

Supporting information

Investigating the efficacy of kidney-protective *Lactobacillus* mixture-containing pet treats in feline chronic kidney disease and its possible mechanism

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Table S1. Model parameters of multiple reaction monitoring of the target

Target	Ion pair	Declustering Potential,	Collision Energy, CE	Internal standard	Electrospray ionization mode
	Q1 (parent)→Q3 (fragment)	DP (volts)	(volts)		
TMAO	76→58*	70	25	TMAO-d9	Positive
	76→59	70	17		
PCS	187→107*	−41	−30	PCS-d7	Negative
	187→80	−41	−30		
IS	212→80*	−55	−40	IS-d4	Negative
	212→132	−55	−25		
PS	173→93*	−40	−30	PS- ¹³ C6	Negative
	173→80	−40	−25		
TMAO-d9	85→66	70	25		Positive
PCS-d7	194→114	−41	−30		Negative
IS-d4	216→80	−55	−40		Negative
PS- ¹³ C6	179→99	−40	−30		Negative

Table S2. qPCR primers of different microorganisms

Target	Primer	Sequence (5'–3')	Annealing temperature (°C)	Reference
<i>Bifidobacterium</i>	g-Bifid-F	CTCCTGGAAACGGGTGG	55	39
	g-Bifid-R	GGTGTTCTTCCCGATATCTACA		
<i>Enterobacteriaceae</i>	En-Isu-3F	TGCCGTA ACTTCGGGAGAAGGCA	55	40
	En-Isu-3'R	TCAAGGACCAGTGTT CAGTGTC		
<i>Lactobacillus</i>	g-Lact(Rin)-1	AGCAGTAGGGAATCTTCCA	50	41
	g-Lact(Rin)-2	CACCGCTACACATGGAG		
<i>Clostridium perfringens</i>	CIPER-F	AGATGGCATCATCATTCAAC	60	42
	CIPER-R	GCAAGGGATGTCAAGTGT		

Table S3. Chemical stability analysis of probiotic pet treats

Storage duration (week)	Acid value (mg KOH/g food)			Peroxide value (POV) (mEq/kg food)			Moisture (%)		
	0	4	8	0	4	8	0	4	8
CA	1.43	1.43	1.47	1.3	1.6	1.2	5.1	5.5	6.0
CB	1.38	1.31	1.26	1.9	1.9	1.4	4.7	5.3	5.7
CC	1.92	1.96	1.98	2.5	1.2	1.4	5.3	5.5	6.1

*CA: chicken + fish; CB: fish + mutton; CC: chicken

Table S4. Harmful residue and pathogenic bacteria analyses of probiotic pet treats

Inspection item (ppm)		CA	CB	CC	Criterion
Heavy metal	Arsenic (As)	0.66	1.38	0.12	< 2 ppm
	Lead (Pb)	0.15	0.2	0.14	< 5 ppm
	Mercury (Hg)	ND	ND	ND	< 0.4 ppm
	Cadmium (Cd)	0.09	0.15	0.04	< 2 ppm
Aflatoxins	Aflatoxins (ppb)	ND	ND	ND	< 20 ppb
Pesticides	Aldrin and Dieldrin	ND	ND	ND	< 0.01 ppm
	Dichloro-diphenyl-trichloroethane (DDT)	ND	ND	ND	< 0.1 ppm
	Benzene hexachloride (BHC)	ND	ND	ND	< 0.01 ppm
	Endrin	ND	ND	ND	< 0.01 ppm
	Heptachlor	ND	ND	ND	< 0.01 ppm

					Total amount should
Preservatives and antioxidants	Ethoxyquin (g/kg food)	ND	ND	ND	< 150 ppm, and Ethoxyquin < 75 ppm
	Butylated hydroxytoluene (BHT) (g/kg food)	ND	ND	ND	
	Butylated hydroxyanisole (BHA) (g/kg food)	ND	ND	ND	
	Nitrite (NO₂-) (g/kg food)	ND	ND	ND	< 100 ppm
Toxic chemical substance	Melamine	ND	ND	ND	< 2.5 ppm
	Propylene glycol	< 0.01%	< 0.01%	< 0.01%	Should not detected
Pathogenic bacteria	<i>Salmonella</i>	—	—	—	—
	<i>Listeria monocytogenes</i> (Qualitative)	—	—	—	—

Pathogenic *Escherichia*

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Clostridium perfringens

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*CA: chicken + fish; CB: fish + mutton; CC: chicken

*ND: not detected

Table S5. Serum and urinary biochemical parameters of CKD cats during the whole trial

Target	Before Lm intervention	During Lm intervention	After Lm intervention	P value	
	week 0	week 4	week 8	week 0 v.s. 4	week 0 v.s. 8
Hematocrit (%)	41.037 (34.80-48.90)	39.808 (31.95-49.90)	38.458 (25.75-50.70)	0.23	0.236
Hemoglobin (g/dL)	13.467 (11.70-15.90)	13.183 (9.80-16.40)	12.750 (7.60-16.50)	0.401	0.341
P (mg/dL)	4.200 (3.40-5.30)	4.533 (3.20-5.90)	4.633 (3.80-6.00)	0.3025	0.025
Ca (mg/dL)	9.87 (9.20-10.90)	9.52 (8.90-10.10)	9.55 (9.10-10.30)	0.039	0.051
Ca x P	41.425 (31.28-52.47)	43.152 (29.37-56.05)	44.077 (39.13-55.20)	0.689	0.115
UPC	0.152 (0.07-0.26)	0.125 (0.04-0.29)	0.140 (0.06-0.36)	0.107	0.625
Specific gravity	1.0125 (1.005-1.022)	1.0165 (1.009-1.027)	1.0153 (1.009-1.023)	0.128	0.207

Data were presented as mean (minimum and maximum). Variables were tested by the ratio paired t test or Wilcoxon sign rank test.

Table S6. Weight changes in CKD cats

Cat NO./Week	0	4	8
PF-1	4.05	4.08	4.08
PF-2	4.65	4.62	4.64
PF-3	6.30	6.43	6.30
PF-4	3.09	3.18	3.08
PF-5	3.30	3.29	3.26
PF-6	5.09	5.09	4.89
Average	4.413	4.448	4.375

*unit: kg