Toxicon 60 (2012) 1298–1306

Contents lists available at SciVerse ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Responses of yellow catfish (*Pelteobagrus fulvidraco* Richardson) exposed to dietary cyanobacteria and subsequent recovery

Guifang Dong [a,b,c], Shouqi Xie [a], Xiaoming Zhu [a,*], Dong Han [a], Yunxia Yang [a], Lirong Song [a], Lanqin Gan [a], Wei Chen [a]

[a] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei 430072, China
[b] School of Marine Sciences, Ningbo University, Ningbo, Zhejiang 315211, China
[c] Hubei Key Laboratory of Animal Nutrition and Feed Science, Wuhan Polytechnic University, Wuhan, Hubei 430023, China

ARTICLE INFO

Article history:
Received 8 May 2012
Received in revised form 31 July 2012
Accepted 29 August 2012
Available online 7 September 2012

Keywords:
Cyanobacteria
Exposure
Recovery
Pelteobagrus fulvidraco

ABSTRACT

A 120-day toxicity experiment was conducted to investigate the effect of dietary cyanobacteria on the growth and liver histopathology of yellow catfish, and subsequent recovery when the fish were free of cyanobacteria. Three experimental diets were formulated: the control (cyanobacteria-free diet), low-cyanobacteria diet (LCD, 32.3 μg microsystins/g) and high-cyanobacteria diet (HCD, 71.96 μg microsystins/g). Each diet was fed to fish for 60 days and then all fish were free of cyanobacteria for a further 60 days.

The results showed that a significant decrease in the specific growth rate (SGR) was observed in both fish fed with the LCD and HCD after a 1st 30-day exposure period, however, no significant difference in the SGR between the LCD and control groups was observed after a 2nd 30-day exposure period. At the end of the 60 days exposure, all examined liver tissues in both doses exhibited what appeared as dose-dependent histopathological modifications. After a 60-day recovery, there were no significant differences in the SGR among groups, while no obvious histopathological alteration was observed in livers of fish previously fed with the LCD. The results indicate that the LCD-treated fish have a full recovery after a 60-day recovery, but the HCD-treated fish did not.

1. Introduction

Harmful cyanobacterial blooms and cyanobacterial toxins (cyanotoxins) have occurred in many eutrophicated freshwaters including lakes, reservoirs and aquaculture ponds (Chorus and Mur, 1999; Paerl et al., 2001). Cyanotoxins have been reported to result in poisonings and deaths of domestic animals as well as a potential threat to human health (Dawson, 1998; Carmichael, 1994). Cyanotoxins have been proven to be harmful to different fishes (Dong et al., 2009, 2011; Amado and Monserrat, 2010).

The acute toxic effects of cyanotoxin on different fishes have been well studied including oral gavaging or intraperitoneal injection (Carbis et al., 1996; Bury et al., 1997; Li et al., 2005), which cannot reflect the uptake route under natural environments. In addition, the toxins used in these experiments were either purified microsystins (MCs) or cyanobacterial crude extract containing MCs (Li et al., 2005; Hao et al., 2008; Zhang et al., 2008). Generally, cyanobacteria are a normal food component for many fishes (Zurawell et al., 2005) and cyanotoxins were normally intaken by fish through food under natural environments. Therefore, chronic impact of oral exposure to cyanobacteria on fish should receive more investigation and the toxic effects on fish from MCs exposure through oral administration need to be evaluated experimentally, especially if there is long-term, frequent exposure.

Cyanotoxins can accumulate in fish tissues via direct feeding on phytoplankton or uptaking of dissolved toxins

* Corresponding author. Tel.: +86 27 68780060; fax: +86 27 68780667.
E-mail address: xmzhu@ihb.ac.cn (X. Zhu).

0041-0101/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxicon.2012.08.013

through epithelium (gills, skin) (Ibelings and Chorus, 2007). Therefore, the oral exposure route is one of the most important routes for fish accumulation of cyanotoxins (Ernst et al., 2001) through food web of eutrophicated waters (Tencalla and Dietrich, 1997; Magalhães et al., 2001). Accumulation of cyanotoxins in fish through food web could bring potential threat to human food safety. However, little information is available on the chronic toxic effects of cyanobacteria via the oral exposure route on fish species (Zhao et al., 2006; Dong et al., 2009, 2011). It is also unclear if or how much the fish could get recovery when they are free of cyanobacteria.

Yellow catfish (*Pelteobagrus fulvidraco* Richardson) is an omnivorous, freshwater species of fish with increasing interest in Chinese inland aquaculture. In the past 20 years, heavy cyanobacterial blooms have occurred frequently in Chinese lakes (e.g., Lake Dianchi, Lake Taihu and Lake Chaohu), reservoirs and aquaculture ponds during spring to summer and then gradually fade away during autumn to winter. The culture season of yellow catfish overlaps with the occurrence period of cyanobacterial blooms. Therefore, yellow catfish are more likely to be exposed to cyanotoxins via food. Therefore, it is important to investigate the accumulation and depuration of cyanotoxins in tissues of yellow catfish and their public health consequences. The purpose of the present study was to investigate the chronic toxicity of oral cyanobacteria exposure on yellow catfish and then to evaluate if or how much the fish could get recovery when being fed with cyanobacteria-free diet.

2. Materials and methods

2.1. Fish, cyanobacteria and experimental diets

Yellow catfish were obtained from the hatchery of Jingzhou Fishery Institute, Hubei, China and acclimated in two circular fiberglass tanks (diameter: 150 cm, height: 120 cm, water volume: 1500 L) for 30 days prior to the experiment. During the acclimation period, the fish were fed to apparent satiation with the practical diet (containing 42% protein) twice daily (09:00 and 15:00 h).

The fresh cyanobacteria (90% of the algae were *Microcystis aeruginosa*) were collected from Lake Taihu, Jiangsu, China and were air-dried. Three experimental diets were formulated to be approximately isonitrogenous (crude protein: 45%) and isocaloric (gross energy: 20 kJ/g diet). Fish meal and soybean meal were used as the dietary protein source in the control diet. Two different concentrations of cyanobacteria were included to replace soybean meal as the tested diets. The microsystins (MCs) concentrations in LCD and HCD were 32.3 μg MCs/g diet and 71.96 μg MCs/g diet, respectively. The diet formulations and chemical compositions are shown in Table 1. The experimental diets were made into 1 mm pellets using a laboratory pelleting machine, oven-dried at 60 °C, and stored at −4 °C prior to use.

