

Supplementary Materials

Effects of different dietary zinc (Zn) sources on growth performance, Zn metabolism, and intestinal health of grass carp

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Table S1 Formulation and proximate composition of the experimental diets (% dry

matter)

¹⁾	Ingredients (g kg ⁻¹)	ZnSO ₄ • 7H ₂ O	ZnO NPs	Zn-Lac	Zn-Gly
	Fish meal	80	80	80	80
	casein	35	35	35	35
	Rice bran	110	110	110	110
	Soybean meal	200	200	200	200
	Rapeseed meal	250	250	250	250
	Wheat Starch	250	250	250	250
	Fish oil	20	20	20	20
	Soybean oil	10	10	10	10
	Choline chloride	1.5	1.5	1.5	1.5
	Vitamin premix	4	4	4	4
	Mineral premix	7.5	7.5	7.5	7.5
	DL-methionine	0.15	0.15	0.15	0.15
	L-lysine	0.4	0.4	0.4	0.4
	L-threonine	0.4	0.4	0.4	0.4
	Lecithin	1	1	1	1
	Ca(H ₂ PO ₄) ₂ •H ₂ O	20	20	20	20
	Cellulose	9.9	10.008	9.923	9.939
	ZnSO ₄ •7H ₂ O	0.150			
	ZnO NPs		0.042		
	Zn-Lac			0.127	
	Zn-Gly				0.111
	Proximate analysis %, dry weight				
	Moisture	8.57	8.43	8.66	8.86
	Crude protein	30.82	31.09	31.88	31.13
	Crude lipid	6.31	6.63	6.58	6.49
	Ash	7.67	7.54	7.76	7.59
	Zn (mg kg ⁻¹)	81.09	81.12	81.38	81.56

Vitamin premix (mg or IU per kg diet): niacin, 50; calcium pantothenate, 30; pyridoxine hydrochloride, 12; menadione nicotinamide bisulfite, 7; folic acid, 6; thiamine hydrochloride, 6; riboflavin, 3; D- biotin, 1; cyanocobalamine, 0.03. retinylacetate, 10000IU; cholecalciferol, 1000IU; all-rac-a-tocopheryl acetate, 30IU;

Mineral mixture (mg per kg diet): NaCl, 500; FeSO₄•7H₂O, 400; MnSO₄•H₂O, 40; CuSO₄•5H₂O, 12; CaIO₃•6H₂O, 1.5; Na₂SeO₃, 0.45; CoSO₄, 1.

Table S2 The quality of RNA samples.

	Concentration (ng μL^{-1})	A260/280	A260/230
ZnSO ₄ •7H ₂ O	702.54	2.03	2.21
	884.05	2.00	2.29
	918.11	2.03	2.09
ZnO NPs	662.38	1.97	2.29
	584.77	1.95	2.34
	666.06	1.97	2.36
Zn-Lac	797.19	2.01	2.34
	804.34	1.99	2.33
	608.29	1.94	2.33
Zn-Gly	553.56	1.97	2.22
	754.23	1.95	2.37
	814.24	2.02	2.25

Table S3 Primers used for quantitative real-time PCR (qRT-PCR) analysis.

Genes	Forward primer (5'-3')	Reverse primer (5'-3')	Accession no.
<i>atf4</i>	CCTCTCCTCTTCCTAC	CGACATCCAATCCATG	AY437846
	GCTG	CCAG	
<i>atf6</i>	TGACACCTCTGTTCC	CCTCAGCAAAACCAC	KT279356
	TGACC	TCGAG	
<i>β-actin</i>	ACCCTGAAGTACCCC	CAGAGGCATACAGGG	DQ211096.1
	ATCGA	ACAGC	
<i>cat</i>	CCAGAGAGAGTTGT	CTTCACCGCAAATCCT	FJ560431
	GCATGC	CGAG	
<i>claudin 1</i>	TGGCTTCTCTGGGTC	TGAACTTCCCCTGGTA	XM05188046
	TCTTG	GCTG	
<i>claudin 2</i>	CAACATCGTGACAGC	GCACAGGCCATGACA	XM05187679
	GGTAG	GAAAA	
<i>claudin 3</i>	ATCGTGACGGCTCAG	GGACGGTCAGAAGGA	XM05186218
	ATCAT	TGGAT	
<i>claudin 4</i>	GGGTTTGTGGATGAG	CACACCTGAGACGAT	XM05187744

