Supplementary materials

Animals

Heat-Killed *Lactobacillus acidophilus* Promotes Growth by Modulating the Gut Microbiota Composition and Fecal Metabolites of Piglets

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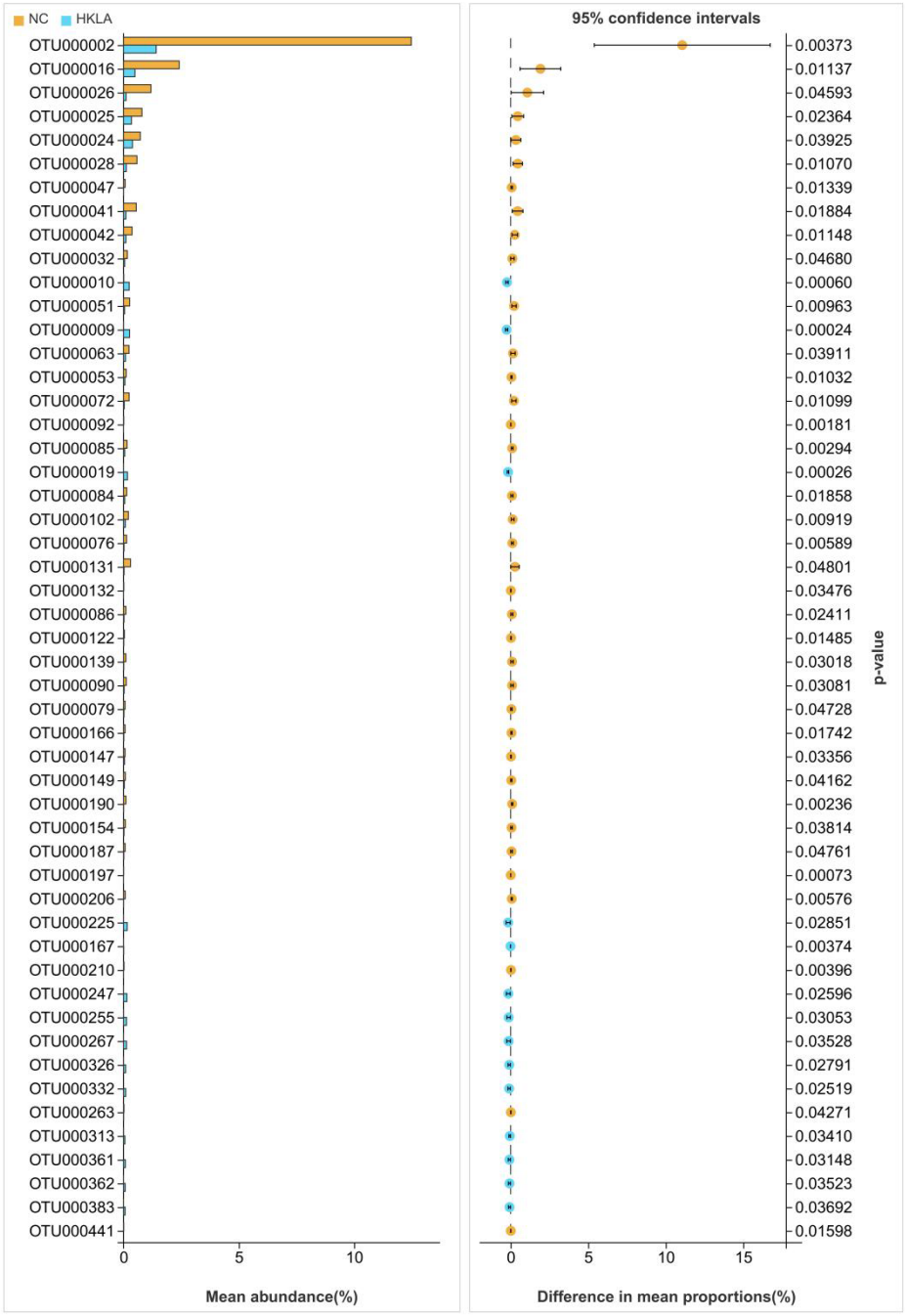
Supplementary figure

Supplementary figure S1. The OTUs differed significantly between the two comparison groups. OTUs = operational taxonomic units; NC = control group; HKLA = test group. The analysis of differences between the two groups used the T-test.



