Electronic Supplementary Material (ESI) for Food & Function.

Supplementary table S1 Baseline data, number of specimens analysed, and health status in four groups

Group	Initial numbers	Final numbers	Health status (No. of specimens)	Sampled number
CD1	25	20	normal	15
CD2	25	24	normal	15
CD3	25	23	gallbladder disease (1)	15
HCD1	25	18	fatty liver (2)	15
HCD2	25	23	normal	15
HCD3	25	18	normal	15
HCB1	25	24	normal	15
HCB2	25	23	normal	15
HCB3	25	22	eye disease (1)	15
HC4B1	25	24	tail hyperaemia (1)	15
HC4B2	25	24	normal	15
HC4B3	25	25	normal	15

The four diet groups are: CD (control), HCD (high-carbohydrate), HCB (betaine supplementation for 16 weeks), HC4B (betaine supplementation for 4 weeks).

Supplementary table S2 Primers for liver qPCR

Target	Sequences	Fragment length (bp)
fmo3	F: TTCGGTAAAGCTGCCCACTC	163
	R: AGGTGGTGTGAATCGGGCTA	
cyp7a1	F: TTGATCGTGCGTTCCCTCTG	242
	R: AGCTGGCAGAGTATTCGCTT	
cyp27a1	F: AGGGTGACAGAGTCCCAACA	239
	R: GCGGTCAGGTTTGAACTTTCG	
oapt1	F: TCGGTCCAGTGTTTGGCTAC	98
	R: TCCTGGGGTGATGCTAATGC	
oapt4	F: GGGCAGCTACATGAAAAGCTC	71
	R: ATCCAATCAGCGAGCTGGGAAT	
mrp2	F: GTTCGATACCGGCCTGAGTT	92
	R: CCAGTTCGACCAACAATGCC	
bsep	F: GGCCGTGAATCTACTAAGGTCA	68
	R: GGCTCCTGAGACACAATTCCA	
fxr	F: CAAACATCGCATCCCTCAGGA	230
	R: TGGTGAAAACCTGGGCCGAA	
tgr5	F: CCATCCTTTCCATTGCTGCG	124
	R: CGCATTTCCAGTCTCCGTCT	

Supplementary table S3 Primers for microbial qPCR

Target	Primer names and sequences	Source
Labeled *16S rRNA*	27f: 6FAM-AGAGTTTGATCMTGGCTCAG 1492r: TACGGYTACCTTGTTACGACT	1
16S rRNA	F: GTGSTGCAYGGYTGTCGTCA R: ACGTCRTCCMCACCTTCCTC	2
Lactobacillus sp.	F: AGCAGTAGGGAATCTTCCA R: CACCGCTACACATGGAG	3
Clostridium sp.	F: GCGTAGGCGGATGTTTAAGTG R: CCTCACGGACTTCGGGTGT	4
cutc	F: TTYGCIGGITAYCARCCNTT R: TGNGGRTCIACYCAICCCAT	5
cnta	F: TAYCAYGCITGGRCITTYAARCT R: RCAGTGRTARCAYTCSAKRTAGTTRTCRAC	6
grdh	F: AYCARTTCTTTGGACAGRTAGG R: CCCATRAAGTTRTCWCCACATA	this study
tora	F: TTYGAYGAYTTYTGGAAYAA R: YTCRAACCACATIGGRTG	this study

Supplementary table S4 *P*-values for pairwise gene expression comparisons among the four groups

