Impact of microcystin containing diets on physiological performance of Nile tilapia (*Oreochromis niloticus*) concerning detoxification

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Nile tilapia (*Oreochromis niloticus*) were fed by diets supplemented with cyanobacteria containing in part the cyanotoxin microcystin-LR (MC-LR) to determine the potential impacts on detoxification. Four different diets were prepared based on a commercial diet: (1) control, (2) MC-5% (containing 5% dried *Microcystis* sp. biomass with 4.92 μg MC-LR g^{-1} diet), (3) MC-20% (containing 20% dried *Microcystis* sp. biomass with 19.54 μg MC-LR g^{-1} diet), and (4) Arthr-20% (containing 20% dried *Arthrospira* sp. biomass without MC-LR). Blood and liver samples were taken after one, 7, and 28 days and protein has been determined in plasma and liver. In the liver, impacts on detoxification were measured by glutathione-S-transferase (GST) activities and gene expression of multi drug resistance protein (MDRP). Plasma protein did not change between all four diets at any sampling time whereas liver protein was significantly elevated already after one day in Arthr-20% and after 28 days in both, MC-20% and Arthr-20%. Biochemical measurements of GST activities revealed no significant impact at any sampling time. In order to characterize the potential effect of MC-LR on MDRP, RT-qPCR method was established. However, as for GST activities no significant changes in MDRP gene expression have been observed. Thus, in summary, oral exposure of MC-LR containing cyanobacteria to Nile tilapia *via* feed ingestion did not impact significantly detoxification in liver concerning GST activities and MDRP expression despite biochemical composition concerning liver protein was significantly elevated by the diets containing 20% cyanobacteria biomass, regardless whether they contained MC-LR or not.

Introduction

Biomass development of photosynthetic prokaryotic organisms generally named cyanobacteria has been becoming a worldwide issue in fresh and saline waters for several years.[1] Besides negative effects such as deterioration of physicochemical parameters of aquatic habitats accompanied by high pH due to photosynthetic activities, oxygen depletion and bad odor from decaying cyanobacterial biomass, cyanobacteria are able to produce a wide range of bioactive compounds.[2] The natural functions of these cyanobacterial secondary metabolites, for example, in cell signaling, environmental signaling and defense against zooplanktic predators still remain unclear. These metabolites are called cyanotoxins and can be classified into five groups: the hepatotoxic oligopeptide microcystins (MC) and nodularins, the neurotoxic alkaloids, such as anatoxin, cytotoxic cylindrospermopsin, the irritant lyngbyatoxin that are produced by several cyanobacterial species and furthermore the pyrotoxic endotoxin lipopolysaccharides which are constituents of the cell wall of gram-negative bacteria, including cyanobacteria.[3] MC toxicity is based on the specific inhibition of protein phosphatases type 1 and 2A.[4]

Despite the fact that fishes are primarily exposed to cyanobacteria in their environment, most studies concerning cyanotoxins, especially MC-LR, were performed using mammalian

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Environmental impact

Cyanobacteria blooms including their toxic metabolites are one of the most discussed topics in ecotoxicology. Cyanobacteria as primary producers are a worldwide abundant biomass and therefore the question arises whether it might be feasible to make at least in part use of that natural biomass in aquaculture as a natural source for fish diet. The aim of this study was to evaluate the physiological impacts of cyanobacteria used in fish diet on the omnivorous fish, Nile tilapia, concerning detoxification by measuring glutathione-S-transferase activity as well as of gene expression of multi drug resistance protein (MDRP). MDRP mRNA expression was established for the first time to evaluate detoxification of biotransformation phase three after oral application of a fish diet containing toxic cyanobacteria.

model species. The few studies in fish deal mainly with exposure to MC *via* intraperitoneal injection, which is not a natural exposure route. Thus it is required to investigate how fish, especially commercially important aquaculture species such as tilapia,[5] can cope with cyanobacteria *via* natural occurring oral application by feeding them cyanobacteria containing diets, addressing the digestion of these compounds. Recently, only few studies investigating potential impacts of cyanobacteria containing diets on bioaccumulation of MC and growth exist.[6–9]

