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Effect of inclusion of blue-green algae meal on growth and accumulation of microcystins in gibel carp (*Carassius auratus gibelio*)

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Summary

Six isonitrogenous (crude protein content: 38%) and isoenergetic (gross energy content: 17 kJ g^{-1}) diets were formulated to investigate the effects of inclusion of blue-green algae meal on gibel carp (*Carassius auratus gibelio*). In each diet, 15% of the protein was supplied by fishmeal; the remainder was supplied by soybean meal and blue-green algae meal. Diet 1 was used as control with no blue-green algae meal whereas the content in diets 2–6 was 15.15, 29.79, 44.69, 59.58 and 74.48%, respectively. Each diet was fed to five groups of gibel carp for 12 weeks in a flow-through system. Final body weight and specific growth rate (SGR) of fish fed diet 5 were significantly lower than the control diet ($P < 0.05$). Mortality of gibel carp increased with increase in algae meal inclusion ($P < 0.05$), but there was no significant difference between fish fed diets 3–6 ($P > 0.05$). Feed conversion efficiency (FCE) decreased with the increase in algae meal inclusion ($P < 0.05$). Fish-fed diet 6 showed the highest feeding rate ($P < 0.05$), while there were no significant differences among the other groups ($P > 0.05$). Apparent digestibility coefficient of dry matter, protein, and energy decreased with increasing algae meal inclusion in the diets ($P < 0.05$). Aspartate aminotransferase (GOT) activity in the liver was not significantly different among groups ($P > 0.05$). Liver alanine aminotransferase (GPT) activity of fish-fed diets 4, 5 and 6 was significantly lower than the control diet (diet 1; $P < 0.05$). Microcystins in the muscle, liver, gallbladder, and spleen increased with increasing algae inclusion ($P < 0.05$).

Introduction

Mass growths of cyanobacteria occur in freshwater lakes, ponds, reservoirs, slow-flowing rivers, brackish water lakes, estuaries, and shorelines throughout the world and result in water blooms (Codd, 1995; Herath, 1997; Codd et al., 1999). Bloom cyanobacteria often release cyanobacterial toxins (Kaya, 1996). Microcystins (MC), which are heptacyclic peptide hepatotoxins (Kunimitsu, 1996; Dawson, 1998), appear most frequently and have the most serious impact on animals (Baganz et al., 1998). They are poisonous to animals and result in weakness, pallor, cold extremities, heavy breathing, recumbency, vomiting, and diarrhea, and are also promoters of liver tumors (Dawson, 1998; Codd, 2000). Water pollution has recently become increasingly serious, with frequent outbreaks of water blooms (Dawson, 1998). Many methods have been introduced to decrease these water blooms. Lam et al. (1995), however, found that the chemicals used to control phytoplankton blooms induced the release of phytotoxins, increasing the potential health risks in drinking water supplies. Datta and Jana (1998) thought that control through the stocking and management of herbivorous fishes was the most ecologically sound management strategy. The main methods of water bloom control in China are through collecting cyanobacteria and/or stocking filtering fishes such as silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) to intake the cyanobacteria (Xie, 2003).

Shao et al. (2001) reported that cyanobacteria (*T. thiebautii*) contained 30.6% crude protein and could thus be a potential in fish diets, since protein sources are very limited throughout the world. Magalhães et al. (2001) reported that the intake of cyanobacteria could result in accumulation of MCs in the fish body. However, there is still no direct relationship of body MC accumulation in relation to dietary MC content.

Snyder et al. (2002) found that MCs were likely to produce an extended period of chronic mortality rather than immediate acute losses. There are many reports about the acute effect of MCs on fish (Phillips et al., 1985; Tencalla et al., 1994), but few reports are available with regard to the chronic effect.

The present study was designed to investigate the effect of blue-green algae powder inclusion in diets on the growth and accumulation of MCs in gibel carp (*Carassius auratus gibelio*) and to evaluate the potential of blue-green algae meal as a fish dietary protein and to establish the relationship between body composition and dietary intake of MCs.

Material and methods

Fish and experimental diets

Gibel carp (*C. auratus gibelio*) were purchased from a local hatchery (Tangxunhu Lake, Hubei province) and stocked in 10 experimental tanks for acclimatization 3 weeks prior to experiment. During the first 2 weeks of acclimatization, fish were fed a practical diet (containing 38% crude protein) to satiation twice a day (9.00 and 15.00 hours). In the last week of acclimatization, the fish were fed an equal mixture of all experimental diets.