	CTGTG	GCATG	3
<i>claudin</i>	TGGGTCTGATCCTAT	ATGGAGTCGTGGATCT	XM05190449
<i>5a</i>	GCGTC	TGCA	2
<i>claudin</i>	ACGGGTCAGATGCA	CAGAGGCACCAACAC	KU200696
<i>5b</i>	GTGTAA	AAACA	
<i>claudin 7</i>	GTGCCCTGATGATTG	AAAGGGCTGCAACAA	KT445866
	TAGCG	TGAGG	
<i>claudin 8</i>	CCGGTCTGGGTCTAC	CATCGATGAGCAAGG	XM05186339
	TGATC	GGTTG	1
<i>claudin</i>	ATGACATCAAGAACT	ACAGGTTATCGTAGCG	XM05186474
<i>12</i>	CGCGC	CTCA	3
<i>elf2a</i>	GTTCAACAGAGTGC	GACGCCTCTCTTCTCT	KJ126860
	ATGCCA	TCGA	
<i>gapdh</i>	GGGAAACTGTGGAG	TGCAGCCTTGACCACT	GQ245759.1
	GGATGG	TTCT	
<i>gpx1</i>	TCTGAAGTATGTCCG	GTTCCAGGCGATGTCA	JX854447
	TCCGG	TTCC	
<i>grp78</i>	CGACGAGAAGAAGG	CTGGTTCTTCGCAGCA	FJ436356
	AAAGCG	TCTC	
<i>ire1</i>	GCAAAGTCCCACAC	AGTAACTCCGTCATGC	MG797683
	ACCAAT	ACCA	
<i>keap1</i>	ACGCAGGAGGAGTT	TAGGTGTGAGTGAGT	XM05189687
	CTTCAA	GGCAG	6
<i>mtf-1</i>	GGCTCCTCCTCCTCC	CCGACTGGTATGGCTG	MK770661
	TACAA	TTGT	
<i>nrf2</i>	CTTGCAGATCCCGTT	TCTAGGCCCCACGA	KY807076
	TACCG	TGTTCTC	
<i>occludin</i>	CACCTTCATCGTTCT	AACTGAACTGATCCGG	KF193855
	GCTCG	ACGT	

<i>perk</i>	CTGGATGGAAGAATC GCAGC	GCCTCCATACTCTCCC TGTC	KX906957
<i>sod1</i>	GTCCGCACTTCAACC CTTAC	TTCCTCATTGCCTCCC TTCC	GU901214
<i>xbp1s</i>	GCACAGACAGCAAG AGACAG	ACCAAACCCAAATCG CTCAC	KU509247
<i>zip1</i>	CTAGATGAGGGCTTC CCGCT	CATTCAACGAGTGCCG TCCA	OP106599
<i>zip4</i>	TCTTGCGGGCGTCTA TTTCT	AAGTTATGGATGCCGT CGCC	OP106600
<i>zip5</i>	TTAGGTGATTTGGCG GTGCT	TGGAGCATTTCGGGC ATCAT	OP106601
<i>zip6</i>	GAAGGTTTGTCCAGC GGTCT	CATACCCGCCTTCAGC AAGA	OP106602
<i>zip7</i>	CCAGTGCAGTCAAA CACGGA	AGAGTGAGAGTGCGA GTGTC	OP106603
<i>zip8</i>	AAAACGGCTCTCTGT CCACG	ATTACCCAACCCAAA GGCGG	OP106604
<i>zip9</i>	TCCAGAAGCAGCAA GGACTG	AGCAGGAGCCTTATG CAACA	OP106605
<i>zip10</i>	TTCTGGTGCCCATCC TCAAC	CCTGCTAGTGCAGTCA GTCC	OP106606
<i>zip13</i>	ATCAGCCCTTACACA GAGCC	TACGAGTGGCTTTCCA ACGG	OP106608
<i>zip14</i>	GCGCTATGCCAACTC CAATG	TAACATCCACCCGTTC CAGC	OP106609
<i>znt1</i>	GCAACCCGATGTGGT GATTG	GGCTGTTGTTGTGATT CCCG	OP106610
<i>znt4</i>	CTCAATCAGTCCGGG	CACACCCTCCAGCAG	OP106612

	CATCT	AATGA	
<i>znt5</i>	ATGTGCTGTCTGGTG	TGATGAAGCGAGGCA	OP106613
	GAGTG	GAGAC	
<i>znt6</i>	CGAGAGGAAAGTGT	ACACCAGATTCAACA	OP106614
	CGCCTT	CGCCA	
<i>znt9</i>	TTGGCGTGGGTTTAT	GGCGATGTAACGCAT	OP106617
	ACGGG	GTTGG	
<i>znt10</i>	ACTGGCTAAAGGAC	CCACCAGGACAACAG	OP106618
	GGAACG	GAGAG	
<i>zo-1b</i>	CCCAAGCACGACAA	GTGGATTCTGGCCCTTG	XM05188508
	AGACAA	ATTC	2
<i>zo-2</i>	GACGTCCATCATTGT	TAATCACGGCTATCCA	XM05190425
	GTCGG	CGCT	2

