Electronic Supplementary Material (ESI) for Food & Function.

Title: Carbohydrate supplementation retains intestinal barrier and ameliorates bacterial translocation in an antibiotic-induced mice model

Supplementary information

Author: Yuan Zhang[1,2], Waleed A.S. Aldamarany[1,3], Liling Deng[4], Geng Zhong[1,2,*]
Author Affiliation:
1 College of Food Science, Southwest University, Chongqing, 400715, China
2 Chongqing Key Laboratory of Speciality Food Co-Built by Sichuan and Chongqing, Southwest University, Chongqing, 400715, China
3 Food Science and Technology Department, Faculty of Agriculture, Al-Azhar University (Assiut Branch), Assiut 71524, Egypt
4 Chongqing Key Laboratory of High Active Traditional Chinese Drug Delivery System, Chongqing Engineering Research Center of Pharmaceutical Sciences, Chongqing Medical and Pharmaceutical College, Chongqing 401331, China
Corresponding author: Geng Zhong
Postal address: College of Food Science, Southwest University, Beibei District, Chongqing, 400715, P.R.China.
E-mail address: zhongdg@126.com

Table S1 Properties of antibiotics

Antibiotics	Class	Predominant spectrum	Mechanism of action
Amoxicillin	β-lactam	Gram+/ Gram- bacteriocidal	Inhibits cell wall synthesis
Neomycin sulfate	Aminoglycoside	Gram- bacteriocidal	Inhibits protein synthesis
Vancomycin	Glycopeptide	Gram+ bacteriocidal	Inhibits cell wall synthesis
Methonidazole	Nitroimidazole	Anaerobes bacteriocidal	Inhibits DNA synthesis

Table S2 Body weight gain and average food intake

	Groups	Body weight gain (%)	Food intake (g/d)
Abx administration period	Control	50.575 ± 5.044 ^{a}	6.092 ± 0.178 ^{a}
	Abx	43.054 ± 4.225 ^{ab}	6.456 ± 0.216 ^{a}
	KGM	35.413 ± 8.189 ^{b}	5.155 ± 0.255 ^{a}
	DKGM	32.937 ± 5.835 ^{b}	5.090 ± 0.657 ^{a}
	KOGM	45.090 ± 8.600 ^{a}	5.610 ± 0.294 ^{a}
Recovery period	CN	8.787 ± 2.875 ^{a}	5.914 ± 0.510 ^{a}
	AN	9.850 ± 1.633 ^{a}	6.321 ± 0.362 ^{a}
	KK	5.771 ± 2.001 ^{a}	5.923 ± 0.311 ^{a}
	KN	7.076 ± 4.935 ^{a}	5.683 ± 0.293 ^{a}
	DD	4.377 ± 0.050 ^{a}	5.891 ± 0.206 ^{a}
	DN	10.967 ± 1.868 ^{a}	5.695 ± 0.385 ^{a}
	KOKO	5.355 ± 0.056 ^{a}	6.341 ± 0.263 ^{a}
	KON	8.958 ± 4.011 ^{a}	5.836 ± 0.161 ^{a}

Note: Data are mean ± SD of ten replicates. Values of different periods in the same column with different letters are significantly different ($P < 0.05$).

