Dietary supplementation of n-3 long chain LCPUFAs prevents salmonellosis in a murine model

Running title: n-3 LCPUFAs prevent *Salmonella* infection

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Supporting Information

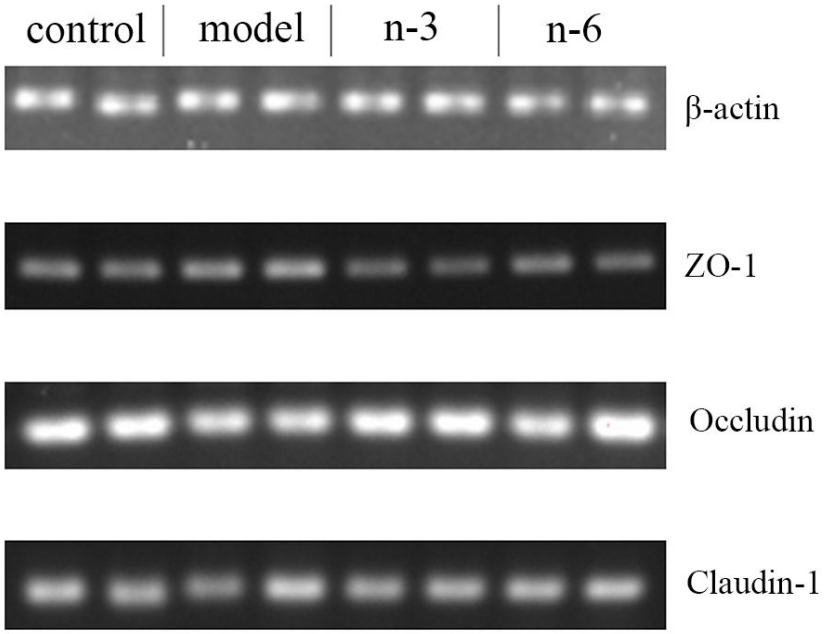

Figure 1 Effects of n-3 and n-6 LCPUFAs on the expression of tight junction proteins. Mice were pretreated with regular diet (control and model), n-3 LCPUFAs-enriched diet (n-3), and n-6 LCPUFAs-enriched diet (n-6) for 7 d. Mice from model, n-3, and n-6 groups were infected with *Salmonella*. When sacrificed at 2 d post-infection, mice colon was dissected, and the mRNA expression of tight junction proteins (β-actin, ZO-1, Occludin, and Claudin-1) were determined by PCR.

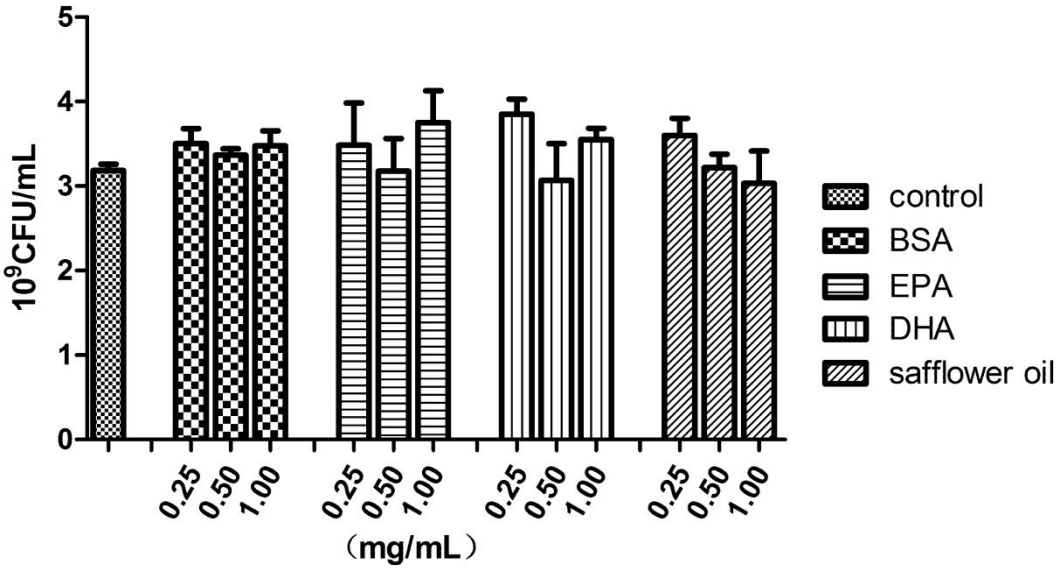

Figure 2 LCPUFAs did not exert antibacterial effects against *Salmonella* growth. EPA, DHA, and safflower oil (LA) were added into culture media broth with final concentrations of 0.25 mg/ml, 0.50 mg/ml, and 1.00 mg/ml. *S.* Typhimurium SL1344 was inoculated in these media and incubated for 12 h. Control: LB broth; BSA: BSA-modified LB broth; EPA: BSA-modified LB broth with EPA supplement; DHA: BSA-modified LB broth with DHA supplement; safflower oil: BSA-modified LB broth with safflower oil supplement.

