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Toxicological Effects of Heavy Oil on Carp by NMR-based Metabolic Profiling of Plasma

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Abstract—Toxicants stress for bunker A heavy oil on freshwater carp, *Cyrinus carpio*, was evaluated using ^{1}H-nuclear magnetic resonance (NMR) metabolomics technique. Dietary exposures of heavy oil to carp were conducted with 5 treatments (control, 0.01, 0.1, 1, and 5%) of bunker A heavy oil for 14 days. Several metabolites were identified such as amino acids, glucose, and lipids in carp plasma on NMR spectrum. By principal component analysis (PCA), using all peaks data derived plasma sample on NMR spectrum, 0.1, 1, and 5% exposure groups could be distinguished from the control while the 0.01% group was not distinguishable. These results suggested that exposure groups from 0.1 to 5% heavy oils received some kinds of effects in the metabolic profiling. By the results of metabolic changing in carp plasma, 0.1% exposure group was slight liver damage. 1% group was suspected that oil exposure was induced liver damage and abnormality of oxygen carry in blood. 5% group was perturbed kidney function addition with above abnormalities.

Keywords: metabolomics, carp, heavy oil, hepatic abnormalities

INTRODUCTION

Oil is frequently spilt into the water system and, in fact, large spill occurred throughout the world, whether sea or river (Lee *et al*., 2002; Koyama *et al*., 2004; Soriano *et al*., 2006; Short *et al*., 2007; Holliday *et al*., 2008). These pollutions have been influenced to aquatic organisms. Oil is complex mixtures of aliphatic and aromatic hydrocarbons, particularly includes *n*-alkanes, branched alkanes, benzene and alkylbenzenes, and polycyclic aromatic hydrocarbons (PAHs) (Wang *et al*., 1999; Wang and Fingas, 2003). PAHs contaminants are often emphasized since some PAHs have potential mutagenic and carcinogenic properties (e.g., Schneider *et al*., 2002; Jeffy *et al*., 2002), endocrine-disrupting activity (Santodonato, 1997; Villeneuve *et al*., 2002), and other toxics. PAHs from oil spills can also persist in sediments for decades of longer and have long

term toxic effects on aquatic organisms (Marty *et al.*, 2003; Peterson *et al.*, 2003; Davoodi and Claireaux, 2007; Thomas *et al.*, 2007; Viarengo *et al.*, 2007). Sometimes, the findings of those effects need the complicated procedure and long time.

Metabolomics of biofluids using ^{1}H-NMR make clear comprehensive biochemical profiles of low molecular weight metabolite reflecting the exposure to toxicants. This new technique is becoming powerful tool in the diagnoses of metabolic and evaluations of toxicity and will be applied in various fields (Robertson, 2005).

In this study, the freshwater carp, *Cyrinus carpio*, was exposed to bunker A heavy oil, frequently causing serious environmental pollutions, and investigated the biochemical profiles and its changes in carp plasma using ^{1}H-NMR Metabolomics technique.

MATERIALS AND METHODS

Exposure conditions

Test fish was obtained from general aquaculture farm, and their weights were 177 ± 30 g. In present study, the oral exposures were carried out with food, were spiked 0.01%, 0.1%, 1%, and 5% bunker A oil (weight %), and were continued for 14 days. In addition, control group, fed heavy oil free food for 14 days, and starvation group, were no feeding for 14 days, were established. In control and exposure groups, the food volumes were 1% of body weight in a day. The carp blood plasma collected from 6 fish at 0, 7, and 14 days.

^{1}H-NMR spectroscopic analysis of metabolite

The collected blood plasma for 250 μL was added 0.1 % 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium (TSP-d_4) for 100 μL, and D_2O for 250 μL. The mixture was transferred to the NMR tube. NMR analysis of metabolite in carp plasma was performed with Carr-Purcell-Meiboom-Gill and Water Suppression Enhanced Through T1 Effect (CPMG-WET) modes.

