

Supplementary material

Effects of spermine on ileal physical barrier, antioxidant capacity, metabolic profile and large intestinal bacteria in piglets

Guangmang Liu, ^{*a,b,c} Weiwei Mo, ^{a,b,c} Wei Cao, ^{a,b,c} Xianjian Wu, ^{a,b,c} Gang Jia, ^{a,b,c} Hua Zhao, ^{a,b,c} Xiaoling Chen, ^{a,b,c} Caimei Wu, ^{a,b,c} and Jing Wang ^d

^a Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu, Sichuan, China

^b Key Laboratory for Animal Disease-Resistance Nutrition, Ministry of Education, Chengdu, Sichuan, China

^c Key laboratory of Animal Disease-resistant Nutrition and Feed, Ministry of Agriculture and Rural Affairs, Chengdu, Sichuan, China

^d Maize Research Institute, Sichuan Agricultural University, Chengdu, Sichuan, China

Table S1 Composition and nutrient level of basal formula milk powder (87.5%

DM basis)

Ingredients	%
Whole-milk powder (24% CP)	58.00
Whey protein concentrate (34% CP)	25.00
Casein	5.70
Coconut oil	10.00
CaH ₂ PO ₄	0.10
Choline chloride (50%)	0.10
Vitamin premix ^a	0.10
Mineral premix	0.50
L-Arg (98.5%)	0.06
DL-Met (98.5%)	0.06
L-Lys.HCl (78.5%)	0.30
L-Thr (98%)	0.03
L-Trp (98%)	0.05
Total	100
Nutrient content	
Digestible energy (kJ kg ⁻¹)	18390
Crude protein (%)	25.30
Ca (%)	1.02

Total P (%)	0.81
Available P (%)	0.67
Digestible Lys (%)	1.93
Digestible Met (%)	0.63
Digestible Arg (%)	0.86

^a Vitamin premix provided per kg powder diet: vitamin A, 0.94 mg; vitamin D3, 0.01 mg; vitamin E, 20 mg; vitamin K3, 1 mg; vitamin B12, 0.04 mg; riboflavin, 5 mg; niacin, 20 mg; pantothenic acid, 15 mg; folic acid, 1.5 mg; thiamin, 1.5 mg; pyridoxine, 2 mg; biotin, 0.1 mg. Mineral premix provided per kg powder diet: Zn, 90 mg; Mn, 4.0 mg; Fe, 90 mg; Cu, 6.0 mg; I, 0.2 mg; Se, 0.3 mg.

Table S2 Primers sequences of target and reference genes selected for analysis by real-time PCR

Genes	Primer	Sequence (5'-3')	Size, bp	Temperature (°C)	Accession No.
β-actin	Forward	TGCGGGACATCAAGGAGAA	58	58	DQ452569.1
	Reverse	GCCATCTCCTGCTCGAAGTC			
SOD1	Forward	GATCAAGAGAGGCACGTTGGA	62	58	AF396674.1
	Reverse	GTGGCCACACCATCTTTGC			
GPx1	Forward	GGCGGCGGGTTCTGA	55	58	NM_214201.1
	Reverse	CGCCATTCACCTCACACTTCT			
CAT	Forward	GGACGTGCAGCGCTTCA	52	58	NM_214
	Reverse	CCGCACCTGGGTGACATTA			
GR	Forward	CAGTAGAGGTCAACGGGAAGAAGT	59	58	AY368271.1
	Reverse	GCCGCCTGTGGCAATC			
GST	Forward	TCCCCACGGTGAAGAAGTTT	57	58	Z69586.1
	Reverse	CGTCAGTGGGAGGCTTCCT			
Nrf2	Forward	GCCCCTGGAAGCGTTAAAC	67	58	XM_003133500.5
	Reverse	GGACTGTATCCCCAGAAGGTTGT			
Keap1	Forward	ACGACGTGGAGACAGAAACGT	56	58	NM_001114671.1
	Reverse	GCTTCGCCGATGCTTCA			
ZO-1	Forward	CCCAACCTCACAAATAGAAAGTGA	70	58	XM_013993251.1
	Reverse	GCGAATAATGCCAGAGCTACGT			
ZO-2	Forward	CGGGTGGTCATGGTTAACG	59	58	NM_001206404.1
	Reverse	TGAACGGCAAAGGAATGGA			
Occludin	Forward	CCTCAGGCAGCCTCATTACAG	61	58	NM_001163647.2
	Reverse	GGGAGCCCGTTTTGAAGAC			

Claudin-1	Forward	GCTCCTGCCCCCGAAA	63	58	NM_001244539.1
	Reverse	AAGGCGAAGGTTTTGGATAGG			
Claudin-2	Forward	TCCTCCCTGTTCTCCCTGATAG	59	58	NM_001161638.1
	Reverse	CCTTGCAAGTGGGCAGGAA			
Claudin -3	Forward	TGGGAGGGCCTGTGGAT	64	58	NM_001160075.1
	Reverse	CGTACACTTTGCACTGCATCTG			
Claudin-12	Forward	GGGCTGTCTGGGATGTTCA	57	58	NM_001160079.1
	Reverse	GAGGCGATTCCACACAGGAA			
Claudin-14	Forward	GAAATAAATGCACCCGGATAATCT	92	58	NM_001161642.1
	Reverse	CCGCGGGAGTCCTAATGA			
Claudin 15	Forward	TGCACGGGAACGTCATCA	63	58	NM_001161643.1
	Reverse	CGGTGGCGCAGCTGTAC			
Claudin 16	Forward	TCCTGTTGGCTTGAATGG	57	58	FJ873105.1
	Reverse	GAGGACAGCACCCAGCCAAA			
MLCK	Forward	AAGGCCAACATCGTCATGGT	66	58	XM_021070241.1
	Reverse	GTCAATGATGCGCTCGAACA			
Total bacteria	Forward:	ACTCCTACGGGAGGCAGCAG	200	60	
	Reverse:	ATTACCGCGGCTGCTGG			
<i>Lactobacillus</i>	Forward:	GAGGCAGCAGTAGGGAATCTTC	126	60	
	Reverse:	CAACAGTTACTCTGACACCCGTTCTTC			
	Probe	AAGAAGGGTTTTCGGCTCGTAAACTCTGTT			
<i>Bifidobacterium</i>	Forward:	CGCGTCCGGTGTGAAAG	121	60	
	Reverse:	CTTCCCGATATCTACACATTCCA			
	Probe	ATTCCACCGTTACACCGGGAA			
<i>Escherichia coli</i>	Forward:	CATGCCGCGTGTATGAAGAA	96	60	
	Reverse:	CGGGTAACGTCAATGAGCAAA			

Probe AGGTATTAACCTTTACTCCCTTCCTC

SOD1 = superoxide dismutase 1; CAT = catalase; GP_x1 = glutathione peroxidase 1; GR = glutathione reductase; GST = glutathione S-transferase; Nrf2 = nuclear erythroid 2-related factor 2; Keap1 = Kelch-like ECH-associated protein; ZO-1 = zonula occludens 1; ZO-2 = zonula occludens 2; MLCK = myosin light chain kinase