Supplementary material

Effects of spermine on liver barrier function, amino acid transporters, immune status, and apoptosis in piglets

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Table S1 Average proportion of ingredients and nutrients levels of the basal formula milk powder (on 87.5% dry matter basis)

	Contents
Ingredients (%)	
Whole-milk powder (24% crude protein)	58.00
Whey protein concentrate (34% crude protein)	25.00
Casein	5.70

Coconut oil	10.00
CaH_2PO_4	0.10
Choline chloride (50% purity)	0.10
Vitamin premix*	0.10
Mineral premix#	0.50
L-arginine (98.5% purity)	0.06
DL-methionine (98.5% purity)	0.06
L-lysine · HCl (78.5% purity)	0.30
L-threonine (98% purity)	0.03
L-tryptophan (98% purity)	0.05
Total	100.00
Nutrients	
Digestible energy (MJ/kg)	18.4
Crude protein (%)	25.3
Calcium (%)	1.02
Total phosphorus (%)	0.81
Available phosphorus (%)	0.67
Digestible lysine (%)	1.93
Digestible methionine (%)	0.63
Digestible arginine (%)	0.86

*Vitamin premix provided per kg formula powder: 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 formula powder: Zn, 90 mg; Mn, 4.0 mg; Fe, 90 mg; Cu, 6.0 mg; I, 0.2 mg; Se, 0.3 mg.

Table S2 Primer sequences of the target and reference genes were used in Real-time PCR.

Genes	Primer sequence (5'-3')	Product (bp)	Annealing temperature (°C)	Accession no.
β-Actin	Forward: TGCGGGACATCAAGGAGAA Reverse: GCCATCTCCTGCTCGAAGTC	58	58	DQ452569.1
ZO1	Forward: CCCAACCTCACAAATAGAAAGTGA Reverse: GCGAATAATGCCAGAGCTACG	70	58	XM_013993251.1
ZO2	Forward: CGGGTGGTCATGGTTAACG Reverse: TGAACGGCAAAGGAATGGA	59	58	NM_001206404.1
Occludin	Forward: CCTCAGGCAGCCTCATTACAG Reverse: GGGAGCCCGTTTTGAAGAC	61	58	NM_001163647.2
Claudin-1	Forward: GCTCCTGCCCCCGAAA Reverse: CCTTGCAGTGGGCAGGAA	63	58	NM_001244539.1
Claudin-2	Forward: TCCTCCCTGTTCTCCCTGATAG Reverse: CCTTGCAGTGGGCAGGAA	59	58	NM_001161638.1

Claudin-3	Forward: TGGGAGGGCCTGTGGAT Reverse: CGTACACTTTGCACTGCATCTG	64	58	NM_001160075.1
Claudin-12	Forward: GGGCTGTCGGGATGTTCA Reverse: GAGGCGATTCCACACAGGAA	57	58	NM_001160079.1
Claudin-14	Forward: GAAATAAATGCACCCGGATAATCT Reverse: CCGCGGGAGTCCTAATGA	92	58	NM_001161642.1
Claudin-15	Forward: TGCACGGGAACGTCATCA Reverse: CGGTGGCGCAGCTGTAC	63	58	NM_001161643.1
Claudin-16	Forward: TCCTGTTGGCTTGGAATGG Reverse: GAGGACAGCACCAGCCAAA	57	58	FJ873105.1
MLCK	Forward: AAGGCCAACATCGTCATGGT Reverse: GTCAATGATGCGCTCGAACA	66	58	XM_021070241.1
SLC1A1	Forward: TTCTTTGTTCCCCACATCTTTCTT Reverse: CCCTTCCCACACTCGACTGA	68	58	NM_00116464
SLC1A5	Forward: CCCGGACCTAGCCTCTTGA Reverse: GGTCCTTGGCTCCGAAAAG	57	58	XM_003127238.4

SLC7A1	Forward: CATCTTTGCCGTGATCATAATTCT Reverse: TTTGTTGACCATGGCTGACTCT	79	58	NM_001012613.1
SLC7A7	Forward: TTTGGTTCCCAAGGTTGCA Reverse: GCAGCTTCCTGGCATTGC	58	58	NM_00111042
SLC7A9	Forward: TTGCCATCATCTGTCTCAGCTT Reverse: GCTGCAGCCTGCGTAGAAG	62	58	EF127857.1
SLC5A1	Forward: TGGCCTGAGACCCTCCTTCT Reverse: CATCAATCAAGACACCTCAAAAAAA	71	58	NM_00116402
SLC6A19	Forward: GCAACGTGACGCAGGAGAA Reverse: GGTCGGAGGCGTTGCA	57	58	XM_003359855.3
SLC15A1	Forward: ACTGCAAGCAACGACCATGA Reverse: CATCTGATCCGGCTGGATTT	61	58	NM_214347.1
TNF-α	Forward: CGACTCAGTGCCGAGATCAA Reverse: GACCTGCCCAGATTCAGCAA	60	58	JF831365.1
IL-1β	Forward: AAGGCCGCCAAGATATAACTGA Reverse: GCCCTCTGGGTATGGCTTTC	71	58	NM_001302388.1

