

Supplemental Materials

Amino acid utilization allows intestinal dominance of *Lactobacillus amylovorus*

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Table S1. Composition and nutrient analysis of experimental diets.

Ingredients, %	Intact casein group	Hydrolyzed casein group
Intact casein	4.50	-
Hydrolyzed casein	-	4.50
Corn	45.00	45.00
Fish meal	1.00	1.00
Soybean oil	2.31	2.31
Corn starch	25.00	25.00
Rice husk powder	4.00	4.00
Choline chloride	0.10	0.10
Dicalcium phosphate	1.99	1.99
Sodium bicarbonate	4.44	4.44
L-Lys, 78.8%	1.00	1.00
DL-Met, 99%	0.18	0.18
L-Thr, 98.5%	0.45	0.45
L-Trp, 98%	0.12	0.12
L-Leu, 98.5%	0.46	0.46
L-Ile, 98.5%	0.54	0.54
L-Phe, 98.5%	0.35	0.35
L-Val, 98.5%	0.37	0.37
L-Asp, 99%	0.56	0.56
L-Glu, 99%	0.73	0.73
L-Ser, 98.5%	0.20	0.20
L-Arg, 98.5%	0.20	0.20
L-His, 80.1%	0.20	0.20
L-Ala, 99%	0.28	0.28
L-Gly, 99%	0.26	0.26
L-Cys, 98.5%	0.25	0.25
Vitamins and minerals Premix ^a	1.00	1.00
Glucose	2.00	2.00
Sucrose	2.00	2.00
Limestone	0.10	0.10
Chromium oxide	0.35	0.35
Zinc oxide	0.05	0.05
Total	100.00	100.00
Nutrition level, %		
NE, MJ/kg	10.08	10.08
Crude protein	13.30	13.30
Crude fiber	2.60	2.60

a: The premix provided the following per kg of diet: vitamin A, 8,000 IU; vitamin D₃, 2,4000 IU, vitamin E, 20 mg, pantothenic acid, 15 mg; vitamin B₆,

5 mg; biotin, 0.3 mg; folic acid, 3 mg; vitamin B₁₂, 0.03 mg; ascorbic acid, 40 mg; Fe, 120 mg; Cu, 25 mg; Mn, 20 mg; Zn, 120 mg; I, 0.5 mg; Se, 0.3 mg.

Table S2. The Primer sequences of AA and peptide transporter genes and AA metabolic enzymes

Items	Primer sequence (F:5'→3')	Primer sequence (R:5'→3')
Bacteria		
Total <i>Lactobacillus</i>	AGCAGTAGGGAATCTTCCA	ATTCCACCGCTACACATG
<i>Limosilactobacillus reuteri</i>	GATTGACGATGGATCACCA GT	CATCCCAGAGTGATAGCC AA
<i>Lactobacillus amylovorus</i>	CAAGCACGATTGGCAAGAT G	ATTGGATTCCGCTTCTGT GG
<i>Ligilactobacillus salivarius</i>	TACACCGAATGCTTGCATTC A	AGGATCATGCGATCCTTA GAGA
<i>Lactobacillus johnsonii</i>	AGAGAGAAACTCAACTGA AATA	CCTTCATTAACCTTAACAG TTAA
<i>Lacticaseibacillus paracasei</i>	CAATGCCGTGGTTGTTGGA A	GCCAATCACCGCATTAAATC G
Bacterial functional genes		
<i>potE6</i> - Amino acid transporter gene	AAAGCACCATTCTCCTCA	GAAATGATTGGCAGGTAT
<i>optB</i> - Oligopeptide ABC transporter permease protein	CGGTACATCCTTCCAATA	GATAACGCCAAGCACAAAC
<i>artJ</i> - ABC transporter arginine-binding protein	GGCAGACAATGACCAAGA	CTAAGCAAGTCGCCCAA
<i>bcaP</i> - Branched-chain amino acid permease	ATACATCGGCTTTGACTC	GTGAACATCCCAAGAACG
<i>gluA</i> - Glutamate transport ATP-binding protein	TACGCTGCTCCGCACTTT	AGCCTGCTTTCTAACCTC ATCT
<i>metN</i> - Methionine import ATP-binding protein	AGAACGATTAACCTCCTG	AATAACTGTTTGCCTC
<i>lysXY</i> - Amino acid transport ATP-binding protein	ATCAAGGCTAAACCACAA	ATACACGGTCTGAAATGC
<i>metK</i> - S-adenosylmethionine synthetase	ACTGTGCCGTAATGGTTG	CTTCTGCATCGGTTTGAG
<i>dapA</i> -4-hydroxy-tetrahydrodipicolinate synthase	GTGGGAGGAACAACCTGGA	TGATTGGCATTGCGTCT
<i>dapB</i> -4-hydroxy-tetrahydrodipicolinate reductase	CTAAAGAAGCGGCTAAAT	CCTTGATAATCTCCACCAC
<i>sda</i> - L-serine dehydratase	CCCTTTCTGGCGACACGA	TGCGTTCCCAGCACCAAT
<i>bcat</i> - branched-chain amino acid aminotransferase	CGCAAGACTCTACTATCACT	ATCATAAATGGACGAAGG
<i>ackA</i> - acetate kinase	AAAGGTAGTGGCATTCCGG	GTGCTTCTGCTGGGTTAT
<i>ldh</i> - lactate dehydrogenase	AGCCAGGTGAAACTCGTC	ATCTTCCAAGTTGCGTGA
<i>RecA</i> (reference gene)	TTTCAACAGTGCCAACCG	CAATGTCCACGCCAGAG