Six experimental diets were formulated to be isoenergetic and isonitrogenous. The diets contained about 38% crude protein (in dry matter) and around 17 kJ g^{-1} gross energy (in dry matter). Diet 1 contained 68.81% soybean meal as the main protein source and used as control. Diets 2, 3, 4, 5 and 6 contained 15.15, 29.79, 44.69, 59.58 and 74.48% algae meal, respectively. In diets 2–5, ratios of soybean meal protein to algae meal protein were 4 : 1, 2 : 3, 3 : 2 and 4 : 1, respectively. Diet 6 contained no soybean meal. Cr_2O_3 (1%) was added to diets as an indicator for digestibility determination.

Diet formulation and chemical composition of experimental diets are shown in Table 1. All diets were made into 1 mm pellet by extrusion, wind-dried, and stored at 4°C.

Algae were collected from Dianchi Lake, Yunnan, China. The algae (90% *Microcystis aeruginosa*) were then sun-dried. The MC content of dry algae meal was MC-RR (Arg, Arg): 0.008%, MC-YR (Try, Arg): 0.001%, MC-LR (Leu, Arg): 0.002%.

Experimental procedure

The growth trial was carried out in a flow-through system consisting of 30 cylindrical fiberglass tanks (volume 98 L each). Fresh water continuously flowed into each tank (10 ml min^{-1}) after aeration and dechlorination in a reservoir tank.

At the beginning of the experiment, healthy fish (initial body weight about 1.22 g) were batch weighed after 24 h feed deprivation and randomly distributed into the 30 tanks (40 fish per tank). The tanks were randomly assigned the six diets. Three samples (20 fish for each) were taken from the remaining fish and frozen for initial fish body chemical analysis.

During the experiment, continuous aeration was supplied to each tank; water temperature changed with time from 18 to 29°C (Fig. 1) through the seasonal trend; however, for at least 50 experimental days the temperature was above 22°C. The photoperiod was 12D : 12L with a light period from 8.00 to 20.00 hours supplied by three fluorescent lamps. Dissolved oxygen, ammonia nitrogen, and residual chlorinate (>5 mg L^{-1}, <0.5 mg L^{-1} and <0.05 mg L^{-1}, respectively) in water were measured once a week, pH was around 7.0.

Fish were fed twice a day (9.00 and 15.00 hours) and uneaten feed was collected by siphoning 1.5 h after feeding. Fresh and intact fish feces were collected by siphoning at about 14.00 hours daily [about 5 h after the first feeding; previous studies on this species showed this to be the peak time of feces discharge (Shen et al., 1995)] and dried at 60°C for chemical analysis. Leaching rates of uneaten feeds were measured by putting about 10 g of each diet into tanks without fish and re-collected after 1.5 h, then dried and weighed. The leaching rate was used to calibrate the amount of uneaten feed.

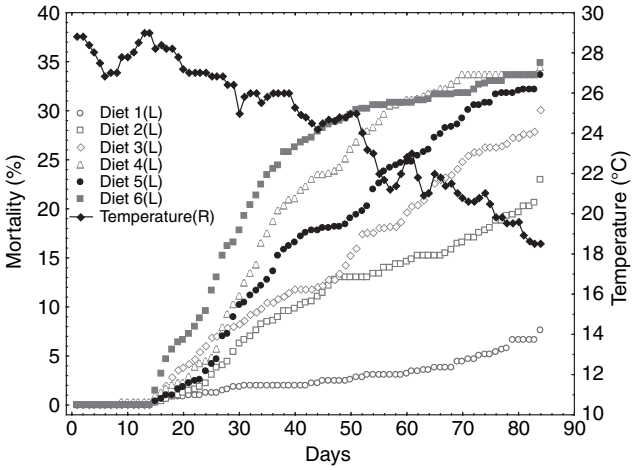

Fig. 1. Cumulative mortality (left *Y*-axis, in number) combined for five replicates of each treatment (diets 1–6) for representing diets with increasing levels of algae meal contained in the feed (for details see Table 1) and water temperature variation (right *Y*-axis)

The growth trial lasted for 12 weeks. At the end of the experiment, fish were batch weighed from each tank after 24 h feed deprivation. Three fish from each tank were killed by a heavy blow to the head. One fish was dissected on an ice pan and the liver was removed for enzymatic analysis; the two other fish from each tank were dissected on an ice pan and the muscle, liver, gallbladder, and spleen were taken for MC accumulation analysis. The remaining fish in each tank were taken for final fish body composition analysis.