Therefore there is an urgent need to extend our knowledge by investigating impacts of MC containing diets on fish concerning the most important physiological parameters associated with MC toxification, characterizing stress, growth and detoxification of MC. In principle, this would provide a better understanding for the mechanisms of MC action on fish during a natural exposure route, thereby helping to clarify whether fish can cope with cyanobacteria supplemented diets, particularly with regard to detoxification pathways.

Metabolism of toxicants is comprised of a set of metabolic pathways modifying the structures and chemical characteristics of xenobiotics. These pathways can be divided into three groups, which are present in all major groups of organisms.[10] The first two phases aim at the biotransformation of toxicants, firstly *via* activation and secondly *via* conjugation, whereas the third phase is characterized by excretion of the parental compounds or their metabolites obtained.[11] MC-LR is known as one of the most abundant cyanotoxins and in fish the impact of MC-LR has been investigated by intraperitoneal injection as well as immersion and application *via* diet concerning uptake kinetics, toxicodynamics, cellular and histopathological damage, as well as biotransformation and antioxidative enzymes amongst others.[12–14] MC-LR has also been researched in fish concerning biotransformation by phase I[15] and II enzymes.[16–18] In conclusion, only biotransformation phase II *via* glutathione-S-transferase (GST) seems to play a major role in detoxification of MC-LR in aquatic organisms.[19] However, no information is available on whether biotransformation phase III, *e.g.* multi drug resistance protein, might also be involved in detoxification of MC-LR in fish.

The aim of the present study was to determine the potential impact of MC-LR containing diets on the physiology of the omnivorous Nile tilapia (*Oreochromis niloticus*) addressing potential impact on detoxification in liver by determining GST activities and for the first time gene expression of MDRP. In parallel, the effects on stress and growth have been already assessed and revealed only moderate to negligible impacts by MC-LR containing diets[7] and thus it has been of great importance to investigate whether and which detoxification mechanisms are involved remarkably to metabolize MC-LR upon oral uptake. In addition, as potential indicators of physiological stress or biochemical changes, protein levels were determined in the plasma and in the liver.

Experimental

Fish and experimental set up

In order to avoid great variability of all physiological parameters determined by inter or intra gender specific social interferences only female Nile tilapia (*Oreochromis niloticus*) were chosen for the experiment and obtained from the fish farm České rybářství Mariánské Lázně s.r.o. (Czech Republic). Two weeks prior to the start of the experiment female Nile tilapia weighing between 30 and 55 g were distributed into twelve 60 L-aquaria each containing 8 individuals to obtain comparable weights of all groups. One day before starting the feeding experiment the individual weights of Nile tilapia were measured for all 12 aquaria and resulted in mean values ± SD ranging from 41.7 ± 10.5 g to 45.9 ± 10.6 g having no significant differences concerning weight between all aquaria.

Four experimental diets were used based on a commercial diet (DanEx 1344, Danafeed, Denmark): (1) control, (2) MC-5% (containing 5% dried *Microcystis* sp. biomass consisting of 90% *Microcystis aeruginosa*, 5% *Microcystis flos-aquae*, 5% *Microcystis ichthyoblabe*) resulting in a final concentration for MC-LR of 4.92 μg g^{-1} diet, (3) MC-20% (containing 20% dried *Microcystis* sp. biomass) resulting in a final concentration for MC-LR of 19.54 μg g^{-1} diet, and (4) Arthr-20% (containing 20% dried *Arthrospira sp*. biomass) without detectable MC-LR concentration, which is referred to as a positive control.[8]