Analysis of PAHs in carp

The analysis of PAHs in carp muscle was confirmed to official analytical method for PAHs, were established by Japanese Environment Agency. The measurement was performed with GC/MS. The quantities of target PAHs were performed in SIM mode using standard solution of 18 PAHs standard mixture (Naphthalene, acenaphthylene, acenaphthene, fluorene, dibenzothiophene, phenanthrene, anthracene, fluoranthene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene, and benzo(g,h,i)perylene. The index total PAHs was calculated as the sum of the total quantity of all these compounds.

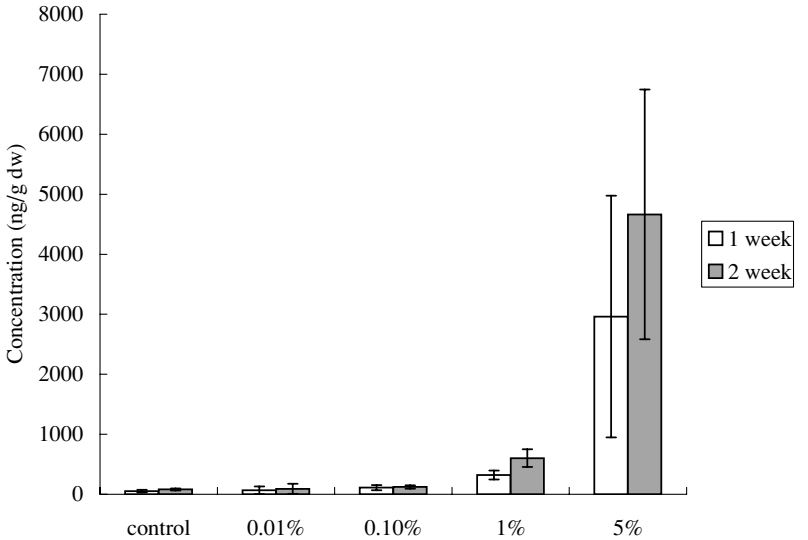


Fig. 1. Changes of ∑PAHs in *C. carpio* at control and exposure groups.

Data reduction and principal component analysis

After NMR analysis, each spectrum was segmented at every 0.04 ppm between 0.0 and 10.0 ppm. But the bins between 4.2–6.0 ppm were removed, since there was the residual water peak, which was not helpful for metabolomics analysis. The integration values from each bin were analyzed by principal component analysis (PCA) using Simca P+ (Ver. 11.5, Umetrics, Umea, Sweden), and the differences between exposure and control groups were investigated.

RESULTS AND DISCUSSIONS

Total PAHs in carp

The concentrations of PAHs, are a group of chemicals included heavy oil, were increased with increments of exposure concentrations and exposure period. Figure 1 shows the change of total PAHs (∑PAHs) in each group for 2 weeks. This results strongly suggested heavy oils were absorbed and accumulated by carp with oral exposures. The metabolic abnormalities in exposure group could be induced to heavy oil exposure, as will hereinafter be described in detail.

PCA scores plots

On the scores plot, 0.01% exposure group could not be distinguished from control group at 7 and 14 days and their individuals were not affected by heavy oil. On the other hand, 0.1%, 1% and 5% exposure group formed different group