Table S3. The concentration of amino acids in the medium before batch culture (mmol/L)

AA	Total amino acids		Free amino acids		Peptide-bound amino acids	
	Casein-AA	Casein-PEP	Casein-AA	Casein-PEP	Casein-AA	Casein-PEP
Ala	7.93	6.49	4.24	4.81	3.69	1.68
Arg	8.35	12.99	3.71	2.59	4.63	10.41
Asp	13.69	10.87	6.09	1.62	7.61	9.25
Glu	19.46	16.84	17.69	2.43	1.77	14.41
Gln	9.25	8.60	8.40	4.51	1.15	4.08
Gly	7.67	6.43	3.56	1.76	4.11	4.68
His	9.55	10.42	8.37	2.17	1.18	8.16
Ile	10.52	9.10	3.29	2.48	7.23	6.62
Leu	11.27	13.23	5.75	5.50	5.52	7.73
Lys	11.01	11.08	5.15	2.26	5.85	8.83
Met	3.28	3.61	1.72	1.29	1.56	2.31
Phe	4.25	4.74	2.56	1.97	1.70	2.77
Pro	8.44	10.33	7.63	3.57	0.80	6.76
Ser	5.74	3.20	3.60	1.48	2.14	1.73
Thr	7.45	9.74	1.25	0.31	6.21	9.43
Tyr	7.50	8.10	5.70	1.73	1.80	6.36
Val	8.63	7.57	4.35	2.15	4.29	5.42
Total	153.99	153.34	93.06	42.62	60.93	110.72

The substrate in Casein-AA group was provided with casein acid hydrolysate, which was rich in free amino acid source. The substrate in Casien-PEP group was provided with casein enzymatic hydrolysate, which was rich in peptide source.

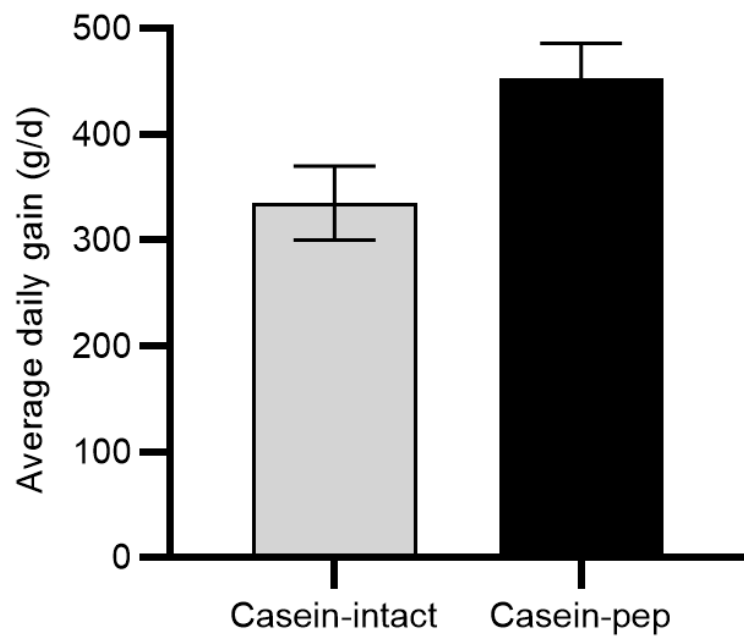


Figure S1 The average daily growth of pigs (n = 8).