For each treatment group, namely control, MC-5%, MC-20%, and Arthr-20%, three aquaria were connected to separated, closed recirculation systems. Fish were kept at a temperature of 26.0 ± 0.5 °C under natural light conditions and fed twice per day at 9 a.m. and at 3 p.m. at 1.5% of the whole body weight per day. Water parameters covering pH, oxygen, temperature, N–NH_4^+ and N–NO_2^- were measured daily at 10 a.m.[20] Every second day, half of the water was replaced to maintain stable water conditions.[8]

Sampling took place after one, 7, and 28 days starting at 9 a.m., using 8 individuals at every time point for each experimental group. After sacrificing the animals by a blow on the head and transection of the spinal cord, fish were weighted and blood was drawn immediately from the caudal vein within one min, centrifuged for one min at 2,000 *g* to obtain plasma which was snap frozen in liquid nitrogen and stored at −80 °C until further analyses. One g of liver was prepared for biochemical and enzymatic analyses. For gene expression analyses about 50 mg of liver tissue were sampled, immediately snap frozen in liquid nitrogen and stored at −80 °C until further processing.

Protein determination in blood plasma and liver tissue

For protein determination, plasma was diluted 1 : 100 with double-distilled water. Protein content of 25 μl of diluted samples was analyzed in triplicates photometrically using the Bradford protein assay (Biorad, London, UK).[21] Protein content of liver tissue was determined after extraction of protein based on the method described by Munro and Fleck (1966), as modified by Binner *et al.* (2008).[22,23]

Glutathione-S-transferase (GST) activities

Enzyme preparation. Enzyme preparation for measuring microsomal (mGST) and soluble GST (sGST) was carried out according to Wiegand *et al.* (2000).[24] In brief, samples were homogenized using 0.1 M potassium phosphate buffer (pH 6.5, 20% (v/v) glycerol, 1 mM ethylene-diamine-tetraacetic acid (EDTA), 1.4 mM dithioerythritol). Cell debris was removed by

centrifugation (10 min at 13,000 *g*), followed by centrifugation at 105,000 *g* for 60 min to separate the membrane fraction (for mGST) and resuspension in 1 mL 20 mM sodium phosphate buffer (pH 7.0, 20% (v/v) glycerol) resulting in preparation of microsomal GST (mGST). Soluble proteins (including sGST) were concentrated by ammonium sulfate precipitation (80% (w/v) saturation), re-dissolved in 20 mM sodium phosphate buffer, pH 7.0, and desalted by passage through NAP-10 columns (Pharmacia, Uppsala, Sweden).

Determination of enzyme activities. Activities of sGST and mGST were determined according to Habig *et al.* (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate, kinetics were followed at 340 nm for five minutes.[25] Enzyme activity was normalised to the protein content of the sample determined by Bradford (1976) at 595 nm, using bovine serum albumin for calibration.[26] Enzyme activity is reported in kat/mg protein, where 1 kat is the conversion of 1 mol of substrate per second.

Gene expression of multi drug resistance protein (MDRP) by RT-qPCR

Gene expression of the multi drug resistance protein (MDRP) and of the housekeeping gene polymerase α (pol) was determined by two-step RT-qPCR.

RT-qPCR quantification. Using hot start Platinum Taq polymerase (Invitrogen), qPCR was carried out with SYBR Green as fluorescent dye with a Stratagene MX3000P cycler under the following conditions: 94 °C initial denaturation for 5 min 40 s, followed by 40 cycles, involving denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 40 s [final concentration: 3 mM $MgCl_2$, 1x PCR buffer, 1x SYBR Green, 0.4 μM primer, 0.3 mM dNTP, 1 U Taq, 4 μl cDNA]. The primer sequences for MDRP (accession number EU878755.1) were CAGATGGGAATAAAGAAGGCGA and TGTCGATACTGGGGACGTGAT and for pol as described by Ziková *et al.* (2010).[8] For each run, relative quantification was calculated from a co-amplified standard dilution series (cDNA obtained from pooled liver), compensating for lot-to-lot and run-to-run variations as described by Wuertz *et al.* (2007).[27] All samples were measured in duplicate.

