Effects of supplemental zinc source and level on antioxidant ability and fat metabolism-related enzymes of broilers[1]

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ABSTRACT The objective of the present study was to investigate the effects of dietary supplemental Zinc (Zn) source and level on antioxidant ability and fat metabolism-related enzymes of broilers. Dietary treatments included the Zn-unsupplemented corn-soybean meal basal diet (control) and basal diets supplemented with 60, 120, or 180 mg Zn/kg as Zn sulfate, Zn amino acid chelate with a weak chelation strength of 6.5 quotient of formation (Q_f) (11.93% Zn) (Zn-AA W), Zn proteinate with a moderate chelation strength of 30.7 Q_f (13.27% Zn) (Zn-Pro M), or Zn proteinate with an extremely strong chelation strength of 944.0 Q_f (18.61% Zn) (Zn-Pro S). The results showed that dietary supplemental Zn increased ($P < 0.01$) Zn contents in the liver, breast, and thigh muscles of broilers, and up-regulated mRNA expressions of copper and Zn containing superoxide dismutase (CuZnSOD) and metallothioneins (MT) in the liver ($P < 0.01$) and thigh muscle ($P < 0.05$), and also enhanced ($P < 0.05$) CuZnSOD activities in the breast and thigh muscles, which exerted antioxidant ability and a decreased malondialdehyde (MDA) level in the liver ($P < 0.01$) and breast and thigh muscles ($P < 0.05$) of broilers. Furthermore, supplemental Zn increased activities of malate dehydrogenase (MDH) and lipoprotein lipase (LPL) in the abdominal fat ($P < 0.05$), and fatty acid synthetase (FAS) and LPL in the liver ($P < 0.01$), which were accompanied with up-regulation ($P < 0.01$) of the mRNA expressions levels of these enzymes in the abdominal fat and liver of broilers. Dietary Zn source, and an interaction between Zn source and level, had no effects on any measurements. It is concluded that dietary Zn supplementation improved Zn status and resulted in promoting antioxidant ability and activities and gene expressions of fat metabolism-related enzymes of broilers regardless of Zn source and level, and the addition of 60 mg Zn/kg to the corn-soybean meal basal diet (a total dietary Zn of approximately 90 mg/kg) was appropriate for improving the above aspects of broilers.

Key words: Zinc, antioxidant ability, fat metabolism-related enzymes, broilers

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INTRODUCTION

Zinc (Zn) is an essential trace element, which plays important roles in various biological activities of animals. Dietary Zn status exerts a powerful influence on the degree of oxidative damage caused by free radicals (Tupe et al., 2010). Exposure of an organism to Zn on a long-term basis, results in an induction of some other substances, such as the metallothioneins (**MT**). Chronic Zn deprivation generally increases sensitivity to some oxidative stresses (Powell, 2000). Supplemental Zn is shown to have increased liver MT content and expressions of copper and Zn containing superoxide dismutase (**CuZnSOD**) activity in piglets (Wang et al., 2012).

Studies in steers showed that supplemental Zn improved carcass traits, including carcass quality grade,

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yield grade, marbling, and backfat (Greene et al., 1988; Malcolm et al., 2000; Spears et al., 2002). Dietary Zn supplementation was reported to increase the intramuscular fat (**IMF**) content in the breast muscle of broilers in our previous study (Liu et al., 2011). Nevertheless, it is not yet known whether dietary Zn supplementation could affect the antioxidant system as well as fat metabolism-related enzymes of broilers.

Organic Zn was reported to be superior to inorganic Zn in improving the carcass traits of steers (Greene et al., 1988; Malcolm et al., 2000), but other investigations did not support this (Spears et al., 2002; Liu et al., 2011). The difference in the results might be attributed to dietary Zn sources and levels. Our previous results indicated that the absorption and utilization of the organic Zn in broilers were closely related to the chelation strengths [defined as the quotient of formation (**Q_f**), which is a quantitative measurement of chelation or complex strength between metal and ligand (Holwerda et al., 1995)] of the organic Zn sources (Huang et al., 2009, 2013; Yu et al., 2010). Zn from the Zn proteinate with a moderate chelation strength of 30.7 Q_f (13.27% Zn) (**Zn-Pro M**) was the most available to broilers, followed by Zn amino acid chelate with a weak chelation strength of 6.5 quotient of formation (Q_f) (11.93% Zn) (**Zn-AA W**) and Zn sulfate (**$ZnSO_4 \cdot 7H_2O$**), while Zn proteinate with an extremely strong chelation strength of 944.0 Q_f (18.61% Zn) (**Zn-Pro S**) was the least available (Huang et al., 2009, 2013). However, it remains unknown whether these 4 Zn sources could differ in affecting the antioxidant system and fat metabolism-related enzymes of broilers. Therefore, the present study focused on the effects of dietary supplemental Zn source and level on the antioxidant ability and fat metabolism-related enzymes of broilers.