Chemical analysis

The initial and final fish samples were autoclaved at 120°C, homogenized and oven-dried at 70°C. Crude protein, fat, ash, and energy contents were analyzed for fish samples and diets.

Table 1
Diet formulation and chemical composition of experiment diets (percentage in dry matter)

Diet	D1	D2	D3	D4	D5	D6
Soybean meal (oil-extracted)	68.81	54.81	41.29	27.52	13.76	0.00
Algae meal	0.00	15.15	29.79	44.69	59.58	74.48
White fishmeal (USA)	15.00	15.00	15.00	15.00	15.00	15.00
Fish oil	0.00	0.39	1.35	2.33	2.72	2.75
Corn starch	5.79	4.99	2.91	0.80	0.00	0.00
Carboxymethyl cellulose	3.74	3.00	3.00	3.00	2.28	1.11
Vitamin premix[1]	0.55	0.55	0.55	0.55	0.55	0.55
Vitamin C	0.01	0.01	0.01	0.01	0.01	0.01
Mineral premix[2]	5.00	5.00	5.00	5.00	5.00	5.00
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Cr_2O_3	1.00	1.00	1.00	1.00	1.00	1.00
Chemical composition (in dry matter)						
Crude protein (%)	39.63	39.04	37.40	37.85	37.91	37.70
Crude lipid (%)	2.78	2.59	5.24	8.33	7.79	8.39
Ash (%)	13.11	13.44	13.89	14.88	14.97	15.50
Water (%)	6.76	6.94	6.76	6.64	7.21	5.50
Gross energy (kJ g^{-1})	16.96	16.99	17.05	17.14	17.45	17.11
Microcystins (ng g^{-1})	0	39.12	124.14	174.5	203.03	228.92

[1]Vitamin premix (mg kg^{-1}): thiamin, 20; riboflavin, 20; pyridoxine, 20; vitamin B_{12}, 2; vitamin A, 1.83; vitamin D, 0.5; vitamin K, 10; folic acid, 5; Ca-pantothenate, 50; inositol, 100; vitamin E, 10.
[2]Mineral premix (g kg^{-1}): NaCl, 0.8; $MgSO_4 \cdot 7H_2O$, 12; $NaH_2PO_4 \cdot 2H_2O$, 20; KH_2PO_4, 25.6; $Ca(PO_4)_2 \cdot H_2O$, 16; $FeSO_4$, 2; $(CH_2CHCOO)_2Ca \cdot 5H_2O$, 2.8; $ZnSO_4 \cdot 7H_2O$, 0.028; $MnSO_4 \cdot 4H_2O$, 0.013; $CuSO_4 \cdot 5H_2O$, 0.0025; $CoCl_2 \cdot 6H_2O$, 0.0008; $KIO_3 \cdot 6H_2O$, 0.0024; cellulose, 0.36.

Feces samples were analyzed for chromic oxide, protein, and energy content.

Crude protein content was determined by the Kjeldahl method (AOAC, 1984). Fat content was measured by chloroform-methanol extraction (Lambert and Dehnel, 1974). Ash content was measured after 6 h at 550°C in a muffle furnace. Gross energy content was measured by combustion in a microbomb calorimeter (Phillipson microbomb calorimeter, Gentry Instruments Inc., Aiken, USA). Cr_2O_3 contents in feces and diets were measured as described by Bolin et al. (1952). For each measurement, at least duplicate samples were measured.

Enzymatic and microcystin analysis

The liver samples were homogenized in ice-water. Crude enzyme liquid was obtained after the homogenized slurry was centrifuged. Activity of aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) were measured according to the method described by Hørder and Rej (1981).