Statistical analyses

Results are presented as the mean ± SD, $n = 8$. Data were analyzed for normal distribution by Kolmogorov-Smirnov and equal variance by Kruskal-Wallis One Way Analysis of Variance (passed if $p < 0.05$) using SigmaStat 2.0 software (Jandel Scientific, San Rafael, USA). Pairwise comparison between all treatment groups at each sampling time was carried out by non-parametric Dunn's test or by parametric posthoc Tukey's test.

Results

During the whole time course of the experiments water parameters were successfully maintained in the optimum range for oxygen (89 ± 5%), pH (7.79 ± 0.19), water temperature (26.0 ± 0.5 °C), $N–NH_4^+$ (0.1 ± 0.1 mg l^{-1}), $N–NO_2^-$ (0.25 ± 0.15 mg l^{-1}) confirming good husbandry as indicated also by the 100% survival rate. At the last sampling after 28 d the weights (mean ± SD) of the various treatments were 64.6 ± 8.1 g in control, 55.7 ± 11.0 g in MC-5%, 51.2 ± 6.1 g in MC-20%, and 60.1 ± 7.7 g in Arthr-20%. The only statistical difference was between Ctrl and MC-20% indicating moderate inhibitory impact of MC-20% diet on weight.

Protein contents in plasma and liver

Plasma protein did not display significant changes between treatments during the time course of the experiment. However, after one week all groups reached slightly elevated values compared to the sampling after day 1 (Fig. 1A, Table 1) remaining similarly also for the values obtained after 28 days of the feeding experiment. In contrast to plasma, in liver protein contents revealed significant changes. After one day, Arthr-20%

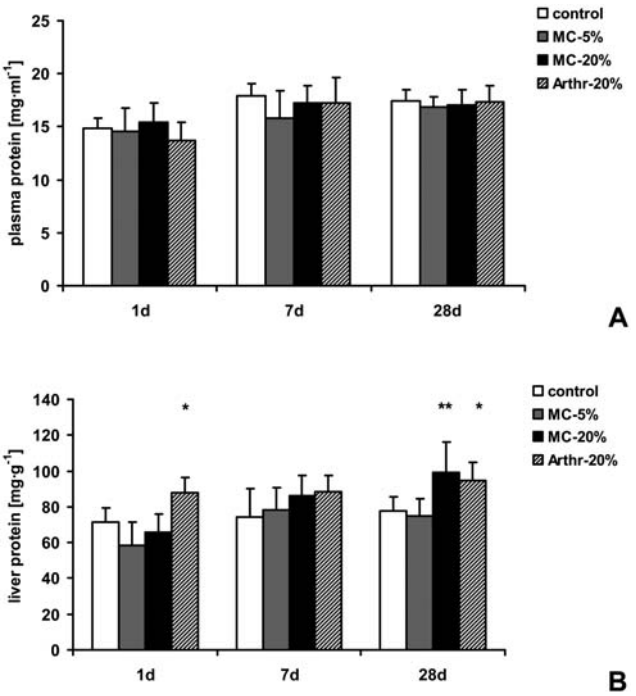

Fig. 1 Impacts of four different diet variants (control, MC-5%, MC-20%, Arthr-20%) on (A) plasma protein levels and (B) liver protein contents in female Nile tilapia. Data represent mean values + SD ($n = 8$) after 1, 7, and 28 days of the feeding experiment. (*, $p < 0.05$; **, $p < 0.01$).

