Supplementary Information

Effects of Zinc-L-Selenomethionine on growth performance in weaned piglets and antioxidant stress mechanism through modulation of PI3K/AKT and Nrf2/Keap1 signaling pathways

Supplementary Table S1. Primer sequences for key genes in oxidative stress-related signaling pathways used for RT-qPCR analysis

Genes	Direction	Primer Sequence (5'-3')
Keap1	Forward	5'-CGTGGAGACAGAAACGTGGA-3'
	Reverse	5'-CAATCTGCTTCCGACAGGGT-3'
NQO1	Forward	5'-CCTCTGGCCAATTCAGAGTGG-3'
	Reverse	5'-TGTGCCCAATGCTGTACGTC-3'
HO-1	Forward	5'-TACCGCTCCCGAATGAACAC-3'
	Reverse	5'-GTCACGGGAGTGGAGTCTTG-3'
ARE	Forward	5'-CGCGCGATCCGCTGAGTCAGC-3'
	Reverse	5'-CTAGAGCGCCCTGTTCCTGGC-3'
NRF2	Forward	5'-TGCAGCTTTTGGCAGAGACA-3'
	Reverse	5'-AGGAGCAATGAAGACTGGGC-3'
AKT	Forward	5'-GCACAAACGAGGCGAGTA-3'
	Reverse	5'-CAGCGGATGATGAAGGTGTT-3'
PI3K	Forward	5'-GAACAAATGAGGCGACCAGA-3'
	Reverse	5'-GATTTCCCAGCTGATGAGCA-3'
Erk1/2	Forward	5'-TCCAACCTGCTGCTCAAC-3'
	Reverse	5'-AGCCTGCTCTACTTCAATCC-3'
JNK	Forward	5'-CATGGCAGAAATGGTCCTCCA-3'
	Reverse	5'-TCTGCAGATGGTGTTCCTAGC-3'
P38	Forward	5'-AAGACTCGTTGGAACCCCAG-3'
	Reverse	5'-TCCAGCAAGTCAACAGCCAA-3'

GAPDH	Forward	5’-GATGGTGAAGGTCGGAGTGAAC-3’
	Reverse	5’-TGGGTGGAATCATACTGGAACA-3’

Supplementary Table S2. Antibodies used for western blot

	Antibody name	Company	product category	Dilution ratio
Primary antibody	Keap1	Abcam,Cambridge, USA	119403	1:500
	NQ O -1	CST, Inc. US	62262T	1:800
	HO-1	CST , Inc. US	70081s	1:800
	Nrf2	Abcam,Cambridge, USA	12721T	1:800
	AKT	Cohesion Biosciences Ltd., UK	CQA8001	1:800
	PI3K	Bioss Biotechnology Co. China	bs-10657R	1:800
	GAPDH	Proteintech Group, Inc.USA	60004-1-AP	1:5000
secondary antibody	HRP-Goat anti Muose	proteintech	SA00002-2	1:10000
	HRP-Goat anti Rabbit	proteintech	SA00002-1	1:10000

Supplementary S1 Materials and Methods

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using Trizol Reagent (TaKaTa, Dalian, China). Then, cDNA was synthesized using using ReverTra Ace reverse transcriptase (Toyobo Co., Osaka, Japan) according to the manufacturer’s instruction. On the StepOne Plus real-time PCR system, RT-qPCR was performed in a final volume of 20 uL with SYBR Green master mix (Applied Biosystems, Singapore). Swine GAPDH was used as an internal control gene, and all samples were analyzed in triplicate. Relative expression levels of selected selenoprotein genes were calculated using 2-ΔΔCt value methods.

Examination of intracellular ROS production

Reactive Oxygen Species Assay Kit（KeyGEN BioTech, Jiangsu, China）was used to detect intracellular ROS. The final concentration of 10 μmol/L was obtained by diluting DCFH-DA 1:1000 with serum-free medium. The cell culture was removed, diluted DCFH-DA was added to the cells, which were incubated for 20 min at 37°C. To remove excess DCFH-DA that had not entered the cells, the cells were then washed three times with serum-free culture medium. Finally, the cells were washed twice with 1 mL of PBS and centrifuged at 1500×rpm for 5 min. The cells were resuspended with 300ul of PBS and examined by fluorescence flow cytometry.

Western blot

Total cellular protein was extracted using the RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). Then cell protein concentration was measured using the BCA protein assay kit (KeyGEN BioTech). Equal amounts of cell proteins were separated by SDS-PAGE and then transferred to the polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, USA). The membranes were incubated with the primary antibodies overnight at 4°C, followed by incubation with secondary antibodies for 2 h at room temperature. Finally, the immunoreactive bands were visualized using ECL luminescence reagent (KeyGEN BioTech, Jiangsu, China).The antibodies used were shown in Supplemental Table S2.