Table 1
Formulation and chemical composition of the experimental diets (g/100 g in dry weight).

Ingredients	Control	LCD	HCD
Algae meal	0.00	6.16	18.48
White fish meal[a]	48.00	48.00	48.00
Soybean meal[b]	18.68	12.42	0.00
Corn starch	13.00	13.00	13.00
Fish oil[c]	5.55	5.80	6.40
α-Starch	6.00	6.00	6.00
Mineral premix[d]	5.00	5.00	5.00
Vitamin premix[e]	0.40	0.40	0.40
Choline chloride	0.11	0.11	0.11
Cr_2O_3	0.50	0.50	0.50
Cellulose	2.76	2.61	2.11
Chemical composition (in dry matter)			
Crude protein (%)	46.56	48.44	51.24
Crude lipid (%)	10.71	10.55	12.47
Ash (%)	10.98	11.45	11.74
Gross energy (kJ/g)	20.34	21.64	21.89
Microcystin (μg/g)	0.00	32.30	71.96

[a] White fish meal: American Seafood Company, Seattle, Washington, USA.
[b] Soybean meal: oil-extracted soybean, Wuhan Coland Feed Co., Ltd., Wuhan, China.
[c] Fish oil: Wuhan Coland Feed Co., Ltd., Wuhan, China.
[d] Mineral premix (mg/kg diet, H440): NaCl, 500; $MgSO_4 \cdot 7H_2O$, 7500; $NaH_2PO_4 \cdot 2H_2O$, 12,500; KH_2PO_4, 16,000; $Ca(H_2PO_4) \cdot 2H_2O$, 10,000; $FeSO_4$, 1250; $C_6H_{10}CaO_6 \cdot 5H_2O$, 1750; $ZnSO_4 \cdot 7H_2O$,176.5; $MnSO_4 \cdot 4H_2O$, 81; $CuSO_4 \cdot 5H_2O$, 15.5; $CoSO_4 \cdot 6H_2O$, 0.5; KI, 1.5; starch, 225.
[e] Vitamin premix (mg/kg diet, NRC, 1993): thiamin, 20; riboflavin, 20; pyridoxine, 20; Ascorbic acid, 110; cyanocobalamine, 2; folic acid, 5; calcium patotheniate, 50; inositol, 100; niacin, 100; biotin, 5; starch, 3226; vitamin A (ROVIMIX A-1000), 110; vitamin D_3, 20; vitamin E, 100; vitamin K_3, 10.

2.2. Oral toxin exposure and sampling methods

The experiment was conducted in a flow-through system containing 27 circular fiberglass tanks (diameter: 70 cm, water volume: 90 L). Ten days prior to the trial, the fish were transferred from the rearing tank to the flow-through system for acclimation. At the beginning of the experiment, fish were deprived of food for 24 h to empty the gut. Fish (initial body weight: 5.32 ± 0.02 g) were randomly selected, individually weighed and transferred into 27 tanks (30 fish per tank). Nine tanks were randomly assigned to each of the three diet groups. During the experiment, oxygen was supplied by aeration, with the minimum level at 7.0 mg/L. The water temperature was determined daily and kept constant at 25 ± 2 °C. Photoperiod was 12 h light/12 h dark with the light period from 08:00 to 20:00 h. Light intensity at the water surface was approximately 200 lx. pH, Ammonia–N and residual chloride were measured weekly, pH was approximately 7.0. Ammonia–N was kept at less than 0.1 mg/L and residual chloride was kept at less than 0.01 mg/L.

During the experiment, fish were hand-fed to satiation twice daily (09:00 and 15:00 h). The daily food supplied was recorded and uneaten diet was collected 1 h after feeding, dried at 60 °C, and reweighed. The leaching rate (potential loss of uneaten diet) of uneaten diet was determined by placing weighed feed in tanks without fish for 1 h and were then collected, dried and reweighed. The average leaching rate was used to calibrate the amount of uneaten diet. Fresh and intact faeces were siphoned 1 h after the collection of uneaten diets from the 11th day to the end of the experiment. The faecal samples were stored at −20 °C until freeze-dried for MCs analysis. The experiment was divided into four periods: 1st 30-day exposure period, 1st

EP; 2nd 30-day exposure period, 2nd EP; 1st 30-day recovery period, 1st RP; 2nd 30-day recovery period, 2nd RP. During the two exposure periods, fish were fed with the three experimental diets based on the dietary treatment and during the two recovery periods, all fish were fed with the control diet. Fish were weighed and sampled once every 30 days. Fish were starved for 24 h prior to each sampling to ensure evacuation of gastrointestinal tract at the end of each period. At each sampling point, fish in each tank were bulk-weighed, six fish from each tank were randomly sampled and sacrificed immediately. The liver and dorsal white muscle (from the posterior edge of the operculum to the end of the dorsal-fin base above the lateral line) of the fish were dissected quickly and stored at −20 °C until freeze-dried for MCs analysis. At the end of the second exposure period (2nd EP) and the second recovery period (2nd RP), one fish from each tank was sacrificed, livers were sampled immediately for histopathological examination.

2.3. Chemical analysis

Crude protein, crude lipid, ash, dry matter and gross energy were determined for the diets (AOAC, 1995): dry matter was determined by drying the sample to constant weight in an oven at 105 ± 1 °C. Nitrogen was determined using the Kjeldahl method, and protein content was calculated from the nitrogen content multiplied by 6.25. Crude lipid was determined via ether extraction using a Soxtec system (Soxtec System HT6, Tecator, Hoganas, Sweden) and ash content was obtained via incineration at 550 °C in a muffle furnace. Gross energy was measured via combustion in a microbomb calorimeter (Phillipson microbomb calorimeter, Gentry Instruments Inc., Aiken, South Carolina, USA). For each variable, at least duplicate samples were determined.

2.4. Histological studies

For histopathological examination, liver specimens were fixed in Bouin's fluid for 12 h, and then the fixed specimens were dehydrated in a graded ethanol series and embedded in paraffin. Tissue blocks were sectioned (7 μm thick) and stained with hematoxylin and eosin (H&E). Tissue sections were examined under a Zeiss photo-microscope with the aid of Axioplan 2 imaging software.