Supplementary tables

Supplementary Table S1. Ingredient composition and nutrient levels of the basal diet for weaned piglets in this experiment (%, as-fed basis).

Ingredients	Content	Nutrient level[1]	Content
Maize	60.13	Digestible energy, MJ/kg	14.57
Soybean meal (46%)	18.30	Dry matter	88.00
Extruded soybean	12.00	Crude protein	20.12
Fish meal (66%)	3.00	Crude fiber	2.35
Soybean oil	1.20	Ash	1.81
Glucose	1.20	Calcium	0.84
Dicalcium phosphate	1.00	Total phosphorus	0.61
Limestone	1.00	Standard total intestinal digestibility of phosphorus	0.41
L-lysine	0.33	Standard ileal digestible lysine	1.12
DL-methionine	0.03	Standard ileal digestible methionine	0.31
L-threonine	0.07	Standard ileal digestible threonine	0.66
Choline chloride	0.04	Standard ileal digestible tryptophan	0.20
Salt	0.60	Standard ileal digestible valine	0.74
Antioxidants 60	0.05		
Vitamin C	0.04		
Acidulant[2]	0.50		

Vitamin premix[3]	0.08
Mineral premix[4]	0.20
Compound enzyme[5]	0.03
Mildew-proof agent[6]	0.10
Feed attractant[7]	0.10
Total	100.00

[1] Nutrients levels were calculated values.

[2] Main ingredients are 2-hydroxy-4-methylthiobutanoic acid.

[3] The vitamin premix provided the following per kilogram of diet: vitamin A, 9 000 IU; vitamin D3, 3 000 IU; vitamin E, 20 IU; vitamin K3, 3.0 mg; vitamin B1, 1.5 mg; vitamin B2, 4.0 mg; vitamin B6, 3.0 mg; vitamin B12, 0.2 mg; niacin, 30 mg; pantothenic, 15 mg; folic acid, 0.75 mg; biotin, 0.1 mg.

[4] The mineral premix provided the following per kilogram of diet: Fe, 100 mg; Cu, 150 mg; Mn, 20 mg; Zn, 100 mg; I, 0.3 mg; Se, 0.3 mg.

[5] The compound enzyme provided the following per kilogram of diet: cellulase, 800 IU; α-amylase, 1 000 IU; protease, 4 000 IU; phytase, 1 000 FTU.

[6] Main ingredients were mepartricin.

[7] Main ingredients were monosodium glutamate, ethyl maltol, and flavor enhancer.

Supplementary Table S2. The unique OTUs (Tag ≥ 10) based on Venn analysis of the test.

OTU	Tota	NC-1	NC-2	NC-3	NC-4	NC-5	NC-6	HKLA-	HKLA-	HKLA-	HKLA-	HKLA-	HKLA-

	l							1	2	3	4	5	6
OTU000106	1116	537	546	10	4	7	7	1	3	0	0	1	0
OTU000019	451	1	0	1	2	0	1	48	100	51	50	93	104
OTU000244	368	0	0	0	0	0	0	220	74	34	39	0	1
OTU000191	367	14	13	19	18	156	142	1	2	0	0	0	2
OTU000224	361	147	204	2	1	2	1	0	4	0	0	0	0
OTU000247	358	0	1	0	0	1	0	101	110	66	77	1	1
OTU000267	306	0	1	0	0	1	0	74	51	80	97	2	0
OTU000332	228	0	0	0	0	0	0	67	57	51	52	1	0
OTU000333	220	0	0	0	0	0	0	2	1	3	203	7	4
OTU000361	189	0	0	0	0	0	0	60	41	52	36	0	0
OTU000362	181	1	0	0	0	0	2	42	57	53	25	1	0
OTU000374	175	0	0	0	0	0	0	0	1	2	2	169	1
OTU000383	168	0	1	0	0	0	1	32	33	46	54	0	1
OTU000417	138	1	0	1	0	2	0	38	19	26	51	0	0
OTU000324	134	0	0	0	0	0	0	74	0	10	0	5	45
OTU000381	133	74	48	3	7	1	0	0	0	0	0	0	0
OTU000455	119	0	0	0	0	0	0	2	5	1	6	34	71
OTU000411	108	2	1	1	1	0	0	48	48	2	1	0	4
OTU000496	108	0	0	0	0	0	0	86	8	2	4	0	8
OTU000488	107	1	0	0	0	0	0	28	29	27	22	0	0
OTU000312	94	0	3	4	0	57	30	0	0	0	0	0	0

