

**Sesamin improved growth and overall health in young animals by enhancing gut-
liver axis function**

Chen Chen^{†a}, Min Qi^{†b}, Zhiran Xu^a, Jincheng Wen^a, Wenjie Tang^{c, d}, Hui Diao^{c,d},
Zhangcheng Li^a, Yunyun Chu^a, Fu Feng^a and Zhiru Tang^{*a}

*^aLaboratory for Bio-feed and Molecular Nutrition, College of Animal Science and
Technology, Southwest University, Chongqing 400715, China*

^bYunnan Animal Husbandry Station, Kunming 650225, China

*^cAnimal Breeding and Genetics Key Laboratory of Sichuan Province, Sichuan Animal
Science Academy, Chengdu 610066, China*

*^dLivestock and Poultry Biological Products Key Laboratory of Sichuan Province,
Sichuan Animtche Group Co., Ltd., Chengdu 610066, China*

† These authors have contributed equally to this work and share the first authorship.

*Corresponding author: Zhiru Tang. E-mail: tangzhiru2326@sina.com; Tel: +86-139-
9619-2900

Food & Function

Supplementary material

Table S1. Composition and nutritional levels of the basal diet for weaned piglets (DM basis %)

Ingredients	Content(%)	Nutritional level	Content
Corn (CP: 7.6%)	43.29%	Crude fat ^c	5.41%
Cracked rice (CP: 7%)	20.20%	Crude fiber ^c	2.11%
Bran	2.02%	Crude ash ^c	3.73%
46% soybean meal	6.06%	Crude protein ^c	17.02%
Extruded soybeans	5.05%	Digestible protein ^b	14.38%
Soy protein concentrate	5.05%	Digestible energy ^b	3482Kcal/kg
Fermented soybean meal	4.04%	Net energy ^b	2482Kcal/kg
Egg yolk powder	2.53%	Calcium ^c	0.49%
Low-protein whey powder	2.53%	Total phosphorus ^c	0.52%
Coconut oil powder (50% fat)	2.02%	Available phosphorus ^b	0.31%
Glucose	1.52%	SID lysine ^d	1.22%
Sucrose	1.52%	SID methionine ^d	0.49%
Limestone powder	0.51%	SID cysteine ^d	0.23%
Dicalcium phosphate	1.01%	SID methionine + cysteine ^d	0.72%
Sodium chloride	0.25%	SID threonine ^d	0.84%
0.2% trace elements for piglets	0.20%	SID tryptophan ^d	0.26%
Complex vitamins for piglets	0.04%	SID valine ^d	0.81%
Benzoic acid	0.40%	SID arginine ^d	0.97%
98% L-lysine	0.61%	SID histidine ^d	0.40%
DL-methionine (99%)	0.25%	SID isoleucine ^d	0.62%
L-threonine (98.5%)	0.30%	SID leucine ^d	1.20%
L-tryptophan (98%)	0.10%	SID phenylalanine ^d	0.68%
L-Valine (99%)	0.15%	SID tyrosine ^d	0.47%
L-Isoleucine (99%)	0.05%	SID (phenylalanine + tyrosine) ^d	1.15%
Mildew inhibitor	0.10%		
Sweetener	0.02%		
50% Choline chloride	0.10%		
Complex enzymes for piglets	0.06%		

^aEach kg of the premix diet provided the following components: V_A: 11,400 IU; V_D₃: 3,200 IU; V_E: 48 mg; V_K₃: 3.2 mg; V_B₁: 4.5 mg; V_B₂: 11.2 mg; V_B₆: 4.8 mg; V_B₁₂: 56 mg; D-biotin: 0.24 mg; folic acid: 2.4 mg; niacin: 56 mg; D-pantothenic acid: 24 mg; Cu: 90.48 mg; Fe: 132.36 mg; Zn: 68.9 mg; Mn: 31.8 mg; Co: 0.72 mg; I: 0.768 mg; Se: 0.344 mg.

^bCalculated values.

^cAnalysed values.

^d Values for the concentrations of SID AA in diets were calculated using standardised ileal digestible coefficients for the various ingredients provided by NRC (2012).