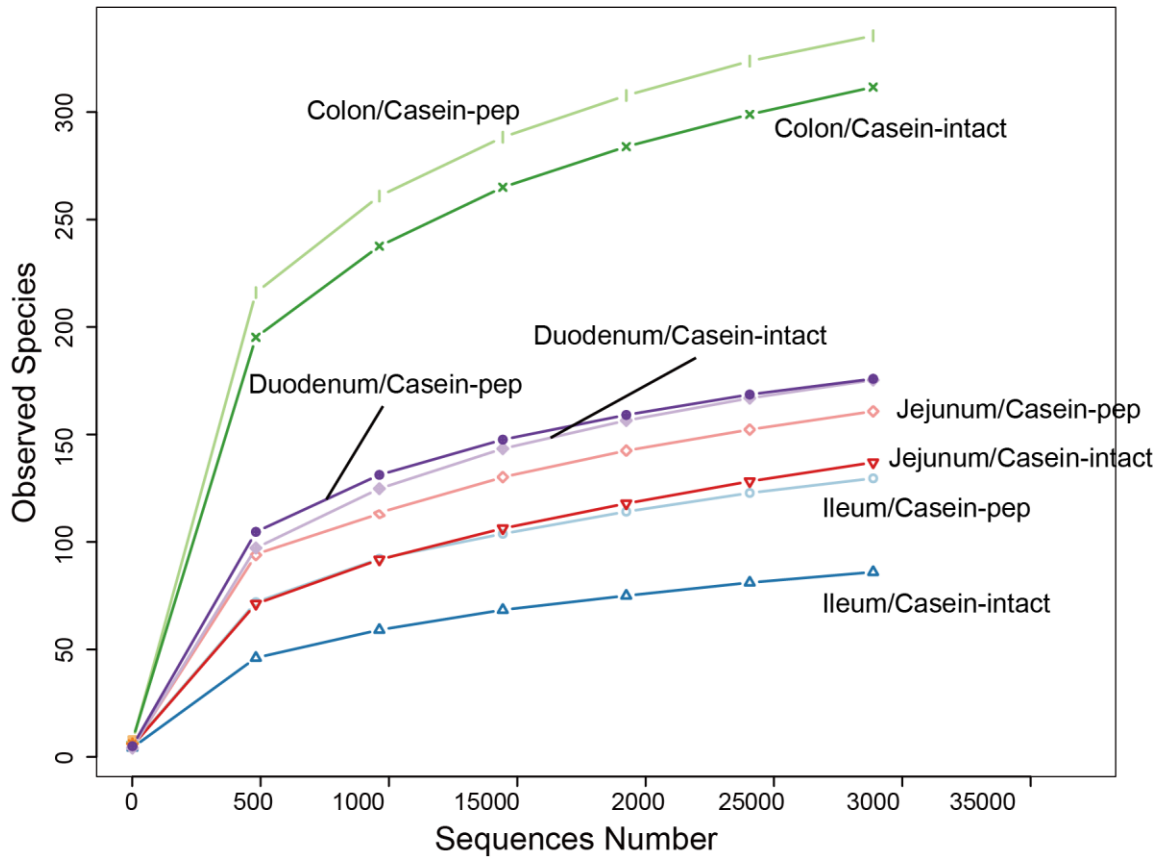


Fig. S2 Rarefaction curves for OTUs of average reads within the same group (n = 8) were calculated for reads exhibiting $\geq 97\%$ sequence identity. IC, intact casein; HC, hydrolyzed casein.