Table 1 Plasma and liver proteins after treatment with four different diet variants (control, MC-5%, MC-20%, Arthr-20%). Data represent mean values + SD ($n = 8$) after 1, 7, and 28 days. Statistical differences ($p < 0.05$) between the groups are marked by various letters

		exposure duration		
		1 d	7 d	28 d
plasma protein/mg ml^{-1}				
treatment	control	14.9 ± 0.9	17.9 ± 1.1	17.4 ± 1.0
	MC-5%	14.5 ± 2.3	15.8 ± 2.6	16.9 ± 1.0
	MC-20%	15.4 ± 1.8	17.2 ± 1.7	17.0 ± 1.4
	Arthr-20%	13.7 ± 1.8	17.3 ± 2.3	17.3 ± 1.6
liver protein/mg g^{-1}				
treatment	control	71.3 ± 8.2[a]	74.3 ± 15.6	77.9 ± 7.8[a]
	MC-5%	58.3 ± 13.2[a]	78.4 ± 12.1	74.6 ± 9.8[a]
	MC-20%	65.7 ± 10.1[a]	86.4 ± 11.3	99.4 ± 17.0[b]
	Arthr-20%	87.8 ± 8.8[b]	88.4 ± 9.0	94.9 ± 10.1[b]

displayed the highest values, significantly different from all other experimental groups which were comparable in terms of significance (Fig. 1B, Table 1). After 7 days, a tendency could be observed with highest protein concentrations in the livers of MC-20% and Arthr-20% groups, which were even significant after 28 days when the control and MC-5% had nearly similar values while both groups with 20% replacement by cyanobacteria were higher compared to control and MC-5% diets.

Glutathione-S-transferase (GST) activities

GST activity integrates enzymatic activities of several isoenzymes and can be determined in target organs such as liver as soluble (cytosolic) (sGST) or microsomal GST (mGST) and GST activities were determined in two subcellular fractions, namely cytosolic (soluble) and microsomal enzyme activity of the liver, considering all potential isoenzymes (Fig. 2, Table 2). After one day, the mean values of both, sGST and mGST, were insignificantly elevated in all treatment groups compared to the control. After 7 days, only sGST of MC treated Nile tilapia displayed a tendency of higher mean values than the control, which remained still insignificantly elevated after 28 days. The pattern of mGST also did not change significantly between all treatments during all sampling points. Concerning mGST activity after 7 days the different diets for MC-20% and Arthr-20% displayed a tendency for equal or slightly lower values and being after 28 days of feeding again slightly higher compared to the control. In general mGST achieved higher values compared to sGST.

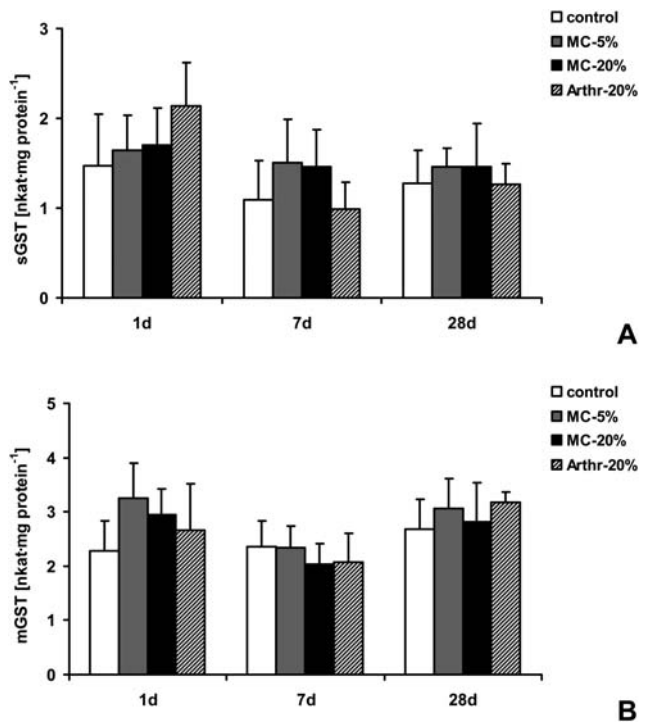

Fig. 2 Impacts of the four different diet variants (control, MC-5%, MC-20%, Arthr-20%) on activities of (A) soluble GST (sGST) and (B) microsomal GST (mGST) measured using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate for liver preparations of female Nile tilapia. Data represent mean values + SD ($n = 8$) after 1, 7, and 28 days of the feeding experiment.