The dissected white muscle (from the back, close to the dorsal fin), liver, gallbladder, and spleen samples were ice-dried and ground. Dried cells after lyophilization were extracted twice with 100% methanol for 30 min with sufficient mixing with a magnetic stirrer. The methanol extract was diluted and redissolved in deionized water and passed through a Sep-pak C18 cartridge (Waters). The cartridge was rinsed with water and 20% methanol in water. The MCs were eluted with 90% methanol in water; the methanol extract was then dried and redissolved in deionized water. The monoclonal antibody (MAB) against MC was kindly provided by Prof. Ueno. Microtiter plates (Costar, USA) were coated with MAB (4.0 μg ml^{-1}) and incubated overnight at 4°C, then blocked with blocking buffer 170 μl (0.5%, w/v) gelatin in phosphate-buffered saline (PBS) for 2 h in the model 237 microplate incubator (Bio-Rad, USA) at 38°C or overnight at 4°C. Various concentrations of MC-LR were pre-incubated in 70 μl at 37°C for 30 min, then an equal volume of biotinylated MC MAB (25 ng ml^{-1}) was added to the coated wells for 30 min. Plates were thoroughly washed with PBS-T three times with a model 1575 immunwash apparatus (Bio-Rad). Horseradish peroxidase (HRP)-streptavidin (Sigma) diluted 1 : 10 000 in dilution buffer [PBS containing 0.5% (w/v) gelatin and 0.05% (v/v)] was added and incubated for 30 min at 37°C. Enzyme reaction was started by adding the substrate solution [0.1 M sodium acetate buffer (pH 5.0)] containing 100 μg ml^{-1} 3,3′,5,5′-tetramethylbenzidine and 0.005% (v/v) H_2O_2 and stopped with 1 M H_2SO_4. The absorbance at 450 nm was measured with a model 550 microtiter plate reader (Bio-Rad).

Calculation and statistical analysis

Specific growth rate (SGR; % day^{-1}) = 100
× [ln final body weight (wet, g)
− ln initial body weight (wet, g)]/Time (days)

Feed conversion efficiency (FCR; %) = 100
× [Final body weight (wet, g)
− Initial body weight (wet, g)]/Feed consumed (dried, g)

Feed rate (FR; % day^{-1}) = 200
× Total feed intake (dried, g)/[Initial body weight (wet, g)
+ Final body weight (wet, g)]/Time (days)

Mortality (%) = 100 × Dead fish in a tank/40

Apparent digestibility coefficient for dry matter (ADCd; %)
= 100 − 100 × % Cr_2O_3in diet/% Cr_2O_3in feces

Apparent digestibility coefficient for protein (ADCp; %)
= 100 − 100 × % Cr_2O_3in diet
× % protein in diet/% Cr_2O_3in feces × % protein in feces

Apparent digestibility coefficient for energy (ADCe; %)
= 100 − 100 × % Cr_2O_3in diet
× % energy in diet/% Cr_2O_3in feces × % energy in feces

Duncan's multiple range test was used to compare the difference between mean values after one-way ANOVA.

Results

Growth and survival

Table 2 shows that the final body weight and SGR of fish-fed diet 5 were significantly lower than those fed the control diet ($P < 0.05$). Mortality of gibel carp increased with the increase in algae meal inclusion ($P < 0.05$), while there was no significant difference among fish-fed diets 3, 4, 5 and 6 ($P > 0.05$). Feed conversion efficiency decreased with the increase in algae meal inclusion ($P < 0.05$). Fish-fed diet 6

Table 2
Mean initial and final wet weight, SGR, mortality, FCE, and FR of gibel carp fed diets containing different levels of algae meal (mean ± SE)*

Diet	Initial body weight (g)	Final body weight (g)	SGR (% day^{-1})	Mortality (%)	FCE (%)	FR (%)
1	1.22 ± 0.02	5.86 ± 0.57 b	1.87 ± 0.13 b	19.0 ± 10.4 a	45.48 ± 2.53 d	3.13 ± 0.17 a
2	1.23 ± 0.03	5.02 ± 0.44 ab	1.67 ± 0.12 ab	57.5 ± 23.3 b	42.75 ± 3.81 cd	2.61 ± 0.45 a
3	1.21 ± 0.02	5.09 ± 0.64 ab	1.70 ± 0.15 ab	75.0 ± 12.9 c	36.91 ± 5.27 c	2.74 ± 0.24 a
4	1.21 ± 0.02	5.17 ± 0.96 ab	1.71 ± 0.23 ab	86.5 ± 7.6 c	29.43 ± 4.56 b	3.17 ± 0.60 a
5	1.22 ± 0.02	4.25 ± 1.10 a	1.46 ± 0.34 a	84.0 ± 5.8 c	25.37 ± 5.59 ab	2.94 ± 0.60 a
6	1.22 ± 0.03	5.32 ± 1.11 ab	1.73 ± 0.21 ab	87.0 ± 1.1 c	21.68 ± 4.82 a	4.70 ± 1.08 b

