

Supplementary material

Putrescine Enhances Intestinal Immune Function and Regulates Intestinal Bacteria in Weaning Piglets

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TABLE S1 | Ingredients and nutrient composition of basal diet (as fed basis) *

Ingredients (g/kg)	
Corn	579.5
Soybean meal	250
Fish meal	50
Dried whey	45

Spray-dried plasma protein	25
Soybean oil	20
Limestone meal	5
Dicalcium phosphate	11
Sodium chloride	3
L-Lysine HCl	1
DL-Methionine	0.5
Vitamin-mineral premix†	10
Analyzed composition (g/kg)	
Digestible energy (MJ/kg)	14.35
Crude protein	229.5
Lysine	14.3
Methionine	3.7
Calcium	9.1
Total phosphorus	7.2

* This formula was provided by NRC (2012)

† Supplied per kilogram of diet: vitamin A, 6,000 IU; vitamin D3, 600 IU; vitamin E, 50 IU; vitamin K3, 1.5 mg; thiamine, 2.0 mg; riboflavin, 8.0

mg; pantothenic acid, 20 mg; niacin, 30 mg; pyridoxine, 3.0 mg; choline, 800 mg; folic acid, 0.6 mg; biotin, 0.10 mg; vitamin B12, 0.04 mg; Zn, 100 mg (ZnSO₄); Cu, 16 mg (CuSO₄•5H₂O); Fe, 125 mg (FeSO₄); Mn, 15 mg (MnSO₄•H₂O); Se, 0.3 mg (Na₂SeO₃); I, 0.2 mg (KI).

TABLE S2 | Primer sequences of the target and reference genes used in Real-time PCR

Genes	Primer sequence (5'-3')	Product (bp)	Annealing temperature (°C)	Accession no.
β-Actin	Forward: TGC GGGACATCAAGGAGAA	58	58	DQ452569.1
	Reverse: GCCATCTCCTGCTCGAAGTC			
TNF-α	Forward: CGACTCAGTGCCGAGATCAA	60	58	JF831365.1
	Reverse: GACCTGCCCAGATTCAGCAA			
IL-1β	Forward: AAGGCCGCCAAGATATAACTGA	71	58	NM_001302388.1
	Reverse: GCCCTCTGGGTATGGCTTTC			
IL-2	Forward: GCCATTGCTGCTGGATTTACA	68	58	FJ543109.1
	Reverse: TGGAGAGATCAGCATTCTCGTAATT			
IL-6	Forward: ATGCTTCCAATCTGGGTTCAA	61	58	AF518322.1
	Reverse: CACAAGACCGGTGGTGATTCT			
IL-8	Forward: ACATCCATGAGGAAGACAGTTTGA	70	58	AB057440.1

	Reverse: CGGGAACTCCACGCTAGATTC			
IL-10	Forward: CCACATGCTCCGGGAACT	63	58	HQ236499.1
	Reverse: TCCTTCGTTTGAAAGAAACTCTTCA			
IL-12	Forward: GCCCAGGAATGTTCAAATGC	60	58	NM_213993.1
	Reverse: TGTTGCTGACGGCCTTCAG			
IgM	Forward: TCTGGCAAACACAGCTCATAACA	69	58	AB699686.1
	Reverse: TCGTTGCATCTGCCATTACTG			
TGF- β 1	Forward: CGAGCCAGAGGCGGACTAC	63	58	NM_214015.1
	Reverse: TTGCCGCTTTCACCATTA			
CD8	Forward: GACCTCCGGCATCCTTCTC	54	58	AB634502.1
	Reverse: CGGCGGTGGCAGATGA			
CD11	Forward: CCGGATGTTCTGCTGAATAATG	64	58	NM_001044608.1
	Reverse: CGATGGCACCAAAGGTGTT			
CD18	Forward: AACTGGCCGAAAGCAACAT	64	58	U13941.1
	Reverse: CACACGTTTTTCACCATTTTCTTGGT			
iNOS	Forward: CAGCGGGATGACTTTCCAA	60	58	NM_001143690.1

	Reverse: TTGCAAGCAAGATCCCCTTT			
Hepcidin	Forward: CGGCTGCTGTCGCAAAG	57	58	AF516143.1
	Reverse: GGTGGGCAGCTCTACGTCTT			
LEAP2	Forward: CATCCCCCCTCAATCAAG	59	58	AF516144.1
	Reverse: GGCAGATGACGAGTACTGCAA			
β -defensin1	Forward: GCTTAAGGAATAAAGGCGTGTGTAT	62	58	NM_213838.1
	Reverse: GCCGATCTGTTTCATCTTTGG			
STAT2	Forward: TGCCTCAAACCTCCACATCGA	65	58	AB004061.1
	Reverse: CCTTGTTCCGGACAGTGAACCTG			
STAT3	Forward: AGCCTCTCCGCAGAGTTCAA	60	58	NM_001044580.1
	Reverse: GCCCCCGTTCCACAT			
JAK2	Forward: CCCAAAGCCAAAAGATAAGTCAA	76	58	AB00601
	Reverse: TGTTGGTGAGGTTGGCACAT			
NF- κ B P65	Forward: CGAGAGGAGCACGGATACCA	60	58	EU399817.1
	Reverse: CCCGTGTAGCCATTGATCTTG			
mTOR	Forward: CAGGCATACGGTCGAGACTTAA	116	58	XM_013998977.1