Table S3 Visceral index of mice in Abx administration period and recovery period

	Viscera Index	Heart	Liver	Spleen	Lung	Kidney	Small intestine	Colon
Abx administration period	Control	0.0066 ± 0.0028[a]	0.0432 ± 0.0046[a]	0.0022 ± 0.0003[a]	0.0054 ± 0.0010[a]	0.0151 ± 0.0018[a]	0.0334 ± 0.007[a]	0.0099 ± 0.0027[a]
	Abx	0.0056 ± 0.0011[a]	0.0442 ± 0.0066[a]	0.0022 ± 0.0005[a]	0.0051 ± 0.0010[a]	0.0152 ± 0.0035[a]	0.0377 ± 0.0059[a]	0.0115 ± 0.003[a]
	KGM	0.0054 ± 0.0009[a]	0.0412 ± 0.0030[a]	0.0023 ± 0.0003[a]	0.0062 ± 0.001[a]	0.0137 ± 0.0032[a]	0.0365 ± 0.0034[a]	0.0123 ± 0.0031[a]
	DKGM	0.0051 ± 0.0009[a]	0.0389 ± 0.0031[a]	0.0022 ± 0.0004[a]	0.0053 ± 0.0014[a]	0.0136 ± 0.0023[a]	0.0354 ± 0.0024[a]	0.0114 ± 0.0034[a]
	KOGM	0.0064 ± 0.0019[a]	0.0405 ± 0.0058[a]	0.0028 ± 0.0011[a]	0.0059 ± 0.0013[a]	0.0156 ± 0.0015[a]	0.035 ± 0.0074[a]	0.0125 ± 0.0029[a]
Recovery period	CN	0.0068 ± 0.0011[a]	0.0369 ± 0.0059[a]	0.0026 ± 0.0008[a]	0.0059 ± 0.0008[a]	0.0159 ± 0.0013[a]	0.0306 ± 0.0019[a]	0.0107 ± 0.0042[a]
	AN	0.0061 ± 0.0011[a]	0.0353 ± 0.0047[a]	0.0023 ± 0.0004[a]	0.0053 ± 0.0015[a]	0.0124 ± 0.0039[a]	0.0309 ± 0.0108[a]	0.0124 ± 0.0052[a]
	KK	0.0062 ± 0.001[a]	0.0374 ± 0.003[a]	0.0023 ± 0.0008[a]	0.0057 ± 0.0007[a]	0.0137 ± 0.001[a]	0.0343 ± 0.0056[a]	0.0118 ± 0.0027[a]
	KN	0.0065 ± 0.0012[a]	0.0373 ± 0.0057[a]	0.0022 ± 0.0004[a]	0.0053 ± 0.0011[a]	0.0133 ± 0.0019[a]	0.0343 ± 0.0056[a]	0.0114 ± 0.0026[a]
	DD	0.0063 ± 0.001[a]	0.0389 ± 0.0041[a]	0.0022 ± 0.0002[a]	0.0057 ± 0.0007[a]	0.0145 ± 0.0024[a]	0.0316 ± 0.0041[a]	0.0113 ± 0.003[a]
	DN	0.006 ± 0.0009[a]	0.0375 ± 0.0034[a]	0.0026 ± 0.0009[a]	0.0054 ± 0.0012[a]	0.0134 ± 0.0022[a]	0.031 ± 0.0033[a]	0.0109 ± 0.0017[a]
	KOKO	0.006 ± 0.0013[a]	0.0333 ± 0.0028[a]	0.0022 ± 0.0002[a]	0.0055 ± 0.0011[a]	0.0147 ± 0.0017[a]	0.0317 ± 0.0042[a]	0.0127 ± 0.0038[a]
	KON	0.0066 ± 0.0019[a]	0.0371 ± 0.0045[a]	0.0029 ± 0.001[a]	0.006 ± 0.0009[a]	0.0152 ± 0.0037[a]	0.0323 ± 0.0037[a]	0.0099 ± 0.0026[a]

Note: Data are mean ± SD of ten replicates. Values of different periods in the same column with different letters are significantly different ($P < 0.05$).

Table S4 Correlation between systemic inflammation cytokines and biomarkers of BT during Abx administration

BT biomarkers	Inflammation biomarkers	spearman R	P value
LBP	IL-17	0.512*	0.030
	IL-10	0.215	0.391
	CRP	0.407	0.094
	TNF-α	0.119	0.639
sCD14	IL-17	0.397	0.060
	IL-10	0.346	0.106
	CRP	0.091	0.703
	TNF-α	0.569**	0.007
sCD163	IL-17	0.503*	0.014
	IL-10	0.051	0.813
	CRP	0.598**	0.005
	TNF-α	0.375	0.094
EndoCab IgM	IL-17	-0.251	0.249
	IL-10	-0.060	0.776
	CRP	-0.467*	0.038
	TNF-α	-0.525*	0.015
EndoCab IgG	IL-17	0.246	0.257
	IL-10	0.387	0.056
	CRP	-0.430	0.058
	TNF-α	-0.030	0.898

* Represents p <0.05 with significant correlation.** indicates p <0.01 with significant correlation.