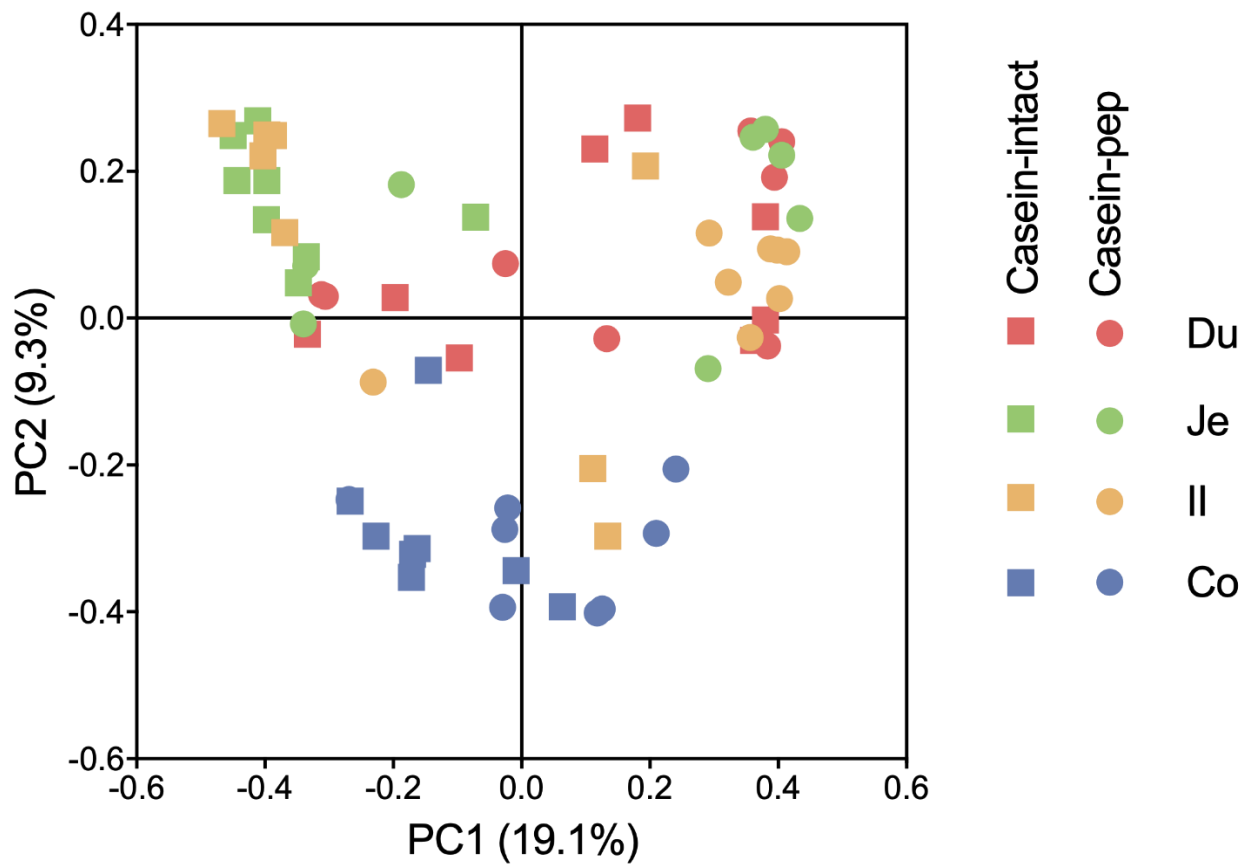


Fig. S3 Principle coordinate analysis (PCoA, based on the weighted Unifrac distance) of all intestinal samples in intact casein (Casein-intact) and hydrolyzed casein (Casein-pep) group (n = 8). Co, colon; Du, duodenum; Il, ileum; Je, jejunum.

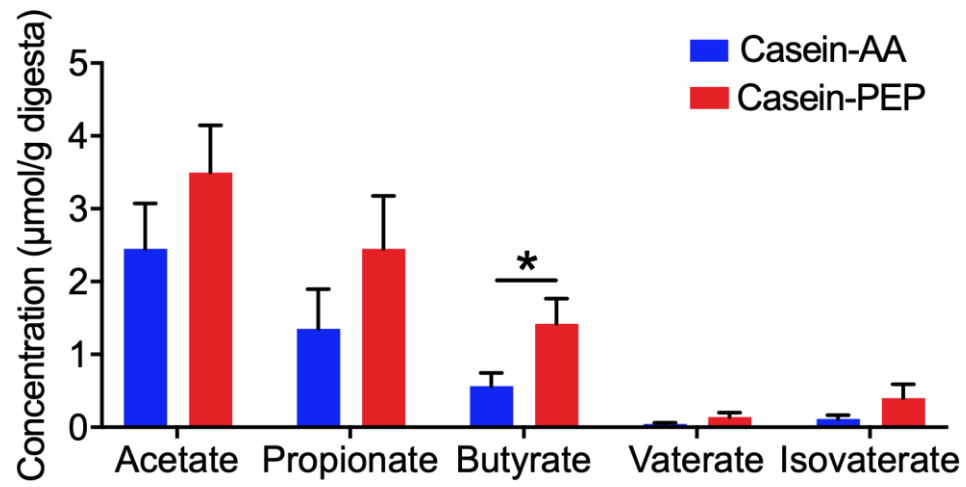


Fig. S4 Concentration of short-chain fatty acids in the jejunum. * $p < 0.05$.