

**Table S1.** Composition and nutrient levels in the basal diets (dry matter basis)

<b>Ingredients</b>	<b>g/kg diet</b>	<b>Nutrient analysis</b>	<b>g/kg diet</b>
Ground corn	543.0	AME/(kcal/kg)	2950
Soybean oil	34.0	CP	210
Soybean meal (44%)	380.2	Calcium	10.1
Lysine (98%)	1.5	Available Phosphorus	4.5
DL-Methionine	2.5	Lysine	11.5
Calcium carbonate	11.4	Methionine	5.0
Di-calcium Phosphate	18.6	Methionine and Cystine	8.6
Sodium chloride	4.0	Manganese (mg/kg) <sup>3</sup>	25.62
Choline chloride	1.5		
Vitamin premix <sup>1</sup>	0.3		
Mineral premix <sup>2</sup>	3.0		
<b>Total</b>	<b>1000</b>		

<sup>1</sup> Provided per kilogram of diet: Vitamin A 8000 IU; Vitamin D<sub>3</sub> 1000 IU; Vitamin E 20 IU; Vitamin K<sub>3</sub> 0.5 mg; Pantothenic acid 10 mg; Niacin amide 35 mg; Folic acid 0.55 mg; Biotin 0.18 mg.

<sup>2</sup> Provided per kilogram of diet: Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O), 8 mg; Fe (FeSO<sub>4</sub>·7H<sub>2</sub>O), 80 mg; Zn (ZnSO<sub>4</sub>·7H<sub>2</sub>O), 70; Se (NaSeO<sub>3</sub>), 0.3 mg; I (KI), 0.7 mg.

<sup>3</sup>Manganese concentration in the basal diet was analyzed by atomic absorption spectrophotometer.

AME, apparent metabolism energy; CP, crude protein;

**Table S2.** The primers for quantitative real-time PCR

Gene	Gene ID	Primer	Sequence (5'-3')	Product Size (bp)
<i>IL-1<math>\beta</math></i>	NM_204524.1	Reverse	gcatcaagggctacaagctc	131
		Forward	caggcggtagaagatgaagc	
<i>IL-6</i>	NM_204628.1	Reverse	ctcctcgcaatctgaagtc	100
		Forward	ccctcacggctcttccata	
<i>IL-8</i>	AJ009800	Reverse	gcttgctaggggaaatgaag	136
		Forward	ggaattaccagtttgctgctg	
<i>IL-12<math>\beta</math></i>	NM_213571.1	Reverse	cctgctgtctgctaagacc	82
		Forward	atcatttgccttggagtc	
<i>IL-18</i>	NM_204608.1	Reverse	tgaaatctggcagtggaatg	144
		Forward	caaccatttcccatgctct	
<i>IFN-<math>\gamma</math></i>	NM_205149.1	Reverse	cagatgtagctgacggtgga	98
		Forward	catcgaacaatctggctca	
<i>TNF-<math>\alpha</math></i>	GU230788.1	Reverse	gccttctctgaaccagatg	71
		Forward	acacgacagccaagtcaacg	
<i>TRAF6</i>	XM_015287208.1	Reverse	atggaagccaagccagagtt	144
		Forward	acagcgcaccagaagggtat	
<i>NF-<math>\kappa</math>B</i>	M86930.1	Reverse	tcaacgcaggacctaaagacat	162
		Forward	gcagatagccaagttcaggatg	
<i>MnSOD</i>	NM_204211.1	Reverse	ttctgacctgcttacgactat	138
		Forward	ccagcgctcttgtatttct	
<i>claudin-1</i>	NM_001013611.2	Reverse	gtctttgggtggcgtgatctt	117
		Forward	tctggtgtaacgggtgtga	
<i>ZO-1</i>	XM_015278981.2	Reverse	ggtcagccagatgtggattt	81
		Forward	ccgaagcattccatcttcat	
<i>Occludin</i>	NM_205128.1	Reverse	gctgagatggacagcatcaa	97
		Forward	cctctgccacatctggat	
<i><math>\beta</math>-actin</i>	NM_205518.1	Reverse	gctacagctcaccaccaca	90
		Forward	tctctgctcgaatccagt	
<i>GAPDH</i>	NM_204305.1	Reverse	tggaagcttactggaatgg	88
		Forward	cttgctggttctccagac	