2.5. Microcystins analysis in fish tissue

The liver and muscle samples were freeze-dried and then all samples were ground into powder (diameter: 250 μm). The recovery of all samples ranged from 89% to 95% and the coefficient of variation (CV) ranged from 2.33% to 11.8%. The MCs extraction procedure for the tissue samples was as follows:

All samples were extracted first using 5% acetic acid (10 mL) for 20 min, and then extracted twice using 80% methanol aqueous solution (20 mL) for 1 h using a magnetic stirrer for sufficient mixing, and then the extracts were centrifuged at 8000 r/min for 15 min, then the residue was re-extracted using 80% methanol aqueous solution (20 mL) for 45 min. The three extracts were then combined, diluted, and passed through a pre-conditioned Sep-Pak C18 cartridge (Waters, Milford, MA). The cartridge was first rinsed with 100% methanol solution and then ultrapure water. MCs were eluted with 10 mL of 100% methanol. After evaporation at 35 °C to dryness using a rotary evaporator, the residue containing MCs was dissolved in 1 mL of ultrapure water. The obtained samples were stored at −20 °C for MCs analysis. The ELISA kit and technology was kindly provided by the Laboratory of Algae Source, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China. The ELISA method was used to determine MCs in all samples as described by Hu et al. (2008) and Dong et al. (2009).

2.6. Calculations and statistical analysis

$$\textit{Hepatosomatic index (HSI, \%)} = 100 \times \textit{hepatopancreas weight (wet weight, g)} / \textit{somatic weight (wet weight, g)}$$

$$\textit{Feeding rate (FR, \% body weight/d)} = 100 \times \textit{total feed intake (dry matter, g)} / \textit{days} / [(\textit{initial body weight (wet weight, g)} + \textit{final body weight (wet weight, g)})/2]$$

$$\textit{Specific growth rate (SGR, \%/d)} = 100 \times [\textit{ln final body weight (wet weight, g)} - \textit{ln initial body weight (wet weight, g)}] / \textit{days}.$$

The normality and homogeneity of variances among groups were tested and then analysis of covariance (ANCOVA) was used to adjust the initial body weight of the fish (the initial body weight at the beginning of each period was used as the covariance). All data were subjected to one-way ANOVA. If significances ($P < 0.05$) were identified, Duncan's multiple range tests were used to determine the differences between experimental groups. Statistica 6.0 for Windows was used for the statistical analysis.

3. Results

No mortality of fish was observed during the whole experiment.

3.1. Growth performance

Table 2 shows that there were no significant differences between body weights at the start of the experiment ($P > 0.05$), and the body weights of fish fed with the LCD and HCD were significantly lower than that of the control during the two exposure periods (1st EP and 2nd EP) ($P < 0.05$). During the 1st RP, body weight of fish previously fed with the HCD was significantly lower than that of the control ($P < 0.05$), but no significant difference between the LCD and control groups was observed ($P > 0.05$), while there were no significant differences in the body weights among all groups during the 2nd RP ($P > 0.05$).

Effect of dietary cyanobacteria on the hepatosomatic index (HSI), feeding rate (FR) and specific growth rate (SGR) of yellow catfish are shown in Figs. 1–3. During the two exposure periods, there was no significant difference in the HSI between fish fed with the LCD and the control groups ($P > 0.05$), while a significant decrease in the HSI was observed only in fish fed with the HCD ($P < 0.05$) (Fig. 1). During the 1st RP, HSI of fish previously fed with the LCD was significantly higher than that of the control ($P < 0.05$), while no significant difference was observed in the HSI between fish previously fed with the HCD and the control groups. During the 2nd RP, there was no significant difference in the HSI among all groups ($P > 0.05$).

Fish fed with the LCD and HCD showed significantly lower FR than that in the control fish during the 1st EP ($P < 0.05$) (Fig. 2). During the 2nd EP, a significant decrease in the FR was observed only in fish fed with the LCD, while a significant increase in the FR was observed between fish fed with the LCD and the control groups ($P < 0.05$). During the 1st RP, fish previously fed with the LCD and HCD diets showed significantly higher FR than that in the control fish ($P < 0.05$). During the 2nd RP, there was no significant difference in the FR among all groups ($P > 0.05$).

During the 1st EP, a significant decrease in the SGR was observed in fish fed with the LCD and HCD ($P < 0.05$) (Fig. 3). During the 2nd EP, there was no significant difference in the SGR between fish fed with the LCD and the control groups ($P > 0.05$), while a significant decrease in the SGR was observed only in fish fed with the HCD ($P < 0.05$). During the 1st RP, fish previously fed with the LCD and HCD diets showed significantly higher SGR than that of the control ($P < 0.05$), while there was no significant difference in the SGR among all groups during the 2nd RP ($P > 0.05$).

3.2. Histological examination

At the end of a 60-day exposure, in the normal livers, hepatocytes and nuclei were uniform in size and shape (Fig. 4A). Liver specimens derived from fish fed with the LCD showed a slight increase in the hepatocyte damages and these alterations were characterized by the non-uniform distribution of hepatocytes, dilation of intercellular space of hepatocytes and vacuolization of hepatocytes (Fig. 4B), while a spindle-shaped form in the lumen of hepatic sinusoids was frequently observed in fish fed with the LCD (Fig. 4C). Unlike the fish in the LCD group, a polar distribution of hepatocytes were widely observed in the HCD group, and abundant serpentine bands with clusters of proliferative cells in the hepatic vein appeared in the HCD group (Fig. 4D). Aside from these alterations, clusters of hepatocytes were attached to the edge of the lumen of hepatic sinusoids and these hepatocytes were arranged in a circular wreath-like pattern in the HCD group (Fig. 4E).

At the end of a 60-day recovery, hepatocytes and nuclei of the control fish showed a normal structure (Fig. 4F). No obvious pathological alterations were seen in livers of fish previously fed with the LCD (Fig. 4G). Fish previously fed with the HCD showed a significant alleviation of liver injury, whereas the dilation of intercellular space and disorganization of the lumen of hepatic sinusoids were still observed (Fig. 4H).

3.3. Microcystins in fish liver, muscle and faeces

At the 1st EP, MCs concentration in livers of fish fed with the LCD was at the similar level as that at the 2nd EP ($P > 0.05$) (Fig. 5A). MCs concentration in livers of fish previously fed with the LCD decreased at the 1st RP, while a significant decrease in the MCs concentration of fish previously fed with the LCD was observed throughout the entire 2nd RP ($P < 0.05$). MCs concentration in livers of fish fed with the HCD at the 1st EP was also at the similar level as that at the 2nd EP. MCs concentration in livers of fish previously fed with the HCD was significantly reduced after the 1st RP and then remained stable throughout the entire 2nd RP ($P < 0.05$).