	Means ± standard deviation				*P*-value					
Parameter	CD	HCD	HCB	HC4B	CD *vs* HCD	CD *vs* HCB	CD *vs* HC4B	HCD *vs* HCB	HCD *vs* HC4B	HCB *vs* HC4B
fmo3	1.03±0.29	1.02±0.03	2.20±0.06***	2.41±0.27***	0.947	0.000	0.000	0.000	0.000	0.238
cyp7a1	1.00±0.06	1.51±0.14$^{\#\#}$	2.48±0.13***	2.73±0.10***	0.002	0.000	0.000	0.000	0.000	0.094
cyp27a1	1.00±0.04	1.21±0.00$^{\#\#\#}$	2.85±0.03***	1.51±0.06***	0.000	0.000	0.000	0.000	0.000	0.000
oatp1	1.00±0.03	8.53±1.30$^{\#\#\#}$	1.13±0.23***	2.95±0.33***	0.000	0.828	0.008	0.000	0.000	0.011
oatp4	1.00±0.04	1.37±0.01	2.23±0.65*	2.33±0.16**	0.211-	0.002	0.001	0.013	0.008	0.715
mrp2	1.00±0.00	0.55±0.04$^{\#}$	0.98±0.27*	0.70±0.02	0.015	0.996	0.097	0.020	0.558	0.134
bsep	1.00±0.10	1.03±0.06	1.27±0.05*	1.24±0.14*	0.776	0.008	0.014	0.012	0.021	0.713
fxr	1.00±0.06	2.02±0.24$^{\#\#\#}$	2.43±0.07	2.99±0.25***	0.001	0.000	0.000	0.087	0.001	0.020
tgr5	1.00±0.06	0.51±0.09$^{\#\#\#}$	1.89±0.04***	2.01±0.10***	0.000	0.000	0.000	0.000	0.000	0.109

The four groups are: CD (control), HCD (high-carbohydrate), HCB (betaine supplementation for 16 weeks), HC4B (betaine supplementation for 4 weeks). Values are presented as means of the entire group with standard deviation. $^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$, compared to the CD group. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$, compared to the HCD group. “0.000” indicates $p<0.001$.

Supplementary table S5 *P*-values for pairwise metabolite concentration comparisons among the four groups

Parameter	CD *vs* HCD	CD *vs* HCB	CD *vs* HC4B	HCD *vs* HCB	HCD *vs* HC4B	HCB *vs* HC4B
Cholesterol	0.414	0.000	0.044	0.000	0.150	0.005
Choline	0.656	0.242	0.972	0.757	0.359	0.104
Betaine	0.028	0.620	0.086	0.083	0.439	0.240
TMA	0.000	0.000	0.000	0.005	0.004	0.876
TMAO	0.067	0.000	0.000	0.000	0.016	0.006

The four groups are: CD (control), HCD (high-carbohydrate), HCB (betaine supplementation for 16 weeks), HC4B (betaine supplementation for 4 weeks). “0.000” indicates $p<0.001$.

Supplementary table S6 Microbial community indices: Shannon-Wiener (H′), Simpson (D′) and Shannon evenness (E′)

	HaeIII			*MspI*		
	H′	D′	E′	H′	D′	E′
CD	3.30±0.09	0.88±0.02	0.76±0.03	3.09±0.40	0.85±0.04	0.67±0.08
HCD	2.59±0.17$^{\#\#}$	0.84±0.07	0.80±0.01	2.41±0.08$^{\#}$	0.84±0.06	0.67±0.06
HCB	2.93±0.19	0.89±0.02	0.83±0.03	2.70±0.18	0.83±0.05	0.72±0.06
HC4B	2.98±0.19*	0.91±0.03	0.84±0.03	2.42±0.09	0.80±0.06	0.69±0.07

H′ and D′ are measures of the diversity of a biological community; E′ is the evenness (equitability of species relative abundances). Groups: CD (control), HCD (high-carbohydrate), HCB (betaine supplementation for 16 weeks), HC4B (betaine supplementation for 4 weeks). Values are presented as means of the entire group with standard deviation. $^{\#}p<0.05$ and $^{\#\#}p<0.01$, compared to the CD group. $^{*}p<0.05$, compared to the HCD group.

Supplementary table S7 *P*-values for pairwise microbial community indices comparisons among the four groups

Parameter		CD *vs* HCD	CD *vs* HCB	CD *vs* HC4B	HCD *vs* HCB	HCD *vs* HC4B	HCB *vs* HC4B
HaeIII	H′	0.002	0.026	0.044	0.058	0.036	0.719
	D′	0.287	0.764	0.378	0.185	0.072	0.551
	E′	0.203	0.025	0.011	0.289	0.141	0.586
MspI	H′	0.039	0.155	0.042	0.275	0.950	0.298
	D′	0.762	0.706	0.305	0.940	0.457	0.501
	E′	0.960	0.379	0.693	0.457	0.762	0.614

H′: Shannon-Wiener, D′: Simpson, E′: Shannon evenness. The four groups are: CD (control), HCD (high-carbohydrate), HBD (betaine supplementation for 16 weeks), HC4B (betaine supplementation for 4 weeks).