*IL*, interleukin; *IFN*, interferon; *TNF*, tumor necrosis factor. *TRAF6*, TNF receptor-associated factor 6. *NF- $\kappa$ B*, nuclear transcription factor kappa B; *MnSOD*, manganese superoxide dismutase; *ZO-1*, zona occludens 1; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

1 **Table S3.** Sequences of the primers used for the determination of the microbial population

<b>Items</b>	<b>Primer</b>	<b>Sequence (5'-3')</b>
Total bacteria	Reverse	gatccgcttgcttcgca
	Forward	catccagtgcaaacctaaagag
<i>Lactobacillus</i>	Reverse	gatccgcttgcttcgca
	Forward	catccagtgcaaacctaaagag
<i>Bifidobacterium</i>	Reverse	taagccatggactttcacacc
	Forward	gggtggtaatgccgatg
<i>Enterococcus</i>	Reverse	act cgttgtacttcccattgt
	Forward	cccttattgtagttgccatcatt
<i>Salmonella</i>	Reverse	aaacggtgaaaaactgagga
	Forward	tcgtcattccattacctacc
<i>Escherichia coli</i>	Reverse	agaacgctttgtggtaaatcagga
	Forward	gtgtgatctaccgcttcgc
<i>Clostridium</i>	Reverse	cccttacaccagtaa
	Forward	gagttgatcmtggctcag
<i>Enterobacter</i>	Reverse	ctctacgagactcaagcttgc
	Forward	cattgacgttaccgcag aagaagc

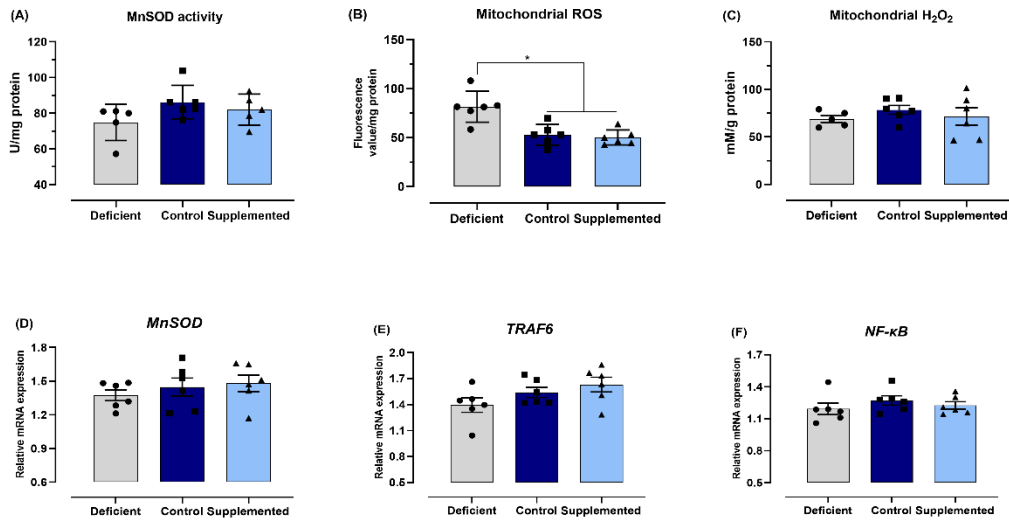
3 **Table S4.** Effects of graded Mn on *MnSOD* and inflammatory cytokine expressions in spleen,  
 4 thymus, and bursa of broilers following oral *S. Typhimurium*