Table S2. Primer sequences for gene amplification in weaned piglets

Gene name ^a	NCBI ID	Sequence of primer (5'→3')	Product size (bp)	Tm ^b (°C)
<i>GAPDH</i>	XM_021091114.1	F: CGAGATCCCGCCAACATCAA R: CCCACCCCTCAAGTGAGC	109	58.0 59.3
<i>OCN</i>	XM_005672525.3	F: CAGTGGTAACTGGAGGCGT R: CCGTCGTGTAGTCTGTCTC	104	57.0 55.0
<i>Claudin-1</i>	NM_001244539.1	F: GGACAAAACCGTGTGGGAAC R: CACTTCCCGTTGGACGAGT	200	57.1 55.3
<i>ZO-1</i>	XM_021098896.1	F: CGTGTCACGCCACTATCA R: TTGTCTTCCAAAGCCCCT	90	55.1 53.7
<i>Mucin-2</i>	XM_021082584.1	F: CTGTGCGACTACAACCTCGC R: AGATGGTGTCTGCTTACC	139	56.7 57.1
<i>Mucin-4</i>	XM_021068274.1	F: AGGATGCCAATGGCTCTACT R: AAGGAGGCTGGTCCGTTGAT	96	60.98 61.73
<i>Villin</i>	XM_001925167.6	F: ACGGCGCAGGAGTATCTCAA R: ACGGCGCAGGAGTATCTCAA	187	58.5 59.1
<i>ChgA</i>	NM_001164005.2	F: AAGGAGAGGTCCCATCAGCA R: CGTCTCGTCTTCATCGTTC	181	58.3 56.5
<i>Lgr5</i>	XM_021090898.1	F: GCCTCGGAATCCTCCGTTTT R: CAGGCTGGAGGAGGAGTTT	105	58.0 59.8
<i>BMI-1</i>	XM_021064000.1	F: CCAGAACAGATTGGATCGGA R: GCTGCTGGGCACCGTAA	90	54.2 59.3
<i>PCNA</i>	NM_001291925.1	F: CCTGTGCAAAAGATGGAGTG R: GGAGAGAGTGGAGTGGCTTT	187	54.0 56.9
<i>β-catenin</i>	XM_021068566.1	F: GGTAAACTCTACATCCACCA R: AGCACGAACCAGCAACTGA	148	53.9 57.3
<i>TCF4</i>	XM_021093592.1	F: CCGGTCTTACTTTCCGCCAA R: CTCGTCGTCGGACTTGATCTC	242	57.9 57.7
<i>c-Myc-a</i>	NM_001005154.1	F: GAACGCCAGAGGAGAAACGA R: GCAGCACGTCTTTTTTCTGACA	175	57.5 56.2
<i>CCND1</i>	XM_021082686.1	F: CTCAAGTGGAACCTGGCGG R: AGGACAGGAAGCTGTTGGAAC	235	59.4 58.5
<i>Nrf2</i>	MH101365.1	F: TGCAGCTTTTGGCAGAGACA R: AGGAGCAATGAAGACTGGGC	119	57.5 57.8
<i>Keap1</i>	NM_001114671	F: AAACCGCTCAACTCAGCAG R: CTGGTCTGACCATCGTAGC	148	58.4 58.1
<i>HO-1</i>	NM_001004027.1	F: TACCGCTCCGAATGAACAC R: GTCACGGGAGTGGAGTCTTG	209	57.6 58.2
<i>NQO1</i>	NM_001159613.1	F: TATCCTCCTCTGGCCAATTC R: AGGCGTTTCTTCCACTCTTC	81	53.8 54.9
<i>GCLC</i>	XM_021098556.1	F: GATCCTCCAGTTCCTGCACA R: GAGAGAGAACCAACCTCGTCG	87	57.2 57.7
<i>GCLM</i>	XM_001926378.4	F: CATCCCTGACATCGAGGCAC R: TGGATTTGATGATGCCTCTGC	89	58.7 55.3
<i>GR</i>	AY368271	F: GTGAGCCGACTGAACACCAT R: CAGGATGTGAGGAGCTGTGT	141	57.9 57.2
<i>Cu/Zn-SOD</i>	NM_001190422.1	F: TGAAGGGAGAGAAGACAGTGTTAG R: TCTCCAACGTGCCTCTCTTG	181	55.6 57.3
<i>Mn-SOD</i>	NM_014127.2	F: GGACAAATCTGAGCCCTAACG	159	55.8