Table S5 Correlation between systemic inflammation cytokines and biomarkers of BT in recovery period

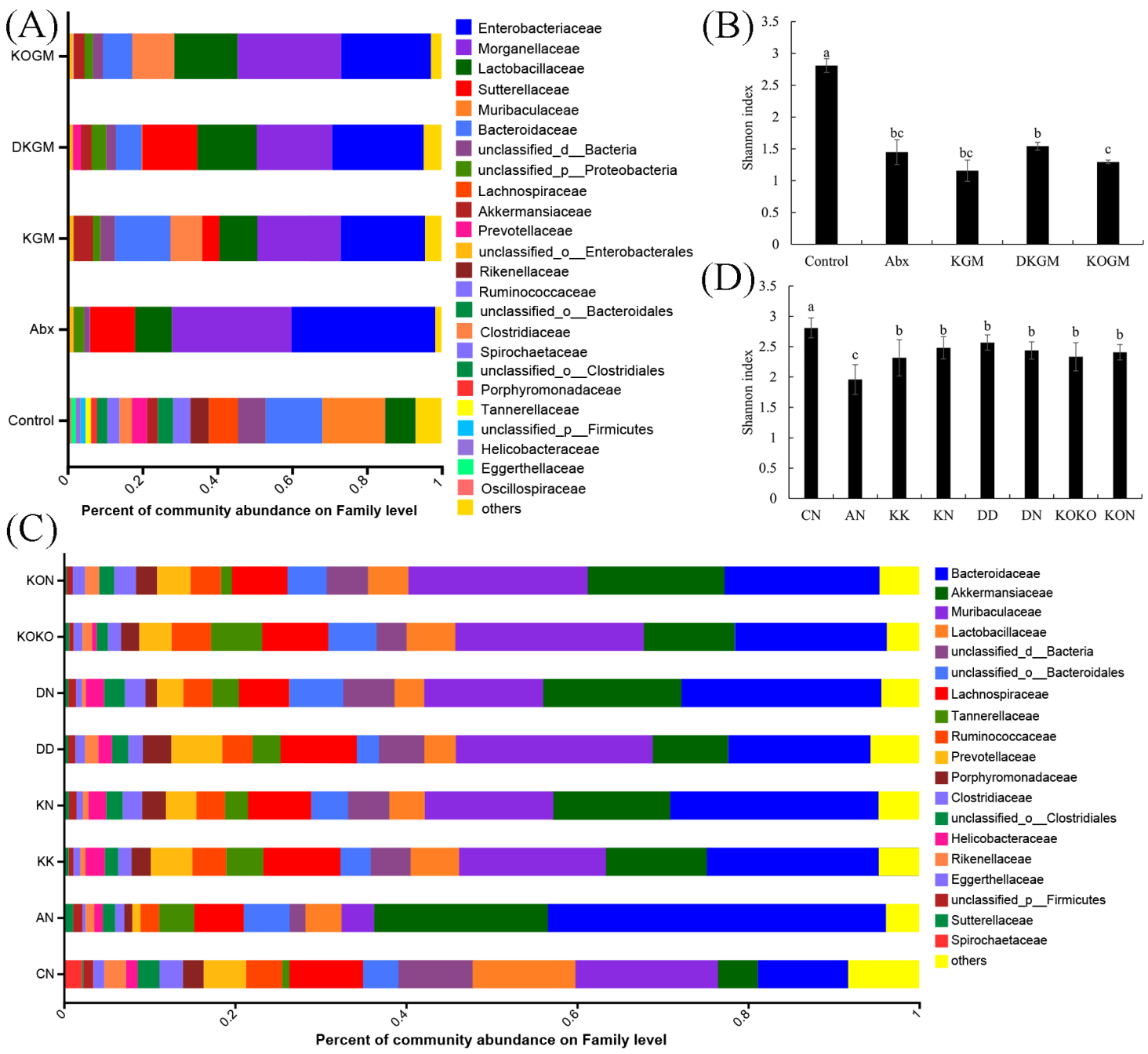
BT biomarkers	Inflammation biomarkers	spearman R	P value
LBP	IL-17	-0.013	0.947
	IL-10	0.232	0.160
	CRP	0.227	0.170
	TNF-α	0.109	0.516
sCD14	IL-17	0.024	0.899
	IL-10	-0.329*	0.044
	CRP	0.735**	0.000
	TNF-α	0.793**	0.000
sCD163	IL-17	0.403*	0.025
	IL-10	-0.264	0.114
	CRP	0.368*	0.025
	TNF-α	0.504**	0.001
EndoCab IgM	IL-17	-0.208	0.262
	IL-10	0.247	0.141
	CRP	-0.518**	0.001
	TNF-α	-0.619**	0.000
EndoCab IgG	IL-17	-0.202	0.277
	IL-10	-0.236	0.154
	CRP	0.117	0.484
	TNF-α	0.069	0.681