Table 2 Soluble (cytosolic) (sGST) and microsomal glutathione-S-transferase (mGST) activities after treatment with four different diet variants (control, MC-5%, MC-20%, Arthr-20%). Data represent mean values + SD ($n = 8$) after 1, 7, and 28 days

		exposure duration		
		1 d	7 d	28 d
sGST [nkat mg protein^{-1}]				
treatment	control	1.5 ± 0.6	1.1 ± 0.4	1.3 ± 0.4
	MC-5%	1.6 ± 0.4	1.5 ± 0.5	1.5 ± 0.2
	MC-20%	1.7 ± 0.4	1.5 ± 0.4	1.5 ± 0.5
	Arthr-20%	2.1 ± 0.5	1.0 ± 0.3	1.3 ± 0.2
mGST [nkat mg protein^{-1}]				
treatment	control	2.3 ± 0.6	2.4 ± 0.5	2.7 ± 0.6
	MC-5%	3.2 ± 0.7	2.3 ± 0.4	3.1 ± 0.6
	MC-20%	3.0 ± 0.5	2.0 ± 0.4	2.8 ± 0.7
	Arthr-20%	2.7 ± 0.9	2.1 ± 0.5	3.2 ± 0.2

However, the activities of neither sGST nor mGST revealed any significant difference at any of the time points between the groups fed with the supplemented diets compared to the control.

Gene expression of multidrug resistance protein (MDRP)

Relative mRNA expression of MDRP did not show significant differences between the treatment groups during the time course of the feeding experiment (Fig. 3, Table 3). After one day of exposure control, MC-5% and Arthr-20% revealed similar expression of MDRP mRNA, only in the MC-20% experimental group, MDRP was insignificantly lower expressed. MDRP mRNA expression in Arthr-20% was increased only as a tendency after 7 days which was correlated to an unusually high SD. After 28 days all experimental variants reached similar values for MDRP expression except the MC-5% group that had slightly higher expression of mRNA. However, no significant changes have been revealed between the four experimental groups at any sampling point.

Discussion

Cyanotoxins derived from cyanobacteria are well known to cause deleterious effects in many organisms. MC, particularly MC-LR, produced by several species of *Microcystis* in considerable amounts during cyanobacterial water blooms are well characterized concerning their hepatotoxic effects in mammals.[28] It is

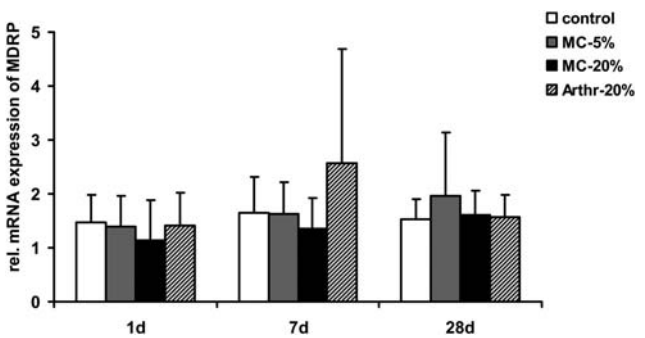

Fig. 3 Impacts of the four different diet variants (control, MC-5%, MC-20%, Arthr-20%) on mRNA expression of hepatic multidrug resistance protein (MDRP) in female Nile tilapia. Data represent mean values + SD ($n = 8$) after 1, 7, and 28 days of the feeding experiment.