OTU000256	91	27	39	15	2	3	1	1	0	0	0	0	3
OTU000547	90	0	1	0	0	0	0	31	25	22	11	0	0
OTU000492	89	2	1	37	46	1	0	0	0	0	2	0	0
OTU000560	89	0	0	1	0	0	0	19	22	24	21	0	2
OTU000562	89	0	1	32	56	0	0	0	0	0	0	0	0
OTU000436	87	47	37	2	1	0	0	0	0	0	0	0	0
OTU000512	85	0	3	2	3	43	33	0	0	0	0	0	1
OTU000585	76	0	1	0	0	1	0	10	21	21	22	0	0
OTU000619	75	1	0	0	0	0	0	19	16	15	24	0	0
OTU000568	73	0	0	0	0	0	0	35	36	0	1	0	1
OTU000627	73	0	0	0	0	0	0	9	19	21	23	1	0
OTU000644	68	0	0	0	0	0	0	1	1	0	2	64	0
OTU000649	68	0	0	0	0	0	0	17	17	18	16	0	0
OTU000513	67	0	0	1	1	0	0	24	41	0	0	0	0
OTU000646	65	0	0	0	0	0	0	23	19	19	4	0	0
OTU000629	63	0	0	0	0	1	1	28	30	0	1	0	2
OTU000664	63	0	0	0	0	0	0	10	20	19	14	0	0

Abbreviations: OTUs = operational taxonomic units; NC = control group; HKLA = test group.

Supplementary Table S3. The main differential metabolites between the two groups.

Compound_ID	MS2_name	Content changes

		NC_vs_HKLA
M217T332_POS	beta-glycerophosphate	down
M373T106_POS	beta-muricholic acid	down
M100T402_POS	2-piperidone	down
M269T177_POS	2'-hydroxy-5'-methyl-2-methoxychalcone	down
M146T48_POS	4-hydroxyquinoline	down
M139T310_2_POS	4-imidazoleacrylic acid	up
M486T163_POS	4-quinazolinamine, 7-[3-(dimethylamino)propoxy]-2-(hexahydro-4-methyl-1h-1,4-diazepin-1-yl)-6-methoxy-n-(1-methyl-4-piperidinyl)-	down
M118T402_2_POS	5-aminovaleric acid	up
M183T113_POS	7-methyluric acid	up
M136T173_1_POS	Adenine	down
M114T41_POS	Agmatine	up
M182T163_POS	Anhydroecgonine methyl ester	down
M187T62_POS	Bis-(acetyl)diaminopentane	down
M387T146_POS	Bufalin	down
M151T175_POS	D-xylose	up
M252T149_POS	Deoxyadenosine	down
M136T319_POS	Dl-octopamine	down
M253T385_POS	His-Pro	down
M415T175_POS	Leukotriene b4 3-aminopropylamide	down

M145T321_1_POS	N-acetylcadaverine	down
M101T402_1_POS	N-nitrosopyrrolidine	down
M521T33_POS	N-palmitoyl-d-sphingosine	up
M159T289_2_POS	Nonanoic acid	down
M297T29_POS	Oleic acid methyl ester	down
M247T163_POS	Oxymatrine	down
M413T106_POS	Palmitoyl 3-carbacyclic phosphatidic acid	down
M209T62_POS	Pilocarpine	down
M146T270_POS	Spermidine	up
M265T367_POS	Thiamine	up
M122T367_POS	Thiamine monophosphate	up
M128T321_POS	Tillam	down
M142T289_POS	Tropine	down
M446T36_POS	Trp-Ile-Lys	down
M182T319_2_POS	Tyrosine	down
M187T251_2_POS	Undecanoic acid	down
M171T118_NEG	(-)-hydroxycitric acid lactone	down
M245T361_2_NEG	(+)-abscisic acid	down
M147T414_1_NEG	(S)-2-Hydroxyglutarate	up
M155T140_NEG	1,3-dimethylbarbituric acid	down
M188T214_NEG	1h-indole-3-propanoic acid	down
M179T364_NEG	2-deoxy-d-ribose	up