Supplementary table S8 Range (%) of frequencies to the total peak height of some T-RF identified by T-RFLP from four groups, and their potential compatible bacteria.

Bacterial taxa (genus)	*HaeIII*/*MspI*（bp）	CD	HCD	HCB	HC4B
Salicola sp.	38/75	0.61-3.98	0.43-2.46	0.13-3.05	0.00-0.38
fenthion degrading bacterium sp.	38/80	0.00-3.98	0.76-2.46	0.12-3.05	0.00-0.38
Pseudomonas/ Azotobacter/ Oceanobacillus/ Azotobacter/ Cronobacter/ Klebsiella/ Enterobacter sp.	38/82	3.98-37.81	2.46-35.16	3.05-28.06	0.38-24.46
Halomonas sp.	38/126	1.80-3.98	0.00-2.46	0.18-3.05	0.38-1.42
Methylosinus/Bradyrhizobium/Empedobacter sp.	38/152	3.98-30.70	2.46-28.17	3.05-39.07	0.38-46.34
Sphingobacterium sp.	38/188	0.25-3.98	0.00-2.46	0.21-3.05	0.00-0.38
Sphingomonas sp.	63/152	0.71-30.70	1.93-28.17	2.70-39.07	1.92-46.34
Lactobacillus sp.	66/188	0.25-2.75	0.00-0.98	0.21-0.32	0.00-0.53
Clostridium sp.	115/188	0.00-0.25	0.00-0.9	0.21-0.93	0.00-1.22
Kineococcus sp.	224/33	0.39-1.62	1.51-2.60	0.42-2.58	2.08-3.26

Streptomyces sp.	224/33, 224/126	0.39-1.80	0.00-1.51	0.18-0.42	1.42-2.08
Rhizobium sp.	224/82, 224/126	1.62-1.80	0.00-1.51	0.18-0.42	1.42-3.26
Mesorhizobium sp.	224/126	1.62-1.80	0.00-1.51	0.18-0.42	1.42-3.26
Arthrobacter/Paenibacillus sp.	224/152	1.62-30.70	1.51-28.17	0.42-39.07	3.26-46.34
Granulicatella sp.	277/68	0.00-16.06	0.29-17.45	0.31-14.24	0.10-13.57
Exiguobacterium sp.	277/82	16.06-37.81	17.45-35.16	14.24-28.06	13.57-24.46

Four groups: CD (control), HCD (high-carbohydrate), HCB (betaine supplementation for 16 weeks), HC4B (betaine supplementation for 4 weeks). The values of line of *HaeIII/MspI* represent the T-RF lengths from the *HaeIII and MspI* restriction enzymes respectively. Bacterial taxa may be assigned to one or more T-RFs on the basis of a single restriction enzyme digestion result, and similarly, one T-RF length may be assigned to one or more bacteria taxa.

Supplementary table S9 *P*-values for pairwise abundance of microbes and gene expression comparisons among the four groups

	Means ± standard deviation				*P*-value					
Parameter	CD	HCD	HCB	HC4B	CD *vs* HCD	CD *vs* HCB	CD *vs* HC4B	HCD *vs* HCB	HCD *vs* HC4B	HCB *vs* HC4B
Lactobacillus sp.	1.00±0.05	696.80±139.67$^{###}$	3.70±1.52***	9.36±1.53***	0.000	0.963	0.887	0.000	0.000	0.923
Clostridium sp.	1.08±0.52	0.01±0.00$^{###}$	0.07±0.05	0.03±0.01	0.001	0.001	0.001	0.795	0.945	0.849
cutc	1.06±0.42	0.00±0.00$^{###}$	0.03±0.01	0.03±0.00	0.000	0.000	0.000	0.873	0.869	0.996
cnta	1.01±0.15	1.11±0.39	0.00±0.00***	1.66±0.14	0.930	0.002	0.026	0.001	0.061	0.000
grdh	1.00±0.02	0.69±0.06	2.35±0.76***	0.59±0.15	0.358	0.003	0.235	0.001	0.766	0.001

The four groups are: CD (control), HCD (high-carbohydrate), HCB (betaine supplementation for 16 weeks), HC4B (betaine supplementation for 4 weeks). Values are presented as means of the entire group with standard deviation. $^{###}$ $p<0.001$, compared to the CD group. *** $p<0.001$, compared to the HCD group. “0.000” indicates $p<0.001$.

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