*Mean values with different letter/s are significantly different ($P < 0.05$).
SGR, specific growth rate; FCE, feed conversion efficiency; FR, feed rate.

showed the highest feeding rate ($P < 0.05$), while there was no significant difference in the other groups ($P > 0.05$).

Gibel carp mortality started in the third week of the experiment. Figure 1 illustrates that the cumulative mortality (number of dead fish from all replicates combined) at that time suddenly increased in all tanks with algae meal in the diet, whereas the control (diet 1) displayed only a marginal but gradual increase over time. In most treatments (diets 3, 4, 5 and 6), mortalities were more than 70%. Thus, few fish were available for tissue sampling and body analysis.

Apparent digestibility coefficient

Table 3 shows that apparent digestibility coefficient of dry matter, protein, and energy decreased with increasing algae meal inclusion in diets ($P < 0.05$).

Enzyme activity and microcystin accumulation

Table 4 shows that GOT activity in the liver was not significantly different among groups ($P > 0.05$). Liver GPT activity of fish-fed diets 4, 5 and 6 were significantly lower than in the control diet (diet 1; $P < 0.05$).

Microcystin content in the muscle, liver, gallbladder, and spleen increased with increasing algae inclusion ($P < 0.05$; shown in Table 5). Accumulation of MC in these tissues exponentially increased with increasing algae inclusion in diets. The relationship between contents of MCs in the muscle (MM; ng g^{-1}), liver (ML; ng g^{-1}), gallbladder (MG; ng g^{-1}), spleen (MS; ng g^{-1}), and the content of MCs in diets (MD; ng g^{-1}) can described (Fig. 2a–d) as:

$$\mathrm{MM} = (0.005180 \pm 0.002113) \times \exp[(0.01547 \pm 0.001894) \times \mathrm{MD}], \quad R^2 = 0.8914, \mathrm{P} < 0.05$$

$$\mathrm{ML} = (0.02514 \pm 0.01253) \times \exp[(0.03226 \pm 0.002230) \times \mathrm{MD}], \quad R^2 = 0.9652, \mathrm{P} < 0.05$$

$$\mathrm{MS} = (0.7748 \pm 0.2453) \times \exp[(0.01093 \pm 0.00152) \times \mathrm{MD}], \quad R^2 = 0.8897, \mathrm{P} < 0.05$$

$$\mathrm{MG} = (0.50532 \pm 0.1786) \times \exp[(0.01296 \pm 0.001671) \times \mathrm{MD}], \quad R^2 = 0.9048, \mathrm{P} < 0.05$$

Table 3
Apparent digestibility coefficient of dry matter (ADCd), protein (ADCp), and energy (ADCe) in gibel carp fed different diets (mean ± SE)*

Diet	ADCd (%)	ADCp (%)	ADCe (%)
1	67.18 ± 2.22 d	94.44 ± 0.83 d	84.70 ± 2.30 d
2	55.73 ± 3.77 c	91.16 ± 2.13 c	79.82 ± 2.73 cd
3	49.17 ± 4.50 c	89.42 ± 1.41 c	74.91 ± 4.69 c
4	37.66 ± 6.18 b	84.01 ± 3.41 b	66.43 ± 8.14 b
5	26.59 ± 7.41 a	81.44 ± 1.70 a	53.47 ± 7.89 a
6	24.54 ± 4.74 a	74.87 ± 2.35 a	55.30 ± 5.92 a

*Mean values with different letter/s are significantly different ($P < 0.05$).