Reverse: CGCCTGAACACGTGGTAATAGA

Reverse: GATCCCATGGCTGGTCCTTT

TNF- α , tumor necrosis factor α ; IgM, immunoglobulin M; TGF- β 1, transforming growth factor β 1; CD8, cluster of differentiation 8; CD11, 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- κ B P65, nuclear factor-kappa B P65; S6K1, ribosomal protein S6 kinase 1; 4EBP1, eukaryotic IF4E-binding protein 1; mTOR, mammalian target of rapamycin.

TABLE S3 | Primer sequences of primers and probes for intestinal bacteria

Genes	Primer sequence (5'-3')	Product (bp)	Annealing temperature (°C)	Reference.
Total bacteria	Forward: ACTCCTACGGGAGGCAGCAG Reverse: ATTACCGCGGCTGCTGG	200	60	
<i>Lactobacillus</i>	Forward: GAGGCAGCAGTAGGGAATCTTC Reverse: CAACAGTTACTCTGACACCCGTTCTTC Probe AAGAAGGGTTTCGGCTCGTAAAACCTCTGTT	126	60	
<i>Bifidobacterium</i>	Forward: CGCGTCCGGTGTGAAAG Reverse: CTTCCCGATATCTACACATTCCA Probe ATTCCACCGTTACACCGGGAA	121	60	Chen et al.[35]
<i>Escherichia coli</i>	Forward: CATGCCGCGTGTATGAAGAA Reverse: CGGGTAACGTCAATGAGCAAA Probe AGGTATTAACCTTACTCCCTTCCTC	96	60	

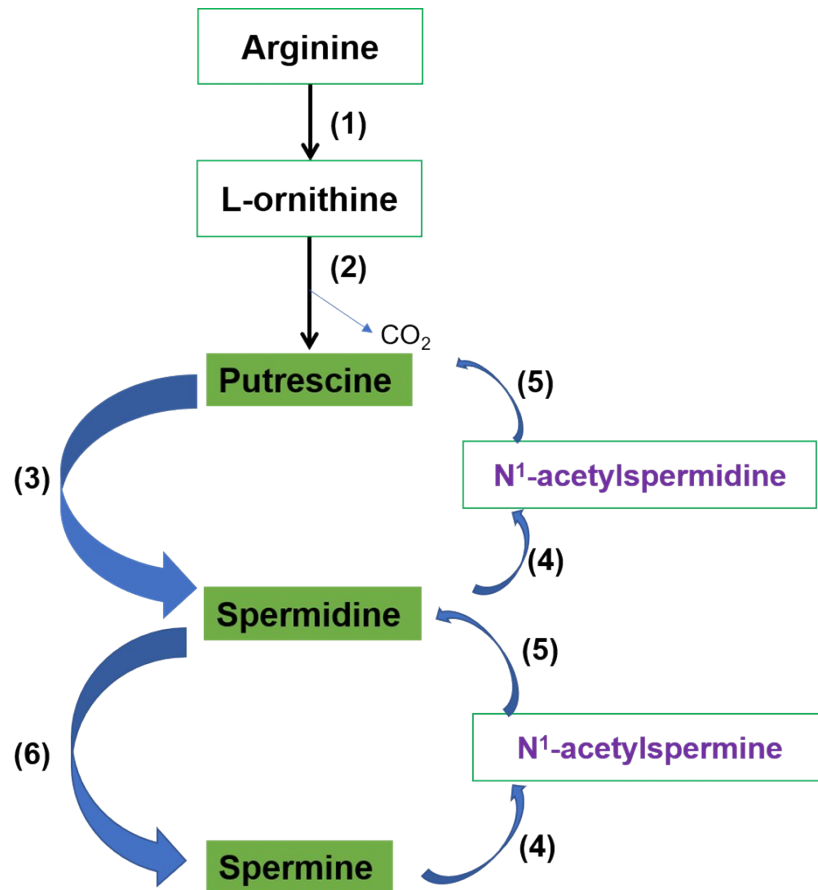


Fig S1 polyamine metabolism pathway. Notes: (1) Arginase; (2) Ornithine decarboxylase; (3) Spermidine synthase; (4) Spermidine/spermine N¹-acetyltransferase; (5) Polyamine oxidase; (6) Spermine synthase