Table 3 Relative mRNA expression of multidrug resistance protein (MDRP) after treatment with four different diet variants (control, MC-5%, MC-20%, Arthr-20%). Data represent mean values + SD ($n = 8$) after 1, 7, and 28 days

		exposure duration		
		1 d	7 d	28 d
MDRP [rel. mRNA exp.]				
treatment	control	1.5 ± 0.5	1.6 ± 0.7	1.5 ± 0.4
	MC-5%	1.4 ± 0.6	1.6 ± 0.6	2.0 ± 1.2
	MC-20%	1.1 ± 0.7	1.3 ± 0.6	1.6 ± 0.5
	Arthr-20%	1.4 ± 0.6	2.6 ± 2.1	1.6 ± 0.4

noteworthy that the toxic impacts of MC-LR are varying greatly due to the chosen exposure route. The highest toxicity was observed following intraperitoneal injections of MC-LR, whereas inhalation or ingestion of MC-LR decreased dramatically the toxic impacts in rodents. The primary targets of naturally occurring MC during cyanobacterial water blooms are aquatic organisms such as fish and several studies have been conducted to investigate the impact of MC on fish. However, these studies mainly addressed the exposure to MC-LR *via* intraperitoneal injection[29] and not *via* natural exposure by ingestion.[30] Information about toxic effects of MC *via* the naturally occurring exposure routes in fish are limited to exposure in water during heavy cyanobacterial blooms[14] but knowledge about effects of cyanobacteria by ingestion[5] is rather limited. However, it seems that the natural occurring exposure routes might be clearly less harmful for fish compared to intraperitoneal application, similar to findings in mammals. Thus the question arises to which extent fish might be able to cope with MC during natural exposure routes especially *via* ingestion with regard to the actual ecological relevance. Only one extensive study addressed the issue of MC containing fish diet where MC containing feed caused surprisingly higher growth rates in Nile tilapia compared to the control diet. This suggested that cyanobacteria dry mass could become an efficient supplementary alternative for fish meal in fish diets.[5] However, the authors measured relatively high concentrations of MC remaining in several tissues including muscle by ELISA suggesting that the MC contents in muscles are too high for human consumption.

Previous determinations of the experiment presented here revealed that MC caused only moderate to negligible stress based on cortisol, plasma glucose, and hepatic glycogen measurements.[8] This was further supported by the observation that growth was only moderately lower in cyanobacteria supplemented diets, and more importantly not only associated with the MC-LR content as revealed by the reduced growth rate of the positive control (Arthr-20% group).[8] The fact that the MC-20% groups after 7 and 28 days had the lowest growth rate[8] suggests that not only the marked feed supplementation by cyanobacterial biomass has an impact acting as a stressor but also the content of the cyanotoxin MC-LR at least at the higher concentration used, which is confirmed by comparing the weights after 28 d demonstrating significant lower weight for MC-20% compared to control. Thus beside the obvious impacts of cyanobacteria supplementation in diets the results suggest that cyanobacteria containing considerable amounts of MC-LR might lead to a more pronounced energy consumption in order to deal with metabolism and detoxification of that compound.

In order to have additional information concerning potential adverse effects, protein levels in plasma and liver as potential stress indicators[31] were measured but no significant change could be observed in plasma at any sampling time. On the contrary liver protein revealed significant elevations for both high supplementation groups, MC-20% and Arthr-20%, indicating slight stress. Thus, it seems that the supplementation of the diet is changing the overall energy charge in liver in a concentration dependent way, resulting in higher protein levels that could be related to marked changes in general energy metabolism indicating minor physiological stress.