Gene	Tissue	Dietary Mn levels		
		Deficient diet (0 mg/kg)	Control diet (40 mg/kg)	Surfeit diet (100 mg/kg)
<i>TNF-α</i>	Spleen	1.03±0.06 <sup>b</sup>	1.33±0.06 <sup>a</sup>	1.33±0.01 <sup>a</sup>
<i>IFN-γ</i>	Spleen	0.91±0.17 <sup>b</sup>	1.48±0.18 <sup>a</sup>	1.51±0.20 <sup>a</sup>
<i>IL-8</i>	Spleen	0.84±0.14 <sup>b</sup>	1.24±0.20 <sup>a</sup>	1.53±0.04 <sup>a</sup>
<i>IL-18</i>	Spleen	1.32±0.07	1.43±0.10	1.54±0.32
<i>IL-1β</i>	Spleen	1.03±0.24 <sup>c</sup>	1.78±0.33 <sup>b</sup>	2.16±0.14 <sup>a</sup>
<i>IL-10</i>	Spleen	1.07±0.20	1.11±0.17	1.09±0.29
<i>IL-12</i>	Spleen	1.19±0.11 <sup>b</sup>	2.72±0.56 <sup>a</sup>	2.38±0.62 <sup>a</sup>
<i>IL-6</i>	Spleen	1.12±0.25	1.87±0.59	2.03±0.90
<i>IL-4</i>	Spleen	1.05±0.48 <sup>a</sup>	0.91±0.25 <sup>ab</sup>	0.60±0.18 <sup>b</sup>
<i>MnSOD</i>	Spleen	1.15±0.03 <sup>b</sup>	1.61±0.11 <sup>a</sup>	1.69±0.14 <sup>a</sup>
<i>TRAF6</i>	Spleen	1.15±0.14 <sup>b</sup>	1.30±0.08 <sup>ab</sup>	1.37±0.16 <sup>a</sup>
<i>NF-κB</i>	Spleen	1.07±0.13 <sup>b</sup>	1.53±0.18 <sup>a</sup>	1.69±0.17 <sup>a</sup>
<i>IL-1β</i>	Thymus	0.97±0.03	0.98±0.08	1.05±0.14
<i>IL-8</i>	Thymus	1.04±0.03 <sup>b</sup>	1.33±0.25 <sup>a</sup>	0.92±0.08 <sup>b</sup>
<i>IL-6</i>	Thymus	1.52±0.04	1.55±0.14	1.48±0.09
<i>TNF-α</i>	Thymus	0.89±0.04	0.91±0.04	0.98±0.04
<i>IL-18</i>	Thymus	1.41±0.09	1.55±0.20	1.62±0.09
<i>IFN-γ</i>	Thymus	0.85±0.09	1.18±0.13	1.07±0.08
<i>IL-12</i>	Thymus	1.03±0.05 <sup>b</sup>	1.73±0.26 <sup>a</sup>	1.68±0.44 <sup>a</sup>
<i>IL-4</i>	Thymus	1.20±0.38	1.44±0.73	1.57±0.36
<i>IL-10</i>	Thymus	0.87±0.06	0.63±0.03	0.91±0.10
<i>MnSOD</i>	Thymus	1.37±0.12	1.44±0.20	1.48±0.18
<i>TRAF6</i>	Thymus	1.39±0.20	1.54±0.14	1.63±0.21
<i>NF-κB</i>	Thymus	1.19±0.13	1.27±0.11	1.22±0.08
<i>IL-1β</i>	Bursa	1.04±0.22	0.97±0.15	1.09±0.06
<i>IFN-γ</i>	Bursa	1.08±0.30	0.91±0.25	1.17±0.21
<i>TNF-α</i>	Bursa	1.18±0.06	1.11±0.04	1.18±0.05
<i>IL-8</i>	Bursa	1.33±1.73	1.28±1.01	1.34±0.65
<i>IL-18</i>	Bursa	1.12±0.30	1.33±0.17	1.45±0.04
<i>IL-12</i>	Bursa	1.16±4.11	1.33±3.12	1.38±0.31
<i>IL-6</i>	Bursa	1.01±0.62 <sup>b</sup>	1.43±1.09 <sup>a</sup>	1.39±0.35 <sup>ab</sup>
<i>IL-10</i>	Bursa	1.04±0.46	1.11±0.29	0.98±0.19
<i>MnSOD</i>	Bursa	1.17±0.08	1.22±0.10	1.23±0.09
<i>TRAF6</i>	Bursa	1.26±0.06	1.32±0.08	1.37±0.10
<i>NF-κB</i>	Bursa	1.31±0.14	1.34±0.05	1.47±0.11

