Supplementary Materials: Nitrite Enhances MC-LR-Induced Changes on Splenic Oxidation Resistance and Innate Immunity in Male Zebrafish

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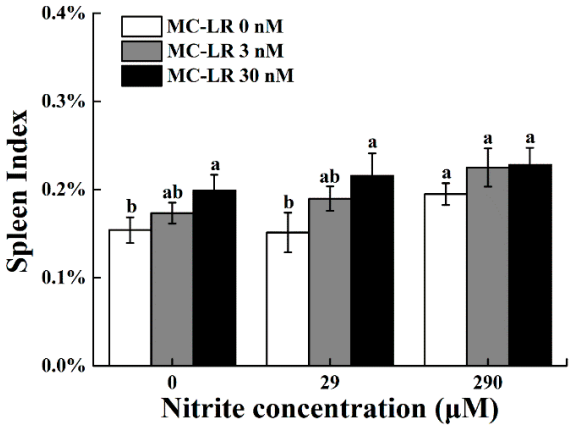

Figure S1. Alterations in the spleen index of male zebrafish exposed to different combinations of nitrite and microcystin-leucine arginine (MC-LR) for 30 d. Different letters above bars represent significant differences ($p < 0.05$).

Table S1. Measured concentrations of nitrite and MC-LR in water samples.

Nominal Concentrations		Measured Concentrations of Nitrite (μM) and MC-LR (nM) [a]
Nitrite (μM)	MC-LR (nM)	
0	0	< MDL [b], < MDL [c]
0	3	< MDL [b], 3.06 ± 0.26
0	30	< MDL [b], 30.88 ± 2.62
29	0	30.3 ± 3.04, < MDL [c]
29	3	29.4 ± 2.61, 3.12 ± 0.23
29	30	29.9 ± 2.32, 29.81 ± 2.98
290	0	303.3 ± 28.8, < MDL [c]
290	3	287.5 ± 29.4, 3.17 ± 0.22
290	30	296.1 ± 29.3, 30.89 ± 2.59

[a] Values are expressed as mean ± standard error (SEM). [b] MDL = minimum detection limit of nitrite (0.29 μM). [c] MDL = minimum detection limit of MC-LR (0.1 nM).

Table S2. Spearman correlation coefficients (r) between antioxidant parameters and innate immune parameters in male zebrafish after exposure [a].

Parameters	MDA	T-AOC	GSH	*cat1*	*sod1*	*gpx1a*
C3	-0.53**	0.62**	0.15	0.15	0.26	0.74**
il1β	-0.39**	0.55**	0.26	0.27	0.51**	0.53**
ifnγ	-0.50**	0.45**	0.17	0.25	0.42**	0.57**
tnfα	-0.51**	0.52**	-0.09	0.56**	0.41**	0.68**
c3b	-0.17	0.23	-0.05	0.57**	0.45**	0.46**
lyz	-0.09	0.19	-0.03	0.56**	0.59**	0.46**

[a] Analysis was conducted separately with 54 samples. $p < 0.01$ (**) indicate significant correlation between parameters. MDA, malondialdehyde; T-AOC, total antioxidant capacity; GSH, glutathione.

Table S3. Primer sequences used for real-time PCR.

Target Gene	Accession No.	Primer Sequences (From 5′ to 3′)	Product Length (bp)	Amplification Efficiency (%)
cat1	BC051626	F: CAAGGTCTGGTCCCATAAA	227	97.6%
		R: TGACTGGTAGTTGGAGGTAA		
sod1	BC055516	F: GTCCGCACTTCAACCCTCA	217	99.4%
		R: TCCTCATTGCCACCCTTCC		
gpx1a	BC083461	F: AGGCACAACAGTCAGGGATT	241	102.3%
		R: CAGGAACGCAAACAGAGGG		
c3b	AF047414	F: CAGTGGGAATATGTTGGCATTG	76	96.6%
		R: TTAGCTGCCCTTCATAACCTGTT		
lyz	NM_139180	F: AGGCTGGCAGTGGTGTTTTT	70	100.5%
		R: CACAGCGTCCCAGTGTCTTG		
il1β	AY340959	F: CATTTGCAGGCCGTCACA	63	98.5%
		R: GGACATGCTGAAGCGCACTT		
tnfα	AY427649	F: CCATGCAGTGATGCGCTTT	68	104.4%
		R: TTGAGCGGATTGCACTGAAA		
ifnγ	AY135716	F: GAATGGCTTGGCCGATACAGGATA	137	93.1%
		R: TCCTCCACCTTTGACTTGTCCATC		
gapdh	BC095386	F: CTGGTGACCCGTGCTGCTT	150	98.2%
		R: TTTGCCGCCTTCTGCCTTA		

Text S1. Pathological studies

Light Microscopic Observation

For the light microscopic study, spleens were first fixed in 10% neutral buffered formalin. After 24 h, samples were dehydrated in 70% ethanol for 30 min, 80% ethanol for 30 min, 95% ethanol for 30 min and 100% ethanol for 15 min. Then, samples were hyalinized in a mixture of xylene and ethanol (*v*/*v*, 1:1) for 15 min and 100% xylene for 15 min. After being immersed in paraffin wax for 60 min at 58 °C, spleen samples

were embedded, sectioned (5 μm) and stained with hematoxylin and eosin (H&E). Histopathological assessment was done on a Nikon H600L Microscope (Tokyo, Japan).

Transmission Electron Microscopic Observation

For the transmission electron microscopic study, samples were diced into 1 mm^3, prefixed in 2.5% glutaraldehyde solution, followed by three 15 min rinses with a 0.1 M phosphate buffer solution (PH 7.4). Post-fixation was in cold 1% aqueous osmium tetroxide for 1 h. After rinsing with the phosphate buffer again, the specimens were dehydrated in a graded ethanol series of 50–100% and then embedded in Epon 812 (Shell Chemical Co., NY, US). Ultra-thin sections were sliced with glass knives on an LKB-V ultramicrotome (Nova, Sweden), stained with uranyl acetate and lead citrate before examination under a HITACHI, HT-7700 electron microscope (Hitachi, Tokoyo, Japan).