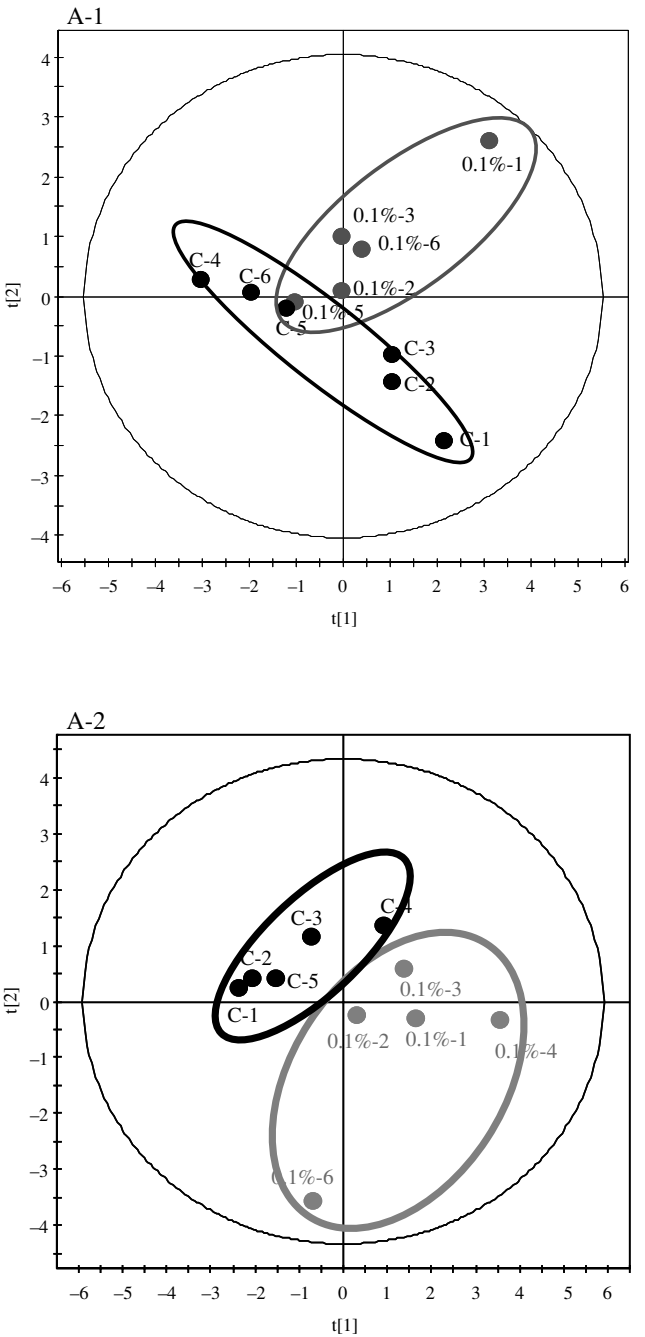
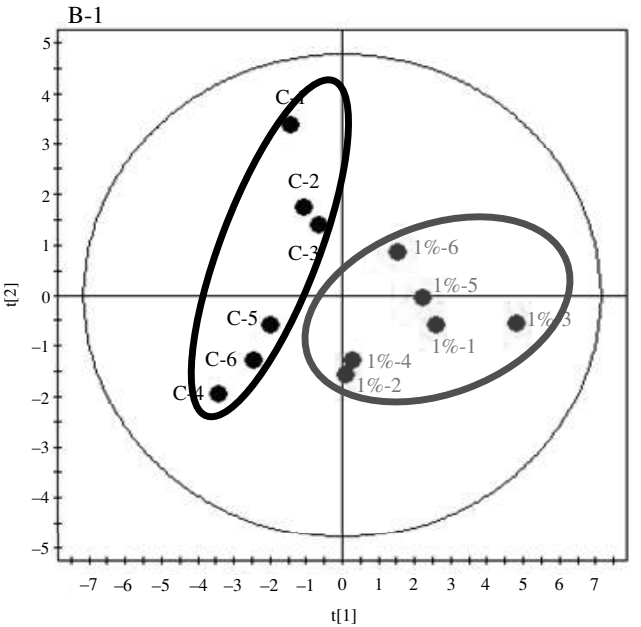


Fig. 2. PCA scores plots mapping between exposure group and control group. A-1: 0.1% for exposure and control at 7 days, A-2: 0.1% at 14 days, B-1: 1% at 7 days, B-2: 1% at 14 days, C-1: 5% at 7 days, C-2: 5% at 14 days.