However, MCs concentrations in muscles of fish fed with the LCD and HCD tended to decrease from the 1st EP to the 2nd EP (Fig. 5B). After the 1st RP, MCs concentration in muscles of fish previously fed with the LCD also tended to decrease and then reached a stable level during the 2nd RP. After the 1st RP, MCs concentration showed a distinct increase in muscles of fish previously fed with the HCD and then kept constant throughout the entire 2nd RP.

Faecal MCs concentration in fish fed with the HCD was significantly higher than that in fish fed with the LCD during the whole exposure period ($P < 0.05$) (Fig. 6).

Table 2
Body weight of yellow catfish at different sampling points (mean ± SE.).

Diets	Body weight (g)				
	Initial	Day 30	Day 60	Day 90	Day 120
Control	5.38 ± 0.02	10.48 ± 0.22[a]	13.75 ± 0.36[a]	14.70 ± 0.60[a]	15.55 ± 0.67
LCD	5.29 ± 0.03	8.42 ± 0.13[b]	11.40 ± 0.4[b]	14.56 ± 0.79[a]	18.47 ± 1.55
HCD	5.25 ± 0.03	6.99 ± 0.16[c]	8.59 ± 0.30[c]	11.36 ± 0.54[b]	15.21 ± 0.78

Means with the different superscripts within the same column are significantly different at $P < 0.05$.

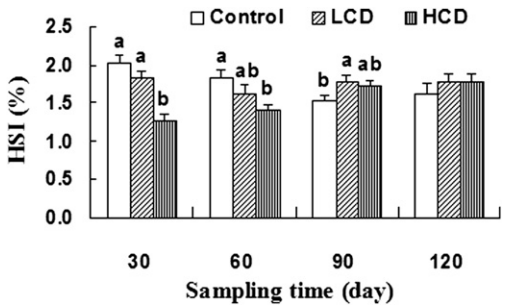

Fig. 1. Hepatosomatic index (HSI) of yellow catfish fed with the experimental diets at different sampling points (mean ± SE). Means with different superscripts at each sampling point are significantly different ($P < 0.05$).

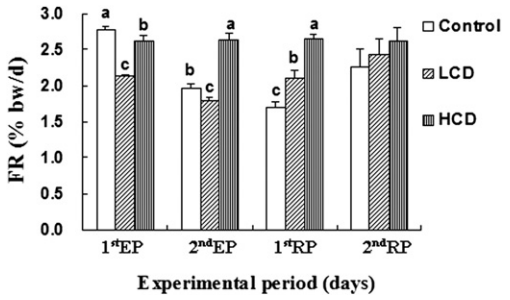

Fig. 2. Feeding rate (FR) of yellow catfish fed with the experimental diets during the whole experiment (mean ± SE). Means with different superscripts during each experimental period are significantly different ($P < 0.05$).

4. Discussion

4.1. Effect of dietary cyanobacteria on the growth performance of yellow catfish

In the present study, no fish mortality was observed in yellow catfish after a 60-day dietary exposure to cyanobacteria. No fish mortality was also reported in hybrid tilapia by exposure to dietary cyanobacteria for 60 days in our previous study (Dong et al., 2009) and no significant difference was also observed in the mortality of Nile tilapia by feeding the fish with the dietary cyanobacteria for 12 weeks (Zhao et al., 2006). However, fish mortalities in rainbow trout and hybrid sturgeon increased with increasing dietary cyanobacteria concentration, while the highest fish mortality was observed in fish fed with the medium-cyanobacteria diet (Zhao, 2006). These discrepancies between results demonstrate that the effects of dietary cyanobacteria on fish mortality vary significantly between different fishes and can be attributed to differences in the consumed amount of MCs. The present results suggested that mortality is not a sensitive marker for sub-chronic dietary toxicity of cyanobacteria in many fishes.

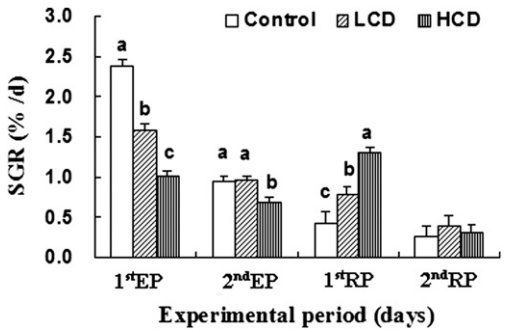

Fig. 3. Specific growth rate (SGR) of yellow catfish fed with the experimental diets during the whole experiment (mean ± SE). Means with different superscripts during each experimental period are significantly different ($P < 0.05$).

In the current study, yellow catfish showed a significant reduction in the growth rate after 30 days dietary exposure to cyanobacteria. Previous studies indicated that a dose-dependent decrease in growth rate was observed after exposure to MCs (Li et al., 2004; Dong et al., 2011). However, Zhao et al. (2006) found that sub-chronic exposure to dietary cyanobacteria increased the growth rate of Nile tilapia. These discrepancies between these results might be due to differences in the administration dose of MCs (Yellow catfish were orally exposed to either 32.3 μg MCs/g or 71.96 μg MCs/g in the present study, but Nile tilapia were only orally exposed to 1.25–5.46 μg MCs/g). In addition, different fishes showed different sensitivities to dietary cyanotoxins. Zhao et al. (2006) also indicated that Nile tilapia might be more tolerable to cyanotoxins.

In the current study, the growth rate of yellow catfish fed with the LCD returned to the normal level compared with that of the control at the end of the 2nd EP. This result suggested that yellow catfish exhibit a stepwise adaptation to the low-cyanobacteria diet and increased tolerance to dietary cyanotoxins during the 2nd EP. In the present study, the increased feeding rate was observed in fish fed with the HCD during the whole exposure period. Similar result was also observed in hybrid tilapia after exposure to high-cyanobacteria diet (Dong et al., 2009). This higher FR in yellow catfish fed with the HCD could be due to the Hormesis (a biphasic dose–response with a low dose stimulation or beneficial effect and a high dose inhibitory or toxic effect) (Calabrese, 1999) and dietary toxins (MCs, aflatoxin B_1 and oxidized fish oil) are sometimes known to have stimulant properties and then resulted in higher FR in our previous study (Zhao et al., 2006; Dong et al., 2009, 2012; Huang et al., 2011). In addition, fish regulate their feed intake depending on their energy requirement (Kaushik and Luquet, 1984). Similar finding was also observed in other fishes after exposure to dietary toxins (MCs, aflatoxin B_1 and oxidized fish oil), which the fishes may increase their feed intake in relation to "energy requirement" for maintaining their physiological requirement or combating the oxidative stress induced by toxins (Zhao et al., 2006; Dong et al., 2009, 2012; Huang et al., 2011).