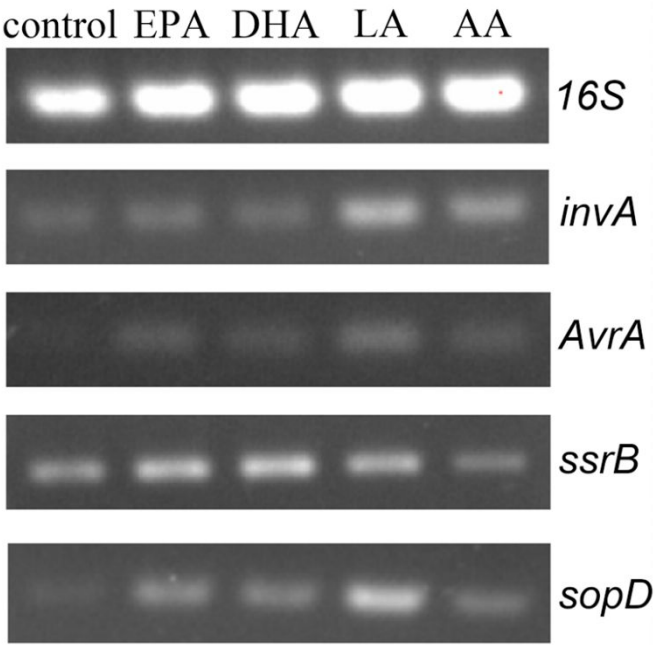

Figure 3 LCPUFAs did not influence the gene expression of virulence effectors of *Salmonella*. EPA, DHA, safflower oil (LA), and arachidonic acid (AA) were added to the culture medium of HT-29 cells, and cells were cultured in these modified media overnight. HT-29 cells were infected with *S.* Typhimurium SL1344 for 60 min. Then, the transcription of *invA*, *AvrA*, *ssrB*, and *sopD* was determined by PCR.

Table 1 Fatty acid composition of different diets

Fatty acids	control	n-3	n-6
C14:0	1.69 ± 0.09	1.08 ± 0.00	1.30 ± 0.11
C16:0	18.15 ± 0.20	10.79 ± 0.07	14.80 ± 0.06
C16:1	2.21 ± 0.16	1.37 ± 0.01	1.57 ± 0.30
C18:0	4.11 ± 0.34	2.71 ± 0.04	4.42 ± 0.19
C18:1	25.77 ± 0.08	16.42 ± 0.15	22.99 ± 0.25
C18:2（LA, n-6）	37.17 ± 0.24	22.81 ± 0.61	48.98 ± 0.86
C18:3	2.93 ± 0.10	1.77 ± 0.07	2.59 ± 0.55
C21:4	ND	0.65 ± 0.03	ND
C22:4	ND	1.88 ± 0.04	ND
C21:4	ND	0.94 ± 0.01	ND
C20:5（EPA, n-3）	3.28 ± 0.18	18.27 ± 0.06	2.53 ± 0.22
C22:6（DHA, n-3）	3.83 ± 0.30	21.33 ± 0.67	2.71 ± 0.11

ND: not detected.

Table 2 Gene primers of mouse tight junction proteins and cytokines

genes	Forward primer（3’-5’）	Reverse primer（3’-5’）
β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
ZO-1	TGAGTGAAGAACTGTCAGGCATTG	CCCCATTTACTGGCTGGTATTTT
Claudin-1	TGAGTGGCTGTGCTGCTAACC	TTGACAGATGGGAAAACGAGGTA
Occuludin	AGAGGATGGTGGGAGATTATGACA	CAATACTGGAGATAGGAAACTGATGGA
TNF-α	AGGGTCTGGGCCATAGAACT	CCACCACGCTCTTCTGTCTAC
IL-1β	CTGAACTCAACTGTGAAATGC	TGATGTGCTGCTGCGAGA
IL-6	CTCTGCAAGAGACTTCCATCCAGT	GAAGTAGGGAAGGCCGTGG
IL-10	CCCTTTGCTATGGTGTCCTT	TGGTTTCTCTTCCCAAGACC
IFN-γ	TTTGAGGTCAACAACCCACAGG	ACAACCCCGCAATCACAGTCT

Table 3 Gene primers of *Salmonella* virulence effectors

genes	Forward primer（3’-5’）	Reverse primer（3’-5’）
16S	CGATGTCTACTTGGAGGTTGTG	CTCTGGAAAGTTCTGTGGATGTC
ssrB	AACCTCATTCTTCGGGCACAG	ACGAGCCTGACATACTTATCCTTGA
AvrA	AAACACCGAAGCATTGACCTGT	CCGATGTCTTTCCGTCCATAACT
invA	GAAATGGCAGAACAGCGTCG	CAACCAGATAGGTAGGTAATGGAATGA
sopD	TCGAAGATGACCTGGCACCC	TGAGTCGTAGGCTCAAAGTTACCG