Electronic Supplementary Material (ESI) for Toxicology Research.

Supporting information

Insight into biological system responses in goldfish (*Carassius auratus*) to multiple doses of avermectin exposure by integrated ^{1}H NMR-based metabolomics

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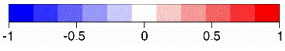
Table S1 Assignments of NMR signals for endogenous metabolites in plasma of goldfish, their fold change values (Low *vs.* CTRL, Medium *vs.* CTRL and High *vs.* CTRL) and associated P-values.

Metabolites	Assignments	Chemical shift[a] (ppm)	Low		Medium		High	
			Fold[b]	P[c]	Fold	P	Fold	P
LDL/VLDL	$CH_3(CH_2)_n$, $CH_3CH_2CH_2C$=	0.86(m), 1.20-1.30(m)	1.40	**	1.30	***	1.23	***
Isoleucine	δ-CH_3, γ-CH_3, α-CH	0.94 (t), 1.01 (d), 3.64 (d)	0.97		0.90		0.91	
Leucine	δ-CH_3, δ-CH_3, γ-CH, α-CH	0.96 (d), 0.97 (d), 1.70 (m), 3.72 (dd)	0.89		0.96		0.90	
Valine	γ-CH_3, γ-CH_3, β-CH, α-CH	0.99 (d), 1.04 (d), 2.26 (m), 3.61 (d)	0.95		0.91		0.91	
Lactate	CH3, CH	1.33 (d), 4.12 (q)	1.04		1.05		1.03	
Alanine	CH_3, CH	1.48 (d), 3.78 (q)	1.01		1.03		1.05	
Lysine	δ-CH_2	1.73 (m)	0.87	*	0.86	*	0.83	*
Acetate	CH_3	1.92 (s)	1.02		0.99		1.01	
Acetamide	CH_3	1.97 (s)	1.02		1.05		1.09	
NAGP	CH_3	2.07 (s)	0.93		0.91		0.85	
Methionine	CH_3, β-CH_2, γ-CH_2, α-CH	2.14 (s), 2.07-2.23 (m), 2.65 (t), 3.85 (m)	0.97		0.96		0.95	
Pyruvate	CH_3	2.36 (s)	0.97		0.93		0.86	
Glutamine	β-CH_2, γ-CH_2	2.14 (m), 2.45 (m), 3.78 (t)	0.99		0.97		0.98	
Succinate	CH_2	2.40 (s)	0.81		0.65	*	0.66	**
Dimethylamine	CH_3	2.73 (s)	1.08		1.02		1.16	
Sarcosine	CH_3, CH_2	2.76 (s), 3.60 (s)	0.99		1.02		1.04	
Cr/PCr	CH_3, CH_2	3.04 (s), 3.93 (s)	0.70	*	0.60	**	0.63	***
Malonate	CH_2	3.13 (s)	0.95		0.87		0.85	
Choline	N^+-CH_3	3.21 (s)	1.25	*	1.28	*	1.43	*
Phosphocholine	N^+-CH_3	3.23 (s)	1.27	*	1.18	*	1.27	*
Taurine	N-CH_2, S-CH_2	3.27 (t), 3.43 (t)	0.75	*	0.71	**	0.69	***
Methanol	CH_3	3.35 (s)	0.94		0.82		0.87	*
Glucose	CH, CH_2	3.7-3.92 (m), 5.24 (d)	1.27	*	1.33	**	1.49	***
Guanosine	O-CH-N, N-CH=N	5.92 (d), 8.00 (s)	0.99		0.97		0.95	
Inosine	N-CH-O	6.10 (d)	0.97		0.96		0.96	
Tyrosine	-CH=	6.91 (d), 7.20 (d)	0.93		0.91		0.86	*
Histidine	N-CH=C	7.13 (s), 7.98 (d)	1.02		1.03		1.04	
Phenylalanine	CH=CH	7.33(m), 7.38(m), 7.43(m)	0.98		0.93		0.89	
Formate	H-CO	8.46 (s)	0.96		0.99		0.94	

[a] Multiplicity: (s) singlet, (d) doublet, (t) triplet, (q) quartets, (m) multiplets, (dd) doublet of doublets, (AB) AB coupling system.

[b] Color coded according to the $\log_2$(Fold), red the increased and blue the decreased in AVM exposed groups. Color bar 

[c] P-values corrected by BH (Benjamini Hochberg) methods were calculated based on a

parametric Student's t-test or a nonparametric Mann-Whitney test (dependent on the conformity to normal distribution). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