Table 4
GOT and GPT activities in the liver of gible carp fed different diets (mean ± SE)*

Diet	GOT (kU g^{-1})	GPT (kU g^{-1})
1	7.13 ± 2.33	5.00 ± 2.51 a
2	5.07 ± 1.46	3.29 ± 1.38 ab
3	7.43 ± 4.24	2.74 ± 1.37 ab
4	6.92 ± 1.53	1.87 ± 1.24 b
5	8.63 ± 2.12	2.10 ± 1.53 b
6	7.81 ± 7.84	1.37 ± 1.27 b

*Mean values with different letter/s are significantly different ($P < 0.05$).
GOT, aspartate aminotransferase; GPT, alanine aminotransferase.

Fish body composition

Dry matter, crude protein, crude lipid, and energy content in the final body of fish fed different diets were significantly higher than in the initial fish ($P < 0.05$), while there was no significant difference among final fish ($P > 0.05$). Fish-fed diet 6 had significant higher final body ash content than fish fed other diets ($P < 0.05$), whereas it was similar to the initial fish ($P > 0.05$; shown in Table 6).

Discussion

In the present trial, algae meal had no obvious effect on growth of gibel carp; only the SGR of the diet 5 group was lower than the control diet. Many authors found that MCs suspend fish growth (Bury et al., 1995; Kamjunke et al., 2002a). Rai (2000) found that *M. aeruginosa* led to poor fish growth. High mortality in this trial could have had an effect on gibel carp growth because of a subsequently lower stocking density compared to the other tanks. Although the initial stocking density in each tank changed with time, the tanks were well enough supplied with flow-through water; all water qualities, including ammonia and dissolved oxygen, remained equally suitable for all fish, which would minimize the different effects.

With the increase of algae meal inclusion in diets, the digestibility decreased while the intake increased. However, the final weight of gibel carp barely differed in the trials. It is suggested that the surviving fish in test groups with algae meal inclusion were comparatively fit, permitting a higher intake of the test diets. Another reason may have been that MCs or algae meal had different effects on growth with different fish species. Gibel carp is an omnivorous fish and can utilize algae much better than can carnivorous fish such as brown trout (Bury et al., 1995).

In the present study, algae meal inclusion led to high mortality of gibel carp. We were unable to identify all factors that affected this mortality. From the data, however, we can only assume that MCs might have played an important role, while lack of confirmation due to unknown deficiencies may have added to the MC effect. Magalhães et al. (2001) found MC concentrations in *Tilapia rendalli* liver reaching 31.1 $\mu g\ g^{-1}$, but no fish mortality was observed. Tencalla et al. (1994) reported that repeated gavage with MC in rainbow trout resulted in only modest to severe liver damage, but no mortality; these findings were different from the high mortality experienced in our gibel carp study. The sensitivity of fish to MC is dependent on the species and is probably influenced by the nature of the normal habitat of the fish (Snyder et al., 2002). Perhaps gibel carp are more sensitive to MCs than tilapia and rainbow trout. Some authors suggested

Table 5
Microcystin-LR equivalents found in tissues of gible carp fed different diets (mean ± SE)*

Diet	Muscle (ng g^{-1})	Liver (ng g^{-1})	Spleen (ng g^{-1})	Gallbladder (ng g^{-1})
1	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
2	0.019 ± 0.003 ab	1.69 ± 0.14 ab	1.47 ± 0.53 ab	1.79 ± 0.32 b
3	0.030 ± 0.002 b	4.14 ± 0.36 bc	3.09 ± 0.22 b	4.14 ± 0.91 c
4	0.062 ± 0.019 c	6.58 ± 0.67 c	5.32 ± 0.99 c	3.56 ± 0.32 c
5	0.147 ± 0.035 d	16.81 ± 1.56 d	7.72 ± 0.77 d	6.25 ± 0.20 d
6	0.171 ± 0.030 d	40.80 ± 6.22 e	8.99 ± 3.02 d	10.56 ± 0.59 e

*Mean values with different letter/s are significantly different ($P < 0.05$).