4.2. Effect of dietary cyanobacteria on the liver histopathology of yellow catfish

In the current study, the reduced growth rate of yellow catfish was accompanied by serious liver lesions. Palíková et al. (2004) and Zhang et al. (2008) reported that the

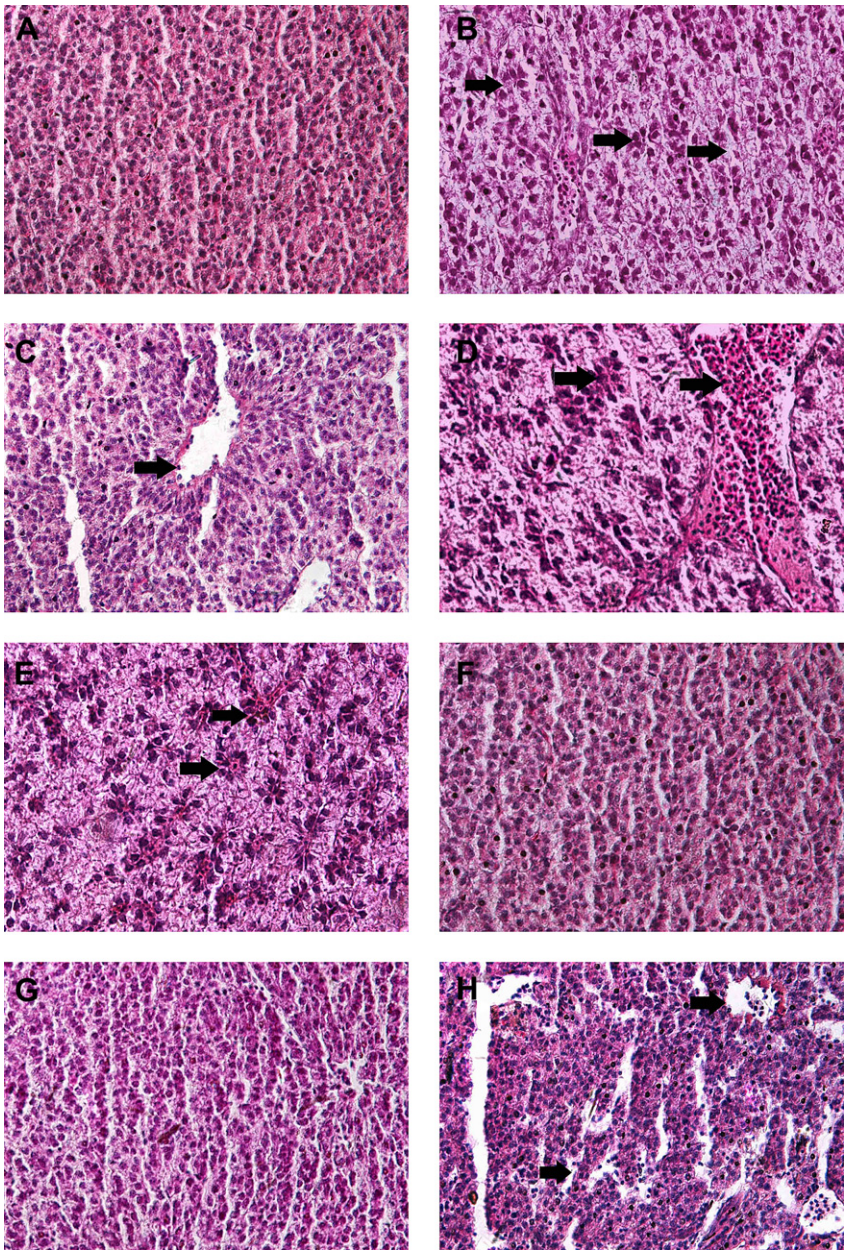

Fig. 4. Histopathological alterations in livers of yellow catfish before and after the whole recovery period (320×). (A): Livers of the control fish at the end of a 60-day exposure. (B) and (C): Liver of fish fed with the LCD at the end of a 60-day exposure. (B) Showing the non-uniform distribution of hepatocytes (Top left arrow), the vacuolization of hepatocytes (Middle arrow) and the dilation of intercellular space of hepatocytes (Bottom right arrow); (C) showing a spindle-shaped form in the lumen of hepatic sinusoids. (D) and (E): Liver of fish fed with the HCD at the end of a 60-day exposure. (D) Showing a polar distribution of hepatocytes (Left arrow) and clusters of proliferative cells in the hepatic vein (Right arrow). (E) Showing clusters of hepatocytes arranged in a circular wreath-like pattern (Arrows). (F): Livers of the control fish at the end of a 60-day recovery. (G): Showing no obvious alteration in livers of fish previously fed with the LCD. (H): Showing the dilation of intercellular space (Bottom left arrow) and the disorganization of the lumen of hepatic sinusoid (Top right arrow) in livers of fish previously fed with the HCD at the end of the 60-day recovery.

characteristic histopathological alterations in fish livers by exposure to cyanotoxins were illustrated as disorganization of cell structure and cellular degeneration with vacuolar hepatocytes. Similar histopathological alterations were also observed in the present study. However, compared to other fishes, yellow catfish fed with the HCD showed remarkable histopathological alterations including clusters of hepatocytes arranged in a circular wreath-like pattern. Fournie and Courtney (2002) reported that both hardhead catfish and gulf killifish exhibited extensive hepatocellular necrosis after the 6 h post injections with 45–300 mg MC-LR/kg. This is most likely due to a more efficient uptake of