IL-2	Forward: GCCATTGCTGCTGGATTTACA Reverse: TGGAGAGATCAGCATTCTCGTAATT	68	58	FJ543109.1
IL-6	Forward: ATGCTTCCAATCTGGGTTCAA Reverse: CACAAGACCGGTGGTGATTCT	61	58	AF518322.1
IL-8	Forward: ACATCCATGAGGAAGACAGTTTGA Reverse: CGGGAACTCCACGCTAGATTC	70	58	AB057440.1
IL-10	Forward: CCACATGCTCCGGGAACT Reverse: TCCTTCGTTTGAAAGAAACTCTTCA	63	58	HQ236499.1
IL-12	Forward: GCCCAGGAATGTTCAAATGC Reverse: TGTTGCTGACGGCCTTCAG	60	58	NM_213993.1
IgM	Forward: TCTGGCAAACACAGCTCATAACA Reverse: TCGTTGCATCTGCCATTACTG	69	58	AB699686.1
TGF-β1	Forward: CGAGCCAGAGGCGGACTAC Reverse: TTGCCGCTTTCCACCATTA	63	58	NM_214015.1
IFN-γ	Forward: CCATTGCCTACAGTAACTCGTTTG Reverse: TCCAGGTTCTCTACCACTTCTATCACT	77	58	HQ026021.1

CD8	Forward: GACCTCCGGCATCCTTCTC Reverse: CGGCGGTGGCAGATGA	54	58	AB634502.1
LFA-1	Forward: CCGGATGTTCTGCTGAATAATG Reverse: CGATGGCACCAAAGGTGTT	64	58	NM_001044608.1
CD18	Forward: AAACTGGCCGAAAGCAACAT Reverse: CATACGTTTTCACCATTTTCTTGGT	64	58	U13941.1
iNOS	Forward: CAGCGGGATGACTTTCCAA Reverse: TTGCAAGCAAGATCCCCTTT	60	58	NM_001143690.1
Hepcidin	Forward: CGGCTGCTGTCGCAAAG Reverse: GGTGGGCAGCTCTACGTCTT	57	58	AF516143.1
LEAP2	Forward: CATCCCCCCCTCAATCAAG Reverse: GGCAGATGACGAGTACTGCAAA	59	58	AF516144.1
β-defensin1	Forward: GCTTAAGGAATAAAGGCGTGTGTAT Reverse: GCCGATCTGTTTCATCTTTGG	62	58	NM_213838.1
STAT2	Forward: TGCCTCAAACTCCACATCGA Reverse: CCTTGTTCGGACAGTGAACTTG	65	58	AB004061.1

STAT3	Forward: AGCCTCTCCGCAGAGTTCAA Reverse: GCCCCCGTTCCCACAT	60	58	NM_001044580.1
JAK2	Forward: CCCAAAGCCAAAAGATAAGTCAA Reverse: TGTTGGTGAGGTTGGCACAT	76	58	AB00601
NF-κB P65	Forward: CGAGAGGAGCACGGATACCA Reverse: CCCGTGTAGCCATTGATCTTG	60	58	EU399817.1
mTOR	Forward: CAGGCATACGGTCGAGACTTAA Reverse: CGCCTGAACACGTGGTAATAGA	116	58	XM_013998977.1
S6K1	Forward: CCCTGCACTCGGTGGTACA Reverse: CCACGTAGCCATCACTAAAAACC	63	58	NM_001243214.1
4EBP1	Forward: GGCGGGCGGTGAAGAG Reverse: GATCCCATGGCTGGTCCTTT	58	58	NM_001244225.1
Bax	Forward: GCCTCAGTGTTCTTGTCTTTGGT Reverse: TCGGGCAACGTGTGGAA	59	58	AM233489.1
Bcl-2	Forward: CCAGCATGCGGCCTCTAT Reverse: GACTGAGCAGCGCCTTCAG	57	58	AB271960.1

Caspase-3	Forward: CCGGAATGGCATGTCGAT Reverse: TGAAGGTCTCCCTGAGATTTGC	60	58	NM_214131.1

ZO-(1,2) zonula occludens (1,2); MLCK, myosin light chain kinase; SLC (7A1, 7A7, 7A9, 5A1, 6A19, 15A1), amino acid transport related factor (7A1, 7A7, 7A9, 5A1, 6A19, 15A1); TNF-α, tumor necrosis factor α; IL-(1β, 2, 6, 8, 10, 12), interleukin (1β, 2, 6, 8, 10, 12); IgM, immunoglobulin M; TGF-β1, transforming growth factor β1; IFN-γ, interferon γ; CD8, cluster of differentiation 8; LFA-1, lymphocyte function-associated antigen 1; CD18, integrin beta-2; iNOS, inducible nitric oxide synthase; LEAP2, liver-expressed antimicrobial peptide 2; STAT2, signal transducer and activator of transcription 2; STAT3, signal transducer and activator of transcription 3; JAK2, Janus kinase 2; NF-κB P65, nuclear factor-kappa B P65; S6K1, ribosomal protein S6 kinase 1; 4EBP1, eukaryotic IF4E-binding protein 1; mTOR, mammalian target of rapamycin; Bax and Bcl-2, apoptosis related factor; Caspase-3, cysteine-aspartic acid protease factor 3; JNK, c-Jun N-terminal kinases.