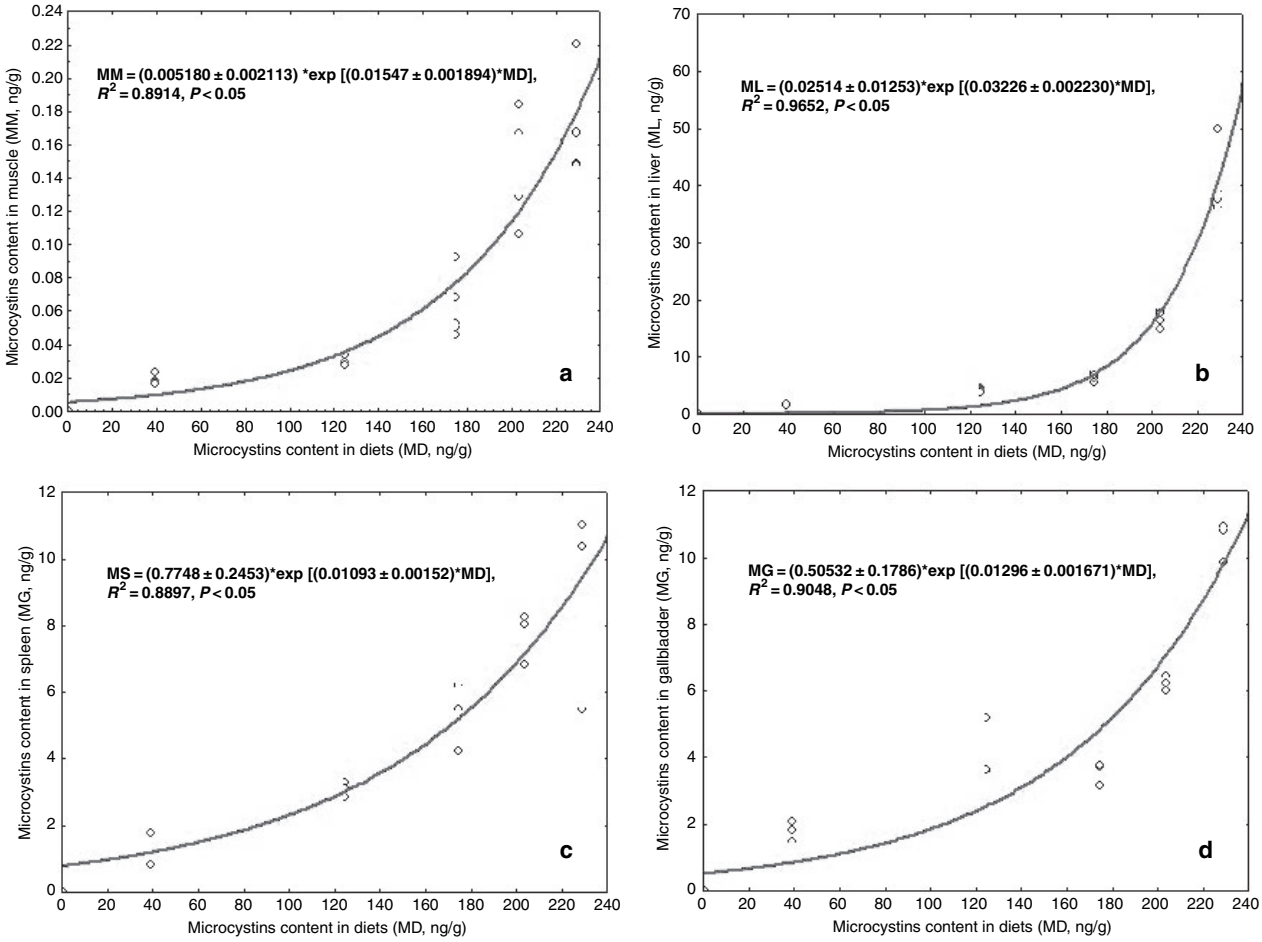

Fig. 2. The relationship between microcystin-LR equivalents content in muscle (a, MM), liver (b, ML), spleen (c, MS), gallbladder (d, MG; MM, ng g^{-1}) and microcystins content in diets (MD, ng g^{-1})

Table 6
Initial and final body composition of gibel carp fed different diets (mean ± SE)*