Abbreviations: *atf*, activating transcription factor; *cat*, catalase; *elf2a*, Eukaryotic translation initiation factor 2A; *gapdh*, glyceraldehyde-3-phosphate dehydrogenase; *gpx*, glutathione peroxidase; *grp78*, Endoplasmic reticulum chaperone BiP; *ire1*, serine/threonine-protein kinase/endoribonuclease IRE1-like protein; *keap1*, kelch-like ECH-associated protein 1; *mtf1*, metal-regulatory transcription factor 1; *nrf2*, nuclear factor erythroid-specific related factor 2; *perk*, Eukaryotic translation initiation factor 2- α kinase 3; *sod*, superoxide dismutase; *xbp*, X-box binding protein; *zo*, tight junction protein.

Table S4 The coefficient of variation for the indices of antioxidant capacity.

	ZnSO ₄ •7H ₂ O	ZnO NPs	Zn-Lac	Zn-Gly
T-SOD	6.41%	1.08%	6.74%	10.09%
Cu Zn-SOD	9.36%	9.26%	7.76%	10.60%
CAT	2.79%	1.33%	2.02%	1.44%
GPX	2.23%	9.17%	8.97%	2.64%
Reduced GSH	5.89%	8.25%	2.72%	11.05%

GSSG:GSH ratio	6.45%	1.59%	0.72%	2.29%
MDA	2.80%	5.31%	23.91%	22.23%

Table S5 The representative loading scores of the principal component analysis (PCA).

feature	PC1	PC2
Protein level of NRF2	-0.323	0.106
mRNA abundance of claudin4	-0.300	-0.075
Activity of T-SOD	-0.130	0.309

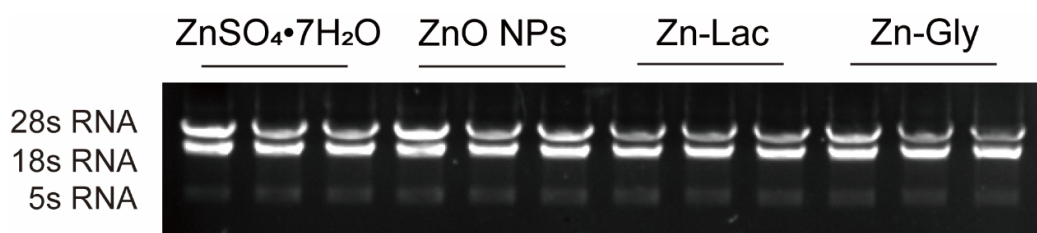


Figure S1. The measurement of RNA integrity in the samples using RNA gel (n=3 replicate tanks).

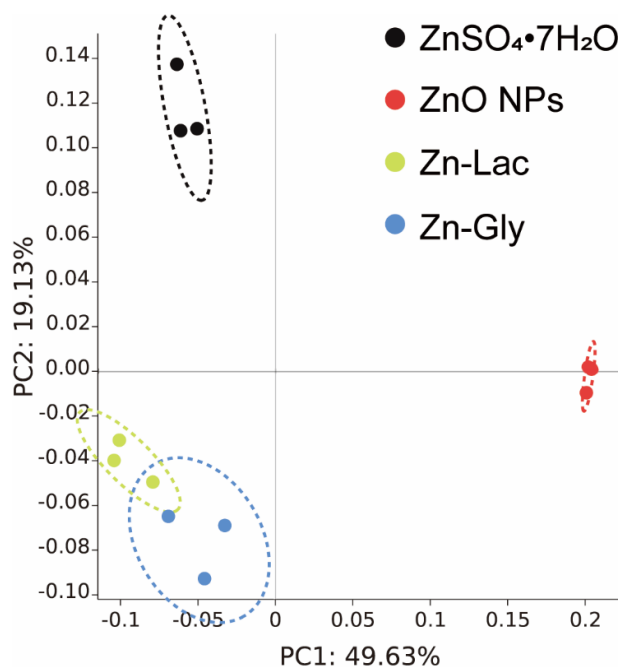


Figure S2. The principal component analysis (PCA) shows the relationship of four dietary Zn sources.