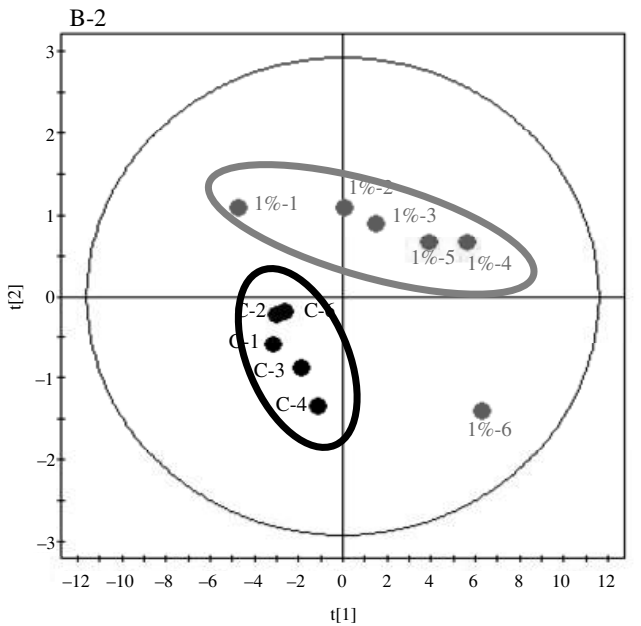
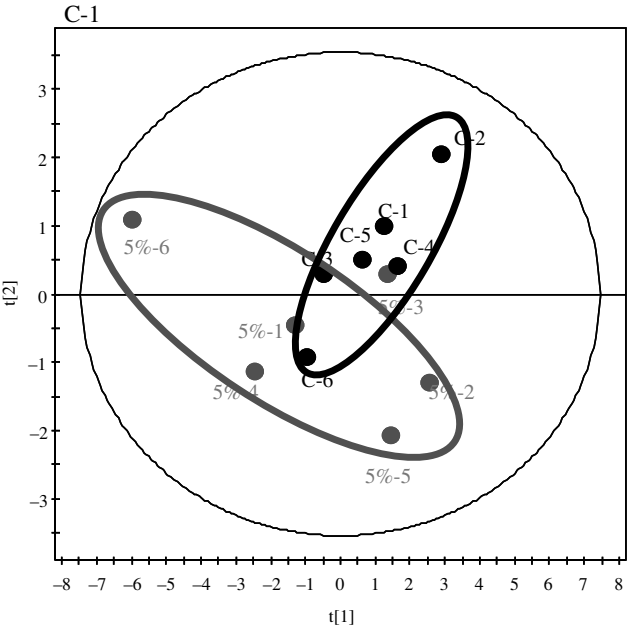


Fig. 2. (continued).



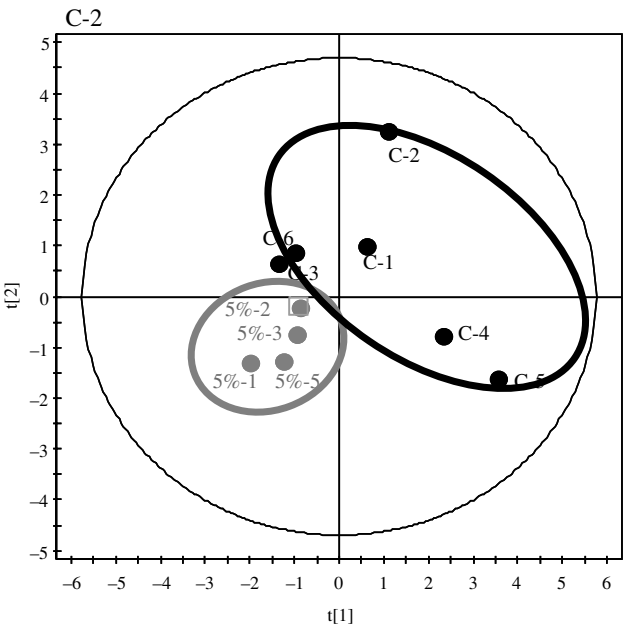


Fig. 2. (continued).

Table 1. Summary of the variations in carp plasma metabolites induced by heavy oil exposure.