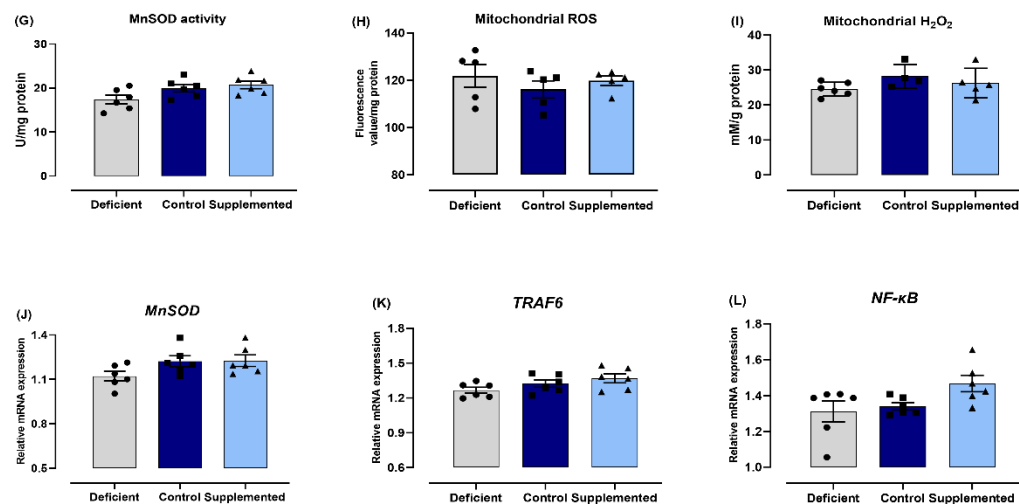
5 Data represent means with standard deviation. <sup>a, b</sup> Mean values with different letters are  
 6 significantly different (one-way ANOVA,  $P < 0.05$ , Tukey's *post hoc* test).

7 *IL*, interleukin; *IFN*, interferon; *TNF*, tumor necrosis factor. *TRAF6*, TNF receptor-associated  
 8 factor 6. *NF-κB*, nuclear transcription factor kappa B; *MnSOD*, manganese superoxide  
 9 dismutase; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

## Thymus



## Bursa



10

11 **Figure S1** Effect of dietary Mn alterations on the activation of redox-sensitive signaling and  
 12 downstream transcription factors in thymus and bursa of birds challenged with *S.*  
 13 *Typhimurium*. (A) and (G) Manganese superoxide dismutase (MnSOD) activity, (B) and (H)  
 14 reactive oxygen species (ROS) level, and (C) and (I) hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) concentration  
 15 were determined in mitochondria isolated from thymus or bursa, respectively. Meanwhile,  
 16 mRNA expression of (D) *MnSOD*, (E) tumor necrosis factor receptor-associated factor 6  
 17 (*TRAF6*), and (F) nuclear factor kappa-B (*NF- $\kappa$ B*) in thymus or bursa were measured by RT-  
 18 PCR, respectively. Data represent means with standard deviation.  $*P < 0.05$ .