Materials and methods

Birds, Diets and Treatments

In this study, a total of 468 commercial one-day-old Arbor Acres male broilers were randomly allotted to 1 of 13 treatments by body weight (6 replicate cages of 6 chicks per cage) in a completely randomized design involving a 4 × 3 factorial arrangement of treatments (4 Zn sources × 3 supplemental Zn levels plus the control with no Zn supplementation). Broilers were raised in electrically heated, temperature-controlled cages with fiberglass feeders and water troughs and a 24 hour constant-light schedule. The experiment lasted for a total of 42 days. Body weight and feed intake were recorded at the end of every 3 weeks. Birds were allowed ad libitum access to experimental diets and tap water containing 106 μg Ca/mL, 72.7 μg Mg/mL, 0 μg Cu/mL, 0 μg Fe/mL, 0.0029 μg Mn/mL, and 0.833 μg Zn/mL. Birds were managed according to guidelines approved by Arbor Acres Farm in Beijing.

Table 1. Composition of the basal diets for broilers.

Ingredient[1] (% unless noted)	Starter (days 1∼21)	Grower (days 22∼42)
Ground yellow corn	53.00	57.72
Corn starch	0.50	0.50
Soybean meal	39.80	35.10
Soybean oil	2.70	3.50
Calcium monohydrogen phosphate	1.94	1.24
Ground limestone	1.20	1.34
Salt	0.30	0.30
D, L-Methionine	0.20	0.07
Micronutrients[2]	0.36	0.23
Nutrient composition[1], %		
DM[3]	89.80	89.50
ME, MJ/kg	12.41	12.83
CP[3]	21.38	20.79
Lysine	1.19	1.08
Methionine	0.53	0.38
Methionine + cysteine	0.89	0.72
Ca[3]	1.02	0.92
Nonphytate P	0.48	0.35
Mn[3], mg/kg	126	124
Fe[3], mg/kg	256	242
Cu[3], mg/kg	17.10	16.20
Zn[3], mg/kg	35.55	32.79

[1]Ingredient and nutrient composition are reported on an as-fed basis.

[2]For starter diets, provided per kg of diet: vitamin A (as all-trans retinol acetate), 15,000 IU; cholecalciferol, 4,500 IU; vitamin E (as all-rac-alpha-tocopherol acetate), 22.5 IU; vitamin K (as menadione sodium bisulfate), 3.0 mg; thiamin (as thiamin mononitrate), 3.0 mg; riboflavin, 10.5 mg; vitamin B_6, 3.0 mg; vitamin B_{12}, 0.015 mg; calcium pantothenate, 15 mg; niacin, 39 mg; folic acid, 1.5 mg; biotin, 0.189 mg; choline (as choline chloride), 700 mg; Cu ($CuSO_4 \cdot 5H_2O$), 8 mg; Fe ($FeSO_4 \cdot H_2O$), 80 mg; Mn ($MnSO_4 \cdot 7H_2O$), 100 mg; I (KI), 0.35 mg; Se (Na_2SeO_3), 0.15 mg.

For grower diets, provided per kilogram of diet: vitamin A (all-trans-retinol acetate), 10,000 IU; cholecalciferol, 3,000 IU; vitamin E (all-rac-α-tocopherol acetate), 15 IU; vitamin K (menadione sodium bisulfate), 2.0 mg; thiamin (thiamin mononitrate), 2.0 mg; riboflavin, 7.0 mg; vitamin B_6, 2.0 mg; vitamin B_{12}, 0.010 mg; calcium pantothenate, 10 mg; niacin, 26 mg; folic acid, 1.0 mg; biotin, 0.126 mg; choline (choline chloride), 500 mg; Cu ($CuSO_4 \cdot 5H_2O$), 8 mg; Fe ($FeSO_4 \cdot H_2O$), 80 mg; Mn ($MnSO_4 \cdot 7H_2O$), 100 mg; I (KI), 0.35 mg; Se (Na_2SeO_3), 0.15 mg.

