and exposed fish were observed during the first 2 weeks of depuration. Fish that were previously exposed to cyanobacteria had slightly lower % of MUFA/PUFA and elevated values of n − 6 FA in comparison with respective control groups. However, these observations were not repeatedly confirmed, and no differences were observed during the first 4 weeks of exposure.

Our findings do not fully correspond to the study of Tadesse et al. (2003) who demonstrated variable effects of different algal and cyanobacterial diets on the content of fatty acids (FA) and the ratio of polyunsaturated fatty acids (PUFA) n − 3/n − 6 in Nile tilapia muscle. Significant

modulation of the fatty acids spectrum was also observed in our previous investigations with common carp and silver carp exposed to a natural cyanobacterial bloom (Mares et al. 2009).

Differences among the studies and various fish species might be attributed to a number of internal and external factors. These may include, for example, different physiological attributes and metabolic rates in various fish (sediment feeders vs. planktivorous vs. carnivorous fish; Mares et al. 2009), or variable sensitivities to general environmental conditions (including toxins). Also, the type of exposure (controlled feeding vs. the exposure to a complex bloom) may significantly affect the outcome of the studies (Tadesse et al. 2003; Mohamed and Hussein 2006).

In summary, exposure of Nile tilapia to a *Microcystis*-dominated water bloom resulted in significant microcystin accumulation in the fish hepatopancreas. However, the edible parts of the fish (i.e., the muscle tissue) were less contaminated. Although some previous studies discussed significant health hazards from microcystins in food (Peng et al. 2010), and suggest the existence of species-specific differences. Our study also demonstrated minor and temporary impacts of a cyanobacterial bloom on the dietetic quality of the tilapia muscle. The results thus contribute to a critical assessment of potential impacts of cyanobacteria on aquaculture. However, further research is required to more fully understand the variability that may occur amongst different fish species with various exposure routes.

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