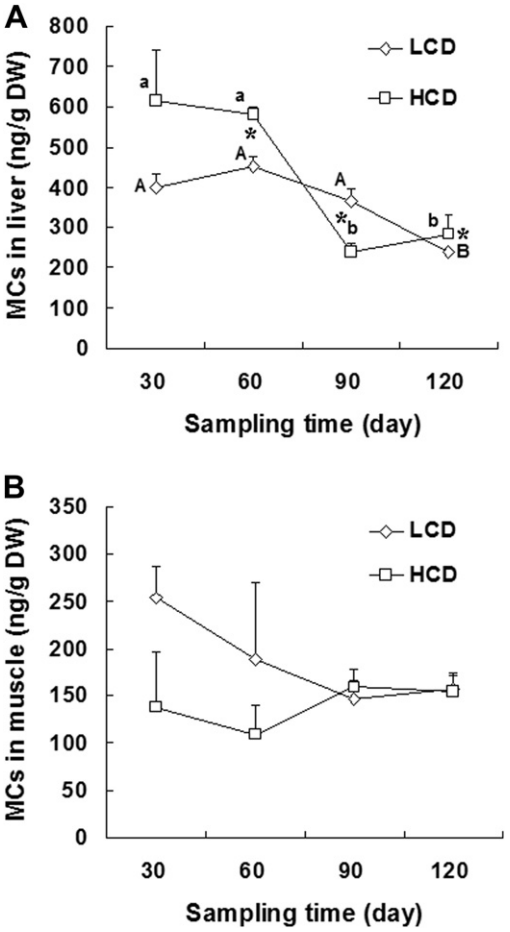

Fig. 5. Microcystins concentration in liver and muscle of yellow catfish at different sampling points. (A) Microcystins concentrations in liver at each sampling point; (B) microcystins concentrations in muscles at each sampling point. Means with different capital letters represent the significance of LCD group between different sampling points ($P < 0.05$); Means with different lowercase letters represent the significance of HCD group between different sampling points ($P < 0.05$). Asterisk (*) represents the significance between fish fed with the LCD and the HCD at each sampling point, respectively ($P < 0.05$). All data were described as mean ± S.E.

toxin after acute exposure to MCs and a slower development of pathological alteration was observed in yellow catfish after sub-chronic exposure to dietary cyanobacteria. Therefore, the conflicting results in the different pathological alterations between the present study and the previous study could be attributed to several factors including the exposure concentration, exposure route and exposure duration.

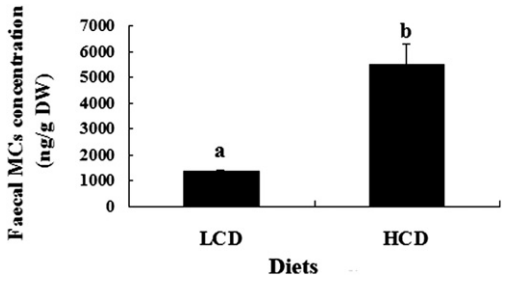

Fig. 6. Faecal microcystins concentration in yellow catfish during the whole exposure period. Means with different superscripts are significantly different ($P < 0.05$).

MCs have been known to be potent and specific inhibitors of PP1 and PP2A, which disturbed the cellular phosphorylation balance and causes hyperphosphorylation of a variety of proteins (Eriksson et al., 1990; Falconer and Yeung, 1992; Runnegar et al., 1993). These proteins (e.g., actin, talin or α-actin) are certainly responsible for the alterations in the loss of normal cell shape, the widening of intercellular spaces and the disassociation of hepatocytes (Dawson, 1998). It is likely that the widening of intercellular spaces observed in livers of yellow catfish in the present study might have resulted from cyanotoxin-induced destabilization of adhesive structures through the increased phosphorylation (Eriksson et al., 1990; Falconer and Yeung, 1992).

There have been only limited studies on the recovery ability at the histological level after sub-chronic exposure to cyanotoxins. Malbrouck et al. (2003) reported that the recovery of tissue structure and regeneration of hepatocytes were observed in goldfish after the 96 h post rejection with 125 mg MC-LR/kg and the reconstruction of initial tissue structure was observed at the end of a 21-day recovery. An excellent recovery rate of the damaged liver of mice was found after 2 months of wash-out followed by every 2 days injections of MC-LR for 1 month (Andrinolo et al., 2008).

Consistent with the previous studies, no obvious histopathological alteration was observed in livers of fish previously fed with the LCD in the present study at the end of 2nd RP. However, a mild pathological alteration was still observed in livers of fish previously fed with the HCD. The present study suggested that yellow catfish showed a rapid and complete recovery in fish previously exposed to the low-dose of dietary cyanotoxins for 60 days, while there was a significant alleviation of symptoms and a slow recovery were exhibited in fish previously exposed to the high-dose of dietary cyanotoxins after a 60-day recovery.

4.3. Microcystins accumulation and depuration in fish tissue

The present study confirmed that MCs accumulated in liver and muscle of aquatic animals, which was consistent with the previous studies in other fishes (Dong et al., 2009, 2011), mussels (Amorim and Vasconcelos, 1999), shrimps (Chen and Xie, 2005) and snails (Chen et al., 2005). Previous study indicated that liver is the major organ for MCs accumulation in fish (Magalhães et al., 2001; Ernst et al., 2001), similar results were also obtained in yellow catfish. Similarly, higher MCs accumulation (423.55–3007.33 ng MCs/g DW) was observed in Nile tilapia after a 12-week dietary cyanobacteria exposure (Zhao et al., 2006). Yellow catfish in the current study accumulated much higher MCs in liver than that in shrimps (4.29 μg MCs/g DW) (Chen and Xie, 2005) and snails (1.06–7.42 μg MCs/g DW) derived from natural environments (Chen et al., 2005). Generally, accumulation of MCs in fish tissue is depended on the concentration of MCs in their food resources. Magalhães et al. (2003) reported that in Sepetiba

Bay, Brazil, a significant correlation was observed between MCs concentration in the seston samples and in the fish muscle. MCs accumulation in fish tissue might also be affected by various factors such as the exposure route, exposure duration and exposure concentration.

In the current study, MCs concentration decreased much faster in liver than in muscle of yellow catfish during the whole recovery period. Xie et al. (2004) also reported that the depuration rate was in the order of liver > muscle in silver carp. A rapid depuration was observed in silver carp (Xie et al., 2004): a depuration period of 20 days resulted in MC-RR declines from 24.63 to 3.21 μg/g DW in liver, and from 1.77 to 0.8 μg/g DW in muscle. A much faster depuration of MC-LR was also observed in rainbow trout in the acute exposure experiment: a depuration period of 3 days resulted in reductions of 91.6% MC-LR in liver (Tencalla and Dietrich, 1997). However, there is a fundamental difference in the depuration kinetics of MCs in tissues between rainbow trout, silver carp and yellow catfish, the difference could be due to the exposure concentration and food habits of fishes under natural environments. In the present study, the highest MCs concentration in muscle was observed in fish previously fed with the HCD after the 1st RP. A similar result was also observed in hybrid tilapia in our previous study (Dong et al., 2009). This could be due to different transferring of MCs from liver to muscle. In addition, MacKintosh et al. (1990) revealed that MC-LR was a potent specific inhibitor of protein phosphatases 1 (PP1) and 2A (PP2A) and there was a strong interaction between MC-LR and the catalytic subunit of PP2A (Honkanen et al., 1990). The MCs determined in the present study could be possible to include the MCs bound to PP1 and PP2A based on the ELISA method. Therefore, muscle MCs referred to free MCs plus bound MCs during the depuration period and total MCs in the muscle tissues are slightly overestimated. Amorim and Vasconcelos (1999) and Soares et al. (2004) also observed higher MCs concentrations in mussels during the depuration period. It could be a consequence of the metabolization of proteins phosphatases, a turnover that would lead to releasing of these toxins, allowing its detection by the ELISA method.