[3]Determined by analysis. Each value based on duplicate determinations.

The basal corn-soybean meal diets (Table 1) were formulated to meet or exceed the requirements for starter and grower broilers (NRC, 1994) except for Zn. Dietary treatments included the basal diets supplemented with 0, 60, 120, or 180 mg Zn/kg as $ZnSO_4 \cdot 7H_2O$, Zn-AA W (Zinpro Corp., Eden Prairie, MN), Zn-Pro M (Fenyahua Bioengineering Co., Changzhi, P. R. China), or Zn-Pro S (Alltech Inc., Nicholasville, KY). All organic Zn sources used in the current study are the same as those in the study of Liang et al. (2008), and their Q_f values and Zn concentrations were determined by Liang et al. (2008). These organic Zn sources were obtained from independent distributors rather than directly from the product manufacturers. Lysine or methionine levels in each treatment were balanced by supplementation of additional lysine or methionine from supplemental organic Zn sources, and added to each dietary treatment through corn starch. Each Zn source was mixed with corn starch to the same weight and then mixed with

Table 2. Added and analyzed Zn concentrations in diets for broilers.[1]

Treatment	Added Zn (mg/kg)	Analyzed dietary Zn (mg/kg)	
		Days 1~21[1]	Days 22~42[1]
Control	0	35.6	32.8
$ZnSO_4.7H_2O$[2]	60	99.3	98.4
	120	160.2	160.3
	180	212.4	212.3
Zn-AA W[2]	60	91.1	92.8
	120	154.2	152.4
	180	209.1	210.3
Zn-Pro M[2]	60	92.8	91.5
	120	150.3	149.9
	180	211.1	210.1
Zn-Pro S[2]	60	91.1	90.4
	120	150.3	150.1
	180	210.9	210.2

[1]See Table 1 for the composition of the basal diets. Values based on chemical analysis of triplicate samples of diets, and reported on an as-fed basis.

[2]$ZnSO_4 \bullet 7H_2O$ = Zn sulfate; Zn-AA W = Zn amino acid chelate with a weak chelation strength of 6.5 quotient of formation (Q_f) (11.93% Zn, Zinpro Corp., Eden Prairie, MN); Zn-Pro M = Zn proteinate with a moderate chelation strength of 30.7 Q_f (13.27% Zn, Fenyahua Bioengineering Co., Changzhi, P. R. China); Zn-Pro S = Zn proteinate with a nearly extremely strong chelation strength of 944.0 Q_f (18.61% Zn, Alltech Inc., Nicholasville, KY). All organic Zn sources used in the present study and their Q_f values and Zn concentrations were determined by Liang et al. (2008). These organic Zn sources were obtained from independent distributors rather than directly from the product manufacturers.

each aliquot of the basal diet. Added and analyzed Zn concentrations are presented in Table 2.

Sample Collections and Analyses

On day 42 of the experiment, 2 broilers were selected from each cage according to the average BW within the cage following a 12 hour fasting, were weighed individually and slaughtered according to the welfare of animal slaughter regulations of China. All experimental procedures were approved by the Animal Research Center at the Veterinarian Office of Beijing. Broilers were killed by cervical dislocation. About 2 g pectoralis major and thigh muscles and liver were removed and immediately frozen in liquid nitrogen for assays of MT and malondialdehyde (**MDA**) contents and the activities of copper-zinc superoxide dismutase (**CuZnSOD**), malate dehydrogenase (**MDH**), hormone sensitive lipase (**HSL**), lipoprotein lipase (**LPL**) and fatty acid synthetase (**FAS**) and the gene expressions *MT, CuZnSOD, MDH, LPL and FAS*. About 10 g pectoralis major and thigh muscles and liver were removed and frozen at -20°C for testing Zn contents.

Zn Concentrations Zn concentrations in diets, water, and tissues were determined by inductively coupled argon plasma emission spectroscopy (Model IRIS Intrepid II, Thermal Jarrell Ash, Waltham, MA) after wet digestion with HNO_3 and $HClO_4$ as described by Huang et al. (2009). Validation of the mineral analysis was conducted using bovine liver powder [GBW (E) 080193, National Institute of Standards and Technology, Beijing, China] as a standard reference material.

Enzyme Activities Activities of CuZnSOD and LPL were analyzed using analysis kits (A001–2 and A067; Nanjing Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's directions. The MDH activity was determined as described by Xu et al. (2003), and the activity of HSL in abdominal fat measured as described by Lu et al. (2006). The FAS activity in the liver was determined as described by Kim and Elson (1981).