Diet	Dry matter (%)	Crude protein (%)	Crude lipid (%)	Ash (%)	Energy (kJ g^{-1})
Initial	17.45 ± 1.39 a	11.45 ± 1.67 a	0.86 ± 0.28 a	3.40 ± 0.28 ab	2.91 ± 0.20 a
Final					
1	28.61 ± 1.38 b	13.48 ± 0.43 b	5.45 ± 0.74 b	2.71 ± 0.20 a	6.13 ± 0.37 b
2	27.98 ± 2.47 b	13.03 ± 0.80 b	5.17 ± 1.10 b	2.76 ± 0.15 a	6.04 ± 0.65 b
3	28.67 ± 1.51 b	14.28 ± 1.17 b	6.09 ± 1.22 b	3.08 ± 0.33 a	6.10 ± 0.58 b
4	27.24 ± 1.15 b	12.96 ± 0.43 b	5.99 ± 0.74 b	3.17 ± 0.15 a	5.81 ± 0.08 b
5	26.93 ± 2.85 b	13.69 ± 1.11 b	5.06 ± 2.33 b	3.16 ± 0.49 a	5.48 ± 0.93 b
6	27.45 ± 1.27 b	12.91 ± 0.66 b	4.61 ± 1.83 b	4.03 ± 0.73 b	5.63 ± 0.39 b

*Mean values with different letter/s (a–c) are significantly different from each other ($P < 0.05$).

that the effect of algae meal on fish could not be fully due to MCs (Bury et al., 1995; Keil et al., 2002). Bury et al. (1995) reported that lysed toxic *M. aeruginosa* cells were more toxic to brown trout than was purified MC-LR. Best et al. (2002) reported that the combined effects of MCs and endotoxins of cyanobacteria such as lipopolysaccharides on glutathione *S*-transferase activities in zebra fish (*Danio rerio*) were greater than the individual effects. Oberemm et al. (1999) reported that, compared to the effects of pure toxins, the effects of aqueous crude extracts of cyanobacteria were much more

pronounced. They suggested that additional important toxins might exist in freshwater cyanobacteria. Other factors such as lipopolysaccharides in algae meal combined with MCs may have led to high mortality of gibel carp in the present study (Best et al., 2003). The lowest consistently lethal dose of MC-LR to common carp was 550 ng g^{-1} through intraperitoneal injections (Råbergh et al., 1991). In our study, gibel carp consumed MCs 1.02–10.76 ng g^{-1} wet weight daily. Even the lowest intake level (1.02 ng g^{-1}) resulted in high mortality of gibel carp with long-term intake.

Liu (1990) reported that silver and bighead carp can digest *Microcystis* but that the digestibility was very poor. Domaizon et al. (2000) found that silver carp had low digestive and conversion efficiency to *M. aeruginosa*. Kamjunke et al. (2002b) reported in cultivation experiments with roach (*Rutilus rutilus*) feces that *Microcystis* was not digested and grew exponentially after passing through the gut due to poor digestibility of *Microcystis*. Although we did not explicitly study *Microcystis* digestibility, we obtained similar results on feed digestibility and FCE.

Microcystins has been reported to impact the liver of fish (Phillips et al., 1985; Råbergh et al., 1991; Rodger et al., 1994). GPT activity in the liver of fish fed the high algae meal diet was lower than that of the fish fed the control diet in the present study. Råbergh et al. (1991) and Navratil et al. (1998) reported that GPT and GOT activities in blood plasma increased after common carp (*Cyprinus carpio* L.) received intraperitoneal injections of MC-LR.

In the present study, MCs accumulated in gibel carp when fed diets with algae meal inclusion. Magalhães et al. (2001) reported MC accumulation in other fishes. Microcystins accumulated to a much higher degree in the liver. Compared to the viscera, few MCs accumulated in the muscle. Bury et al. (1998) researched intestinal transport of MC-LR in rainbow trout and found that if MC-LR was administered orally, then most of the absorbed toxin accumulated in the liver. Our study supports this result.

The World Health Organization (WHO; Chorus and Bartram, 1999) suggests the tolerable daily intake of MCs for human as 0.04 μg kg^{-1} body weight day^{-1}. In our experiment, the highest accumulation of MCs in the muscle was 0.171 ng g^{-1}. Data are based on dry matter, thus the highest MCs in fresh fish muscle in the trial was 0.047 ng g^{-1}. If an adult person weighting 60 kg eats 300 g of fish muscle a day, the daily intake of MCs would only be 0.00024 μg kg^{-1} day^{-1}, which is an amount much lower than the accepted tolerable intake for MCs suggested by the WHO. The accumulation of MCs in the liver did not reach this level.

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