Cyanobacterial blooms have occurred frequently in aquatic systems for aquaculture and fishery. Therefore, commercial fish from those environments could pose a potential threat to public health. WHO has proposed a tolerable daily intake (TDI) of 0.04 μg/kg bw per day for MC-LR (Chorus and Bartram, 1999). The present study showed that muscle MCs concentrations were more than 100 ng MCs/g DW in both of fish fed with the LCD and HCD before and after the whole recovery period. A coefficient of 5 was used to convert dry weight to wet weight. If an adult weighing 60 kg ingested 300 g fish muscle a day, the ingestion of MCs is 0.1 μg/kg body weight/day, which is higher than WHO limitation. Therefore, fish muscle in both dose groups could be still not safe for human consumption after a period of depuration. Although dietary MCs concentrations in the present study were higher than those in natural environments, more detailed study should be conducted to investigate how long time required for the clearance of the toxin in fish with a prolonged recovery period to guarantee the food safety for human consumption.

In conclusions, dietary cyanobacteria resulted in a significant reduction in the growth rate of yellow catfish, while a dose-dependent histopathological modification was observed in livers of fish at the end of a 60-day exposure. After a 60-day recovery, the fish previously exposure to the low-dose group could have a full recovery. However, the fish previously exposure to the high-dose group did not get a complete recovery. Fish muscle in both dose groups could be still not safe for human consumption after a 60-day depuration.

Acknowledgments

The research was supported by the National Basic Research Program (973 No. 2008CB418006) and the Special Fund for Agro-scientific Research in the Public Interest (201303053), and partly by the National water pollution control and management technology major projects (2008ZX07102-005). Authors also should give great thanks to Dr. Haiyan Li, Mr Xiaoming Zhang and Mr Guanghan Nie for their technical helps. We are grateful to Dr. Zhihua Pei, Dr. Dongfang Deng and Dr. Swee Teh for their valuable suggestions to the manuscript. Thanks are also given to the anonymous reviewers for their helpful suggestions.

Ethical statement

All experiments were performed in accordance to the Guidelines for the management of Laboratory Animal in China. Under the scientific principles, numbers of animals were minimized and methods to alleviate the suffering of animals were applied.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

Amado, L.L., Monserrat, J.M., 2010. Oxidative stress generation by microcystins in aquatic animals: why and how. Environ. Int. 36, 226–235.

Amorim, Á., Vasconcelos, V., 1999. Dynamics of microcystins in the mussel *Mytilus galloprovincialis*. Toxicon 37, 1041–1052.

Andrinolo, D., Sedan, D., Telese, L., Aura, C., Masera, S., Giannuzzi, L., Marra, C.A., Alaniz, M.J.T., 2008. Hepatic recovery after damage produced by sub-chronic intoxication with the cyanotoxin microcystin LR. Toxicon 51, 457–467.

AOAC (Association of Official Analytical Chemists), 1995. Official Methods of Analysis of Official Analytical Chemists International, sixteenth ed. Association of Official Analytical Chemists, Arlington, VA.

Bury, N.R., McGeer, J.C., Eddy, F.B., Codd, G.A., 1997. Liver damage in brown trout, *Salmo trutta* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum), following administration of the cyanobacterial hepatotoxin microcystin-LR via the dorsal aorta. J. Fish. Dis. 20, 209–215.

Calabrese, E.J., 1999. Evidence that hormesis represents an "overcompensation" response to a disruption in homeostasis. Ecotoxicol. Environ. Saf. 42, 135–137.

Carbis, C.R., Rawlin, G.T., Mitchell, G.F., Anderson, J.W., McCauley, I., 1996. The histopathology of carp, *Cyprinus carpio* L., exposed to microcystins by gavage, immersion and intraperitoneal administration. J. Fish. Dis. 19, 199–207.

Carmichael, W.W., 1994. Toxins of cyanobacteria. Sci. Am. 270, 78–86.

Chorus, I., Bartram, J., 1999. Toxic Cyanobacteria in Water. In: A Guide to Their Public Health Consequences, Monitoring and Management. Published on behalf of WHO by F & FN Spon, London, p. 416.

Chen, J., Xie, P., 2005. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in two freshwater shrimps, *Palaemon modestus* and *Macrobrachium nipponensis*, from a large shallow, eutrophic lake of the subtropical China. Toxicon 45, 615–625.

Chen, J., Xie, P., Guo, L.G., 2005. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in a freshwater snail (*Bellamya aeruginosa*) from a large shallow, eutrophic lake of the subtropical China. Environ. Pollut. 134, 423–430.

Chorus, I., Mur, L., 1999. Preventive measure. In: Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in Water—a Guide to Their Public Health Consequences, Monitoring and Management. E&FN Spon., London, pp. 235–273.

Dawson, R.M., 1998. The toxicology of microcystins. Toxicon 36, 953–962.

Dong, G.F., Zhu, X.M., Han, D., Yang, Y.X., Song, L.R., Xie, S.Q., 2009. Effects of dietary cyanobacteria of two different sources on growth and recovery of hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). Toxicon 54, 208–216.

Dong, G.F., Zhu, X.M., Han, D., Yang, Y.X., Song, L.R., Xie, S.Q., 2011. Response and recovery of hybrid sturgeon from sub-chronic oral administration of cyanobacteria. Environ. Toxicol. 26, 161–170.

Dong, G.F., Huang, F., Zhu, X.M., Zhang, L., Mei, M.X., Hu, Q.W., Liu, H.Y., 2012. Nutri-physiological and cytological responses of juvenile channel catfish (*Ictalurus punctatus*) to dietary oxidized fish oil. Aquacult. Nutr.. http://dx.doi.org/10.1111/j.1365-2095.2011.00931.x

Eriksson, J.E., Toivola, D., Meriluoto, J.A.O., Karaki, H., Han, Y.G., Hartshorne, D., 1990. Hepatocyte deformation induced by cyanobacterial toxins reflects inhibition of protein phosphatases. Biochem. Biophys. Res. Commun. 173, 1347–1353.