In summary, only the high feed supplementation by dried cyanobacterial biomass independent of MC-LR contents was causing moderate to negligible stress and the most sensitive significant biomarkers for stress indication were liver glycogen, plasma glucose[8] and as determined herewith, liver protein but not the stress hormone cortisol.[8] Unfortunately, data for lipid determination are missing due to the limited amounts of liver tissue and thus a complete picture about the energy sources and their changes in liver of Nile tilapia cannot be given. In zebrafish embryos, the yolk fat storage was increasingly consumed due to microcystin exposure.[32]

In teleost fish, studies dealing with detoxification of MC following intraperitoneal injection revealed that MC provokes phase II biotransformation by oxidative stress responses such as increase of SOD (superoxide dismutase), CAT (catalase), and LPO (lipid peroxidase) as well as GPx (glutathione peroxidase) and GR (glutathione reductase)[32,33] whereas phase I biotransformation such as monooxygenase CYP1A seems not to be involved.[15] Concerning biotransformation of phase II in Nile tilapia it has been shown that intraperitoneal injection of MC elevated GST at the transcriptional level.[18] Activation of the GST due to MC-LR exposure has been shown in zebrafish embryos[16,34] whereas it decreased in adults of goldfish.[17]

Surprisingly, the recent results obtained concerning activities of sGST and mGST in liver of Nile tilapia revealed no significant effects at all comparing the four experimental groups at any sampling time. In contrast to other studies applying MC-LR *via* water to zebrafish[16,34] or using intraperitoneal injection of MC-LR in tilapia,[15] ingestion *via* the feed did not induce hepatic GST activities. Furthermore, the other exposure routes always displayed a pronounced and significant increase of both GST activities. In addition, intraperitoneal injection of MC-LR in tilapia resulted also in significant elevation of further markers for oxidative stress such as SOD, CAT and LPO triggering biotransformation phase II[33] supporting the findings for an involvement of GSTs in detoxification of MC at least after injection of MC-LR.

However, no information has been available in fish whether biotransformation phase III, *e.g.* MDRP, might contribute to MC detoxification. Thus, the recent results represent not only the original establishment of assessment of MDRP gene expression in Nile tilapia but also, in addition, the first investigation of whether MC containing diets might involve biotransformation phase III *via* induction of changes of MDRP mRNA levels.

Determination of MDRP mRNA expression has been established in the present study for the first time. Upon exposure to xenobiotics MDRP expression in fish was reported so far in zebrafish[35] and red mullet[36] but not in tilapia.

Analyses of gene expression in liver of Nile tilapia revealed no significant effect at all of the four diets on MDRP mRNA during the whole time course of the experiment Thus, it seems unlikely that MDRP might be involved in any MC depuration processes under the experimental conditions chosen. Unfortunately, as for measurements of GST activities, no comparative studies are available where MC-LR has been applied intraperitoneally, which might induce stronger detoxification processes including changes in mRNA expression of MDRP. Thus to verify whether MDRP might be involved in detoxification of MC it would be interesting to perform experiments applying MC-LR *via* intraperitoneal injection.

Thus, similarly to the recent findings concerning stress the impact of the four diet variants on detoxification parameters indicated no or only negligible effects on GST activities and MDRP expression.

Conclusions

In summary, the recent findings presented here together with previous measurements[8] investigating effects of cyanobacteria containing diets, especially these of MC-LR containing variants, on physiological performance of Nile tilapia led to suggest only minor to negligible impacts on bioaccumulation,[8] stress[8] and detoxification and moderate effects on growth.

The slight physiological changes induced by the various diets seemed to be due to behavioral changes induced by starting to use different diets, which might affect taste.[11] Oral ingestion of MC-LR containing diets did not induce significantly hepatic detoxification mechanisms associated with biotransformation phase II or phase III indicating that neither GST nor MDRP are remarkably involved in metabolism of MC-LR despite MC-LR has been bioaccumulated moderately in liver. Thus it seems like the amounts of MC-LR given orally *via* diets under these conditions might become sufficiently metabolized under normal conditions without inducing significantly higher GST levels or increase of MDRP gene expression.

Thus as a general conclusion it seems to be at least in part feasible that MC-LR containing dried cyanobacterial biomass might be even used in fish diets for Nile tilapia as a nutrient. This clearly needs further research in more detail to address this important issue for aquaculture and its potential implications for human consumption.

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