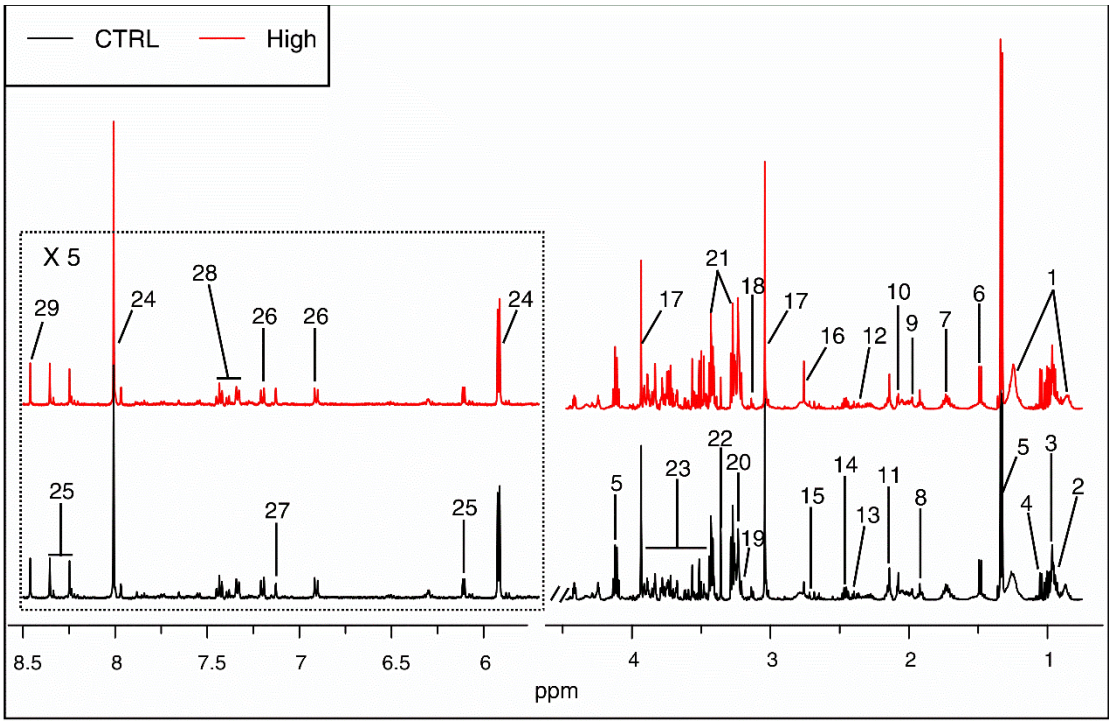


Fig. S1 Typical 500 MHz ^{1}H NMR spectra of plasma obtained from the CTRL groups (black lines) and the high dose of AVM groups (red lines). Metabolites: 1. Low density lipoprotein/very low density lipoprotein (LDL/VLDL), 2. Isoleucine, 3. Leucine, 4. Valine, 5. Lactate, 6. Alanine, 7. Lysine, 8. Acetate, 9. Acetamide, 10. NAGP, 11. Methionine, 12. Pyruvate, 13. Succinate, 14. Glutamine, 15. Dimethylamine (DMA), 16. Sarcosine, 17. Creatine/Phosphocreatine (Cr/PCr), 18. Malonate, 19. Choline, 20, Phosphorylcholine, 21. Taurine, 22. Methanol, 23. Glucose, 24. Guanosine, 25. Inosine, 26. Tyrosine, 27. Histidine, 28. Phenylalanine, 29. Formate.

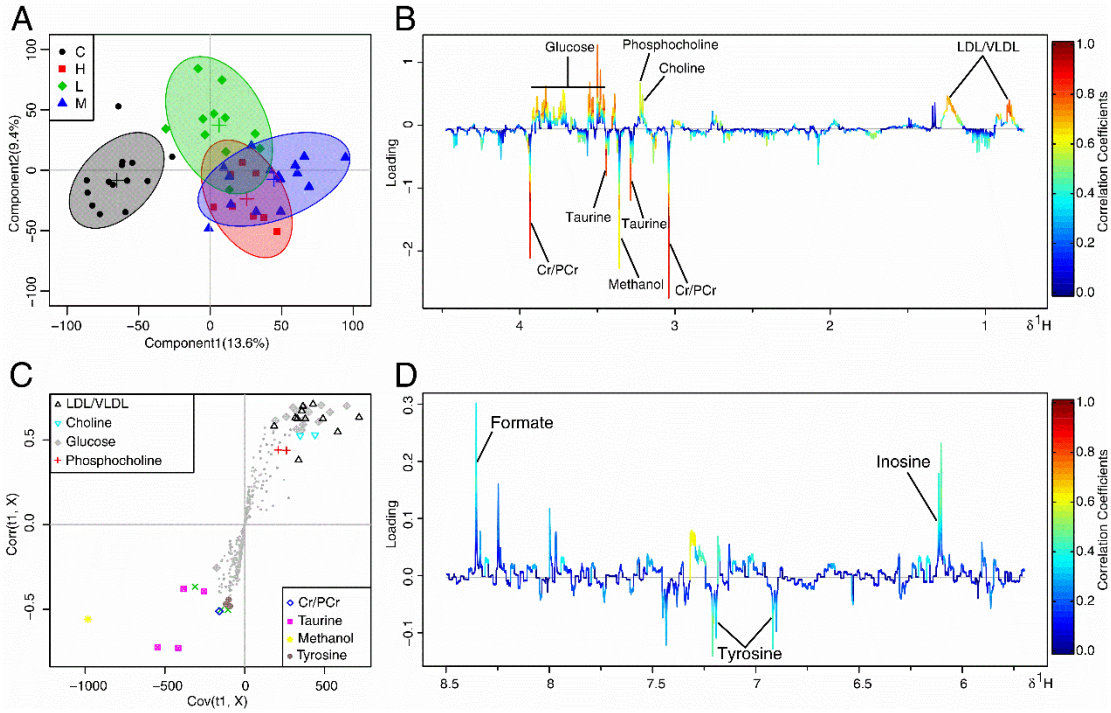


Fig. S2 OSC-PLS-DA scores plot (A), S-plot (B) and color-coded coefficient loadings plots (C, D) of metabolomic profiles between CTRL group and AVM dosed groups in plasma. Symbols of ● (black filled circle), ■ (red filled square), ▲(blue filled triangle) and ◆(green filled rhombus) represented CTRL group, high dose group, medium dose group and low dose group, respectively.