Ernst, B., Hitzfeld, B.C., Dietrich, D., 2001. Presence of *Planktothrix* sp. and cyanobacterial toxins in Lake Ammersee, Germany and their impact on whitefish (*Coregonus lavaretus* L.). Environ. Toxicol. 16, 483–488.

Falconer, I.R., Yeung, D.S.K., 1992. Cytoskeletal changes in hepatocytes induced by *Microcystis* toxins and their relation to hyperphosphorylation of cell proteins. Chem. Biol. Interact 81, 181–196.

Fournie, J.W., Courtney, L.A., 2002. Histopathological evidence of regeneration following hepatotoxic effects of the cyanotoxin microcystin-LR in the hardhead catfish and gulf killifish. J. Aquat. Anim. Health 14, 273–280.

Hao, L., Xie, P., Fu, J., Li, G.Y., Xiong, Q., Li, H.Y., 2008. The effect of cyanobacterial crude extract on the transcription of GST mu, GST kappa and GST rho in different organs of goldfish (*Carassius auratus*). Aquat. Toxicol. 90, 1–7.

Honkanen, R.E., Zwiller, J., Moore, R.E., Daily, S.L., Khatra, B.S., Dukelow, M., Boynton, A.L., 1990. Characterization of microcystin-LR, a potential inhibitor of type 1 and type 2A protein phosphatases. J. Bio. Chem. 265, 19401–19404.

Huang, Y., Han, D., Zhu, X.M., Yang, Y.X., Jin, J.Y., Chen, Y.F., Xie, S.Q., 2011. Response and recovery of gibel carp from subchronic oral administration of aflatoxin B_1. Aquaculture 319, 89–97.

Hu, C.L., Gan, N.Q., He, Z.K., Song, L.R., 2008. A novel chemiluminescent immunoassay for microcystin (MC) detection based on gold nanoparticles label and its application to MC analysis in aquatic environmental samples. Int. J. Environ. Anal. Chem. 88, 267–277.

Ibelings, B.W., Chorus, I., 2007. Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: a review. Environ. Pollut. 150, 177–192.

Kaushik, S.J., Luquet, P., 1984. Relationship between protein intake and voluntary energy intake as affected by body weight with an estimation of maintenance needs in rainbow trout. Z. Tierphysiol. Tierernahr. Futtermittelkd. 51, 57–69.

Li, L., Xie, P., Chen, J., 2005. In vivo studies on toxin accumulation in liver and ultrastructural changes of hepatocytes of the phytoplanktivorous bighead carp i.p.-injected with extracted microcystins. Toxicon 46, 533–545.

Li, X.Y., Chung, I.K., Kim, J.I., Lee, J.A., 2004. Subchronic oral toxicity of microcystin in common carp (*Cyprinus carpio* L.) exposed to *Microcystis* under laboratory conditions. Toxicon 44, 821–827.

MacKintosh, C., Beattic, K.A., Klumpp, C., Cohen, C., Codd, G.A., 1990. Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Lett. 264, 187–192.

Magalhães, V.F., Soares, R.M., Azevedo, S.M.F.O., 2001. Microcystin contamination in fish from the Jacarepaguá Lagoon (Rio de Janeiro, Brazil): ecological implication and human health risk. Toxicon 39, 1077–1085.

Magalhães, V.F., Marinho, M.M., Domingos, P., Oliveira, A.C., Costa, S.M., Azevedo, L.O., Azevedo, S.M.F.O., 2003. Microcystins (cyanobacteria hepatotoxins) bioaccumulation in fish and crustaceans from Sepetiba Bay (Brasil, RJ). Toxicon 42, 289–295.

Malbrouck, C., Trausch, G., Devos, P., Kestemont, P., 2003. Hepatic accumulation and effects of microcytin-LR on juvenile goldfish *Carassius auratus* L. Comp. Biochem. Physiol. 135C, 39–48.

Paerl, H.W., Fulton, R.S., Moisander, P.H., Dyble, J., 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. Sci. World J. 1, 76–113.

Palíková, M., Navrátil, S., Tichý, F., Štěrba, F., Maršalek, B., Bláha, L., 2004. Histopathology of carp (*Cyprinus carpio* L.) larvae exposed to cyanobacteria extract. Acta Vet. (Brno) 73, 253–257.

Runnegar, M.T.C., Kong, S.M., Berndt, N., 1993. Protein phosphatase inhibition and in vivo hepatotoxicity of microcystins. Am. J. Physiol. 265, 224–230.

Soares, R.M., Magalhães, V.F., Azevedo, S.M.F.O., 2004. Accumulation and depuration of microcystins (cyanobacteria hepatotoxins) in *Tilapia rendalli* (Cichlidae) under laboratory conditions. Aquat. Toxicol. 70, 1–10.

Tencalla, F.G., Dietrich, D.R., 1997. Biochemical characterization of microcystin toxicity in rainbow trout (*Oncorhynchus mykiss*). Toxicon 34, 583–595.

Xie, L.Q., Xie, P., Ozawa, K., Honma, T., Yokoyama, A., Park, H.D., 2004. Dynamics of microcystins-LR and -RR in the phytoplanktivorous sliver carp in a sub-chronic toxicity experiment. Environ. Pollut. 127, 431–439.

Zhang, X.Z., Xie, P., Wang, W.M., Li, D.P., Li, L., Tang, R., Lei, H.H., Shi, Z.C., 2008. Dose-dependent effects of extracted microcystins on embryonic development, larval growth and histopathological changes of southern catfish (*Silurus meridionalis*). Toxicon 51, 449–456.

Zhao, M., Xie, S.Q., Zhu, X.M., Yang, Y.X., Gan, L.Q., Song, L.R., 2006. Effect of dietary cyanobacteria on growth and accumulation of microcystins in Nile tilapia (*Oreochromis niloticus*). Aquaculture 261, 960–966.

Zhao, M., 2006. The Comparative Study of Effects of Blue-green Algae Meal Inclusion on Fishes of Different Food Habit. Doctoral thesis, Chinese Academy of Sciences, China, pp. 43–109.

Zurawell, R.W., Chen, H., Burke, J.M., Prepas, E.E., 2005. Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. J. Toxicol. Environ. Health B 8, 1–37.