		R: CCTTGTGAAACCGAGCC		54.3
		F: GAAGTGTGAGGTGAATGGCG		56.6
<i>GPX1</i>	NM_214201.1	R: TGCGATGTCATTGCGACACA	145	57.6
		F: TCACCAATGTGGCCTCTCAA		56.5
<i>GPX4</i>	NM_214407.1	R: CAAGGGAAGGCCAGAATCCG	109	59.1
		F: TCATCCAGGAAGGTTTCCAC		54.5
<i>TLR4</i>	NM_001293316.1	R: TGTCTCCCACTCCAGGTAG	234	58.7
		F: CAGCATCCCTTGGATGTCAGG		58.6
<i>MyD88</i>	NM_001099923.1	R: GGATATCGCTGGGGCAGTAG	101	58.1
		F: CGGGGACTACGACCTGAATG		58.1
<i>NF-κB</i>	NM_001114281.1	R: GCACGGTTGTCAAAGATGGG	117	57.2
		F: CACCAGCAGCAAGTGCCTA		57.6
<i>CCL2</i>	NM_214214.1	R: CCCACTTCTGCTTGGGTTCT	86	57.7
		F: GGAGCAGCAGTGAAGCCTAA		57.7
<i>ADGRE1</i>	XM_021083974.1	R: TGCTTTCCACGCTCTTCAGT	217	56.9
		F: TGGCCCAAGGACTCAGATCA		58.6
<i>TNF-α</i>	NM_214022.1	R: GGCATTGGCATACCCACTCT	113	57.9
		F: CAGGCCATTCAAAGGAGCAT		55.7
<i>IFN-γ</i>	NM_213948.1	R: GAGTTCACTGATGGCTTTGCG	150	57.0
		F: GCAGTGGAGAAGCCGATGAA		57.9
<i>IL-1β</i>	XM_021085847.1	R: TAGACTGCACGTTGGCATCA	148	56.6
		F: TGGCTACTGCCTTCCTACC		59.6
<i>IL-6</i>	NM_001252429.1	R: CACACATCTCCTTCTCATTGC	153	53.8
		F: AGTGCAGAACTTCGATGCCA		56.9
<i>IL-8</i>	NM_213867.1	R: TGGGGTCCACTCTCAATCAC	95	56.9
		F: GGCCGTAAGGCTCTTTACA		57.4
<i>TGF-β</i>	NM_214015.2	R: CCGCTTTCCAGCATTAGCAC	126	57.3
		F: CTGCATCCACTTCCCAACCA		58.0
<i>IL-10</i>	NM_214041.1	R: AGAAACTCTTCACTGGGCCG	77	57.6
		F: AGCAAGCGTGAAGGTGCGGTT		63.1
<i>IL-22</i>	XM_021091968.1	R: GCGGACATCTGGGAGCCCTTT	169	63.2

^a*GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *OCN*, occludin; *ZO-1*, zonula occludens-1; *ChgA*, chromogranin A; *Lgr5*, leucine-rich repeat-containing G-protein coupled receptor 5; *BMI-1*, B-cell specific moloney leukemia virus insertion site 1; *PCNA*, proliferating cell nuclear antigen; *TCF4*, transcription factor 4; *c-Myc-a*, cellular Myelocytomatosis oncogene a; *CCND1*, cyclin D1; *Nrf2*, nuclear factor erythroid 2-related factor 2; *Keap1*, Kelch-like ECH-associated protein 1; *HO-1*, heme oxygenase-1; *NQO1*, *NAD(P)H*:quinone oxidoreductase 1; *GCLC*, glutamate-cysteine ligase catalytic subunit; *GCLM*, glutamate-cysteine ligase modifier subunit; *GR*, glutathione reductase; *Cu/Zn-SOD*, copper/zinc superoxide dismutase; *Mn-SOD*, manganese superoxide dismutase; *GPX1*, glutathione peroxidase 1; *GPX4*, glutathione peroxidase 4; *TLR4*, toll-like receptor 4; *MyD88*, myeloid differentiation primary response 88; *NF-κB*, nuclear factor kappa-light-chain-enhancer of activated B cells; *CCL2*, C-C motif chemokine ligand 2; *ADGRE1*, adhesion G protein-coupled receptor E1; *TNF-α*, tumor necrosis factor-α; *IFN-γ*, Interferon-γ; *IL-1β*, interleukin-1β; *IL-6*, interleukin-6; *IL-8*, interleukin-8; *TGF-β*, transforming growth factor-β; *IL-10*, interleukin-10; *IL-22*, interleukin-22.

^bT_m, melting temperature.