M117T161_2_NEG	2-hydroxy-2-methylbutyric acid	down
M329T326_1_NEG	3,2'-dihydroxy-4,4',6'-trimethoxychalcone	down
M139T334_2_NEG	3,5-dihydroxybenzyl alcohol	down
M780T100_NEG	3.alpha.-hydroxy-7-oxo-5.beta.-cholanic acid	up
M162T45_NEG	5-hydroxy-3,4-dihydro-2(1h)-quinolinone	down
M186T184_NEG	7-keto-8-aminopelargonic acid	up
M435T56_NEG	Artocaprin	down
M784T172_NEG	Chenodeoxycholate	up
M389T100_3_NEG	Cholic acid	down
M103T206_2_NEG	Dl-a-hydroxybutyric acid	up
M297T165_NEG	Enterolactone	down
M337T27_NEG	Eplerenone hydroxy acid	down
M135T128_NEG	Glabridin	down
M448T240_2_NEG	Glycochenodeoxycholate	up
M129T414_NEG	L-2-hydroxyglutaric acid	down
M128T322_3_NEG	L-pyroglutamic acid	up
M375T69_NEG	Lithocholic acid	down
M303T414_2_NEG	Melphalan	up
M309T27_2_NEG	Mestranol	down
M147T149_NEG	Mevalonic acid	up
M766T146_NEG	N-(octadecanoyl)sphing-4-enine-1-phosphocholine	up
M172T214_NEG	N-acetyl-d-norleucine	down

M180T320_2_NEG	N-acetyl-l-tyrosine	down
M504T205_NEG	N-octanoylsphingosine-1-phosphate	down
M782T174_NEG	N-stearoyltaurine	down
M97T86_NEG	Phosphoric acid	up
M231T275_NEG	Thr-Leu	down
M287T259_NEG	Trans-dehydroandrosterone	up
M215T237_NEG	Val-Val	down

Abbreviations: $p < 0.05$ and $|\log_2(FC)| > 2$; NC = control group; HKLA = test group.

Supplementary Table S4. The strains used in this study.

Stains	Preservation number	Function	Source
Bacillus subtilis	CGMCC 1.12938	Activity studies	China General Microbiological Culture Collection Center
Bacillus licheniformis	CGMCC 1.7677	Activity studies	
Bacillus coagulans	CGMCC 1.10823	Activity studies	
Enterococcus faecalis	CGMCC 1.2135	Activity studies	
Lactobacillus acidophilus	CGMCC 1.3342	Activity studies	
Staphylococcus aureus	CGMCC 1.184	Indicator bacteria	
Salmonella enterica	CGMCC 1.755	Indicator bacteria	China Center of Industrial Culture
Escherichia coli	CICC 10899	Indicator bacteria	
Clostridium perfringens	CICC 22949	Indicator bacteria	

Supplementary Materials and methods

Supplementary Materials and methods M1. DNA extraction and PCR amplification. Total fecal microbial DNA was extracted using a Fecal Genomic DNA Extraction Kit (Omega Bio-Tek, Norcross, GA, USA) and was then purified and tested for concentration and purity using a NanoDrop 2 000 (Thermo Fisher Scientific, USA). Bacterial 16S rDNA (V3 and V4 regions) was amplified by PCR using total fecal DNA as a template. Bacterial universal primers were used for the upstream and downstream primers: 338F (5`-ACTCCTACGGGAGGCAGCAG-3`) and 806R (5`-GGACTACHVGGGTWTCTAAT-3`). The amplification system was as follows: 12.5 μL of 2×FastPfu mix, 1.0 μL of each primer (5 μM), 10 ng of template DNA, and ddH_2O supplemented to 25.0 μL. The PCR parameters were as follows: 94 °C for 5 min; 30 cycles of 94 °C for 30 s, 53 °C for 45 s, 72 °C for 50 s; 72 °C for 12 min; and 4 °C cool down. The PCR products were assessed by 2% agarose gel electrophoresis, and the resolved bands were cut, recovered, and sequenced by an Illumina MiSeq PE300.