Note: * Represents p <0.05 with significant correlation. ** indicates p <0.01 with significant correlation.

Table S6 Records of light microscope observation of colon

	Group	Results
Abx administration period	Control	(-)
	Abx	Lesions in mucosal and lamina propria layers (++) Inflammatory cell infiltration (++)
	KGM	(-)
	DKGM	Inflammatory cell infiltration (+)
	KOGM	(-)
Post-antibiotic period	CN	(-)
	AN	Lesions in mucosal and lamina propria layers (+++) Inflammatory cell infiltration (++)
	KK	(-)
	KN	Light lesions in mucosal and lamina propria layers (+) Light infiltration of Inflammatory cell (+)
	DD	Inflammatory cell infiltration (+)
	DN	Lesions in mucosal and lamina propria layers (++) Light infiltration of Inflammatory cell (++)
	KOKO	(-)
	KON	Light lesions in mucosal and lamina propria layers (+) Light infiltration of Inflammatory cell (+)

(+) or (-) indicated that lesions could be observed or not.



Metagenomics: total genomic DNA was extracted from fecal samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's instructions. After library construction, metagenomic sequencing was performed on Illumina NovaSeq (Illumina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Reads after quality control were mapped to the non-redundant gene catalog with 95% identity using SOAPaligner (version 2.21) and gene abundance in each sample was evaluated. Representative sequences of non-redundant gene catalog were annotated based on the NCBI NR database using blastp as implemented in DIAMOND v0.9.19 with e-value cutoff of $1e^{-5}$ using Diamond (version 0.8.35) for taxonomic annotations.

As showed in Fig. S1 A and B, antibiotic treatment caused gut microbial disturbances, characterized with significantly reduced microbial diversity. The dominant microbial family dramatically changed, for example, *Enterobacteriaceae* and *Morganellaceae*, which belong to phylum *Proteobacteria* became predominant in Abx group instead of *Muribaculaceae* and *Bacteroidaceae*, compared with Control. KGM and its derivatives significantly altered the composition of gut microbiota by inhibiting antibiotic-induced flourish of *Enterobacteriaceae* and *Morganellaceae*, and retaining a certain structure of normal intestinal microorganisms, such as *Lactobacillaceae* (SCFAs producer), *Muribaculaceae* (SCFAs producer), *Bacteroidaceae* (SCFAs producer) and *Akkermansiaceae*.

Figure. S1 C and D exhibited the results of microbial composition and diversity after antibiotic administration. When the antibiotic administration was stopped, the microorganisms in each group were recovered to a certain extent after a 14-day recovery period, but the diversity was not completely recovered. Natural recovery (AN group) led to the abnormal proliferation of family *Bacteroidaceae*, while other families did not recover well. *Akkermansiaceae*, from phylum *Verrucomicrobia*, overgrew too in AN group. But the families in phylum *Firmicutes* and *unclassified_d_Bacteria* recovered poorly. The intervention of KGM and its derivatives significantly increased the microbial diversity, decreased the proportion of *Bacteroidaceae* and *Akkermansiaceae*, and promoted the recovery of families with less resilience, such as *Muribaculaceae*, *unclassified_d__Bacteria*, *Lachnospiraceae* and *Prevotellaceae*, thereby boosted the microbial composition ratio to a more normal level.

Figure S1 Analysis of microbial composition and diversity during (A,B) and after (C,D) antibiotic administration .