Chemical shift (δ)	Assignments	Starvation	Exposure group					
			0.1%		1%		5%	
			7 days	14 days	7 days	14 days	7 days	14 days
0.75	Cholesterol	—	—	↓*1	↑*2	↓*1	↑	—
0.85, 0.92	LDL, VLDL	—	—	—	—	—	↓	↓
0.95, 0.96, 1.01, 1.02	Isoleucine	↓	↓	—	↑	—	↑	—
0.97, 0.98	Leucine	↓	↓	—	↑	—	↑	—
0.99, 1.00, 1.05, 1.06	Valine	↓	↓	—	↑	—	↑	—
1.12	Isobuthyrate	—	—	—	↑*2	↑*2	↑*2	↑*2
1.34, 1.35, 4.12, 4.13	Lactate, Threonine	↓	—	—	↑	↑	↓	—
1.48, 1.5	Alanine	↓	—	—	↑	—	—	↓
1.68–1.77	Arginine, Lysine, Leucine	↓	↓	—	—	—	—	—
1.93	Acetate	—	↓	—	↑*2	↑*2	—	↑
1.95–2.07	Glycoproteines lipid, Proline	—	—	↑	—	↑	↓	↓
2.15	Methionine	↓	—	↓	↑	↑	↑	↑
2.29	3-Hydroxybutylate	↑	↑	—	↑	—	↑	—
2.42–2.48	Glutamine	↓	—	—	↓	—	↑	↑
2.77	Dimethylamine	—	↑	↓	—	↓	↓	↓
3.07	Lysine	—	↑	↓*1	—	↓	↓	—
3.04	Creatine	↑	—	—	—	—	—	↑
3.05	Creatinine	↑	—	—	—	—	↑	↑
3.16–3.24	Choline, Phosphatidylcholine	—	—	↑	—	↑	↓	↓
3.24	Glycerophosphorylcholine	—	↓	—	—	—	↓	—
3.26	Arginine, Glucose	—	↑	—	↑	—	—	—
3.27	TMAO	—	↑	↓	↑	—	↑	↓
3.37	Proline	—	—	↑	↓	↑	↓	↓
3.39–3.53, 3.80–3.98	Glucose	↓	—	—	↑	—	↑	—
3.53–3.56	Myo-isotol	—	↓	—	—	—	↑	—
3.57, 3.90	Glycerol	↓	↓	—	↑	—	↑	↑
6.89, 6.90, 7.19, 7.20	Tyrosine	—	↓	—	↑	↑	↑	—
7.04, 7.06	Unkown	—	—	—	—	—	↑	↑
7.34, 7.35	o-Phenylalanine	↓	—	—	↑	↑	↑	↑
7.35, 7.39, 7.4	p-Phenylalanine	—	—	—	↑	—	↑	↑
7.42, 7.43	m-Phenylalanine	↓	—	—	↑	↑	—	—
7.07, 7.75	1-Methylhistidine	↓	—	—	—	—	↓	—
8.45	Formate	↓	↓*1	↓*1	↓*1	↓*1	↓*1	↓*1

*1Metabolites were detected in only control group.
*2Metabolites were detected in only exposure groups.

from control group (Fig. 2). In those groups, the metabolic systems could be changed from no affected conditions by the exposure of heavy oil.

What's happened in carp body?

The detected metabolites in NMR were examined in detail, and a lot of them changed from control groups (Table 1). The metabolites in starvation group also changed from control, but those changing patterns were clearly difference to exposure groups. In exposure groups, the decreasing appetite with heavy oil exposure were largely unaffected on the changing of metabolites, and those were done by the exposure of heavy oil.

From the metabolic changing, as indicated in Table 1, some abnormalities in carp were suggested as follows; in 0.1% exposure group, the capacity of the

Kreb's cycles in liver was exceeding, and of free fatty acid. This effect suggested increasing of 3-hydroxybuthylate. This β-oxidation also caused in 1, and 5% exposures. In 1% exposure group, the abnormality of oxygen carry caused in blood, and as the result, isobutylate was increasing. This abnormality in blood also caused in 5%. In addition, the disorders of renal glomerular filtration induced in carp at 5% exposure group, as increasing of creatinine.

CONCLUSION

Metabolomics of biofluids using ^{1}H-NMR cleared several perturbation in carp bodies, were exposed to heavy oil. 0.1% exposure group was slight liver damage. 5% group was suspected that oil exposure was induced liver damage and abnormality of oxygen carry in blood. 5% group was perturbed kidney function addition with above abnormalities.

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