Malondialdehyde Contents Malondialdehyde (MDA) contents in liver, breast or thigh muscles were determined with analysis kit (A003–1; Nanjing Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's directions.

MT Concentration Metallothioneins concentration in liver was determined by ^{109}Cd-hemoglobin affinity assay (Eaton and Toal, 1982) with inductively coupled argon plasma emission spectroscopy (Model IRIS Intrepid II, Thermal Jarrell Ash, Waltham, MA). The concentration of MT was calculated using a ^{109}Cd-MT binding stoichiometry of 6:1.

Real-Time PCR The total RNA in muscle and liver were isolated using Trizol reagent (15596–026, Invitrogen, Carlsbad, CA) according to the manufacture instructions. Concentrations of total RNA were measured with Nano Drop ND-1000 spectrofluorometer (Nano Drop Technologies, Wilmington, DE). Reverse transcription was performed using the Super Script First-Strand Synthesis System (Invitrogen, Carlsbad, CA), and 1 μg of total RNA was subjected to reverse transcription. Real-time PCR reactions were performed on an ABI7500 real-time PCR system using SYBR-Green PCR Master Mix (Applied Biosystems, Foster City, CA). The details of the primers are listed in Table 3. Every gene was amplified independently in triplicate within a single instrument run. The amplification efficiency of every gene was determined by the standard curve method with 5-fold serial dilutions of cDNA. Relative mRNA expressions of genes were calculated by the $2^{-\triangle\triangle ct}$ method reported by Livak and Schmittgen (2001), and β-actin was chosen as reference to normalize the relative levels of gene expressions.

Statistical Analyses

Statistical analyses of data were performed using SAS software (Release 8.1, SAS Inst. Inc., Cary, NC). Data were analyzed by 2-way ANOVA using the General Linear Models procedure. The model included main effects of Zn source, supplemental Zn level, and their interaction. Cage was the experimental unit. Differences among means were tested by the least significant difference method and $P<0.05$ was considered to be statistically significant.

Table 3. Primer sequences of chicken β-actin, copper-zinc superoxide dismutase (CuZn-SOD), metallothioneins (MT), fatty acid synthetase (FAS), malate dehydrogenase (MDH) and lipoprotein lipase (LPL).

Gene	Genbank Accession	Primer (5'-3')	Product (bp)	Tm (°C)[1]
β-actin	NM_205518.1	F: GAGAAATTGTGCGTGACATCA R: CCTGAACCT CTCATTGCCA	152	60
CuZnSOD	NM_205064	F: GGAGGAGTGGCAGAAGT R: TAAACGAGGTCCAGCAT	159	55
MT	NM_205275.1	F: GCAACAACTGTGCCAAGGGC R: TTTCGTGGTCCCTGTCACCC	138	61
FAS	J03860	F: AGCGTGCTATGCTTGCC R: GTCCGTGACGAATTGCTTTAT	129	60
MDH	XM_419350	F: AAAGACTGTGAAGGTGGTAG R: ATCCAAGCGAGTCAAGC	114	56
LPL	NM_205282	F: TACAGTCTGGGTGCTCAT R: GCATACTCAAAGGTGGG	104	56

[1]Annealing temperature.

Table 4. Effects of dietary zinc source and level on growth performance of broilers.

Zn source	Added Zn level (mg/kg)	ADFI (g)	ADG (g)	F/G (g/g)
Control[1]	0	98.6	54.2	1.72
$ZnSO_4.7H_2O$[1]	60	102.0	57.2	1.69
	120	101.8	56.9	1.69
	180	101.6	57.1	1.69
Zn-AA W[1]	60	100.5	55.3	1.73
	120	102.5	57.3	1.69
	180	99.7	55.4	1.71
Zn-Pro M[1]	60	102.7	57.2	1.70
	120	103.8	57.2	1.71
	180	103.8	57.7	1.70
Zn-Pro S[1]	60	102.5	55.8	1.74
	120	102.8	56.8	1.72
	180	105.8	58.6	1.71
Pooled SE		2.35	1.36	0.016
Zn source[2]	$ZnSO_4.7H_2O$	101	56.4	1.70
	Zn-AA W	100	55.6	1.71
	Zn-Pro M	102	56.6	1.71
	Zn-Pro S	102	56.3	1.72
Pooled SE		1.17	0.678	0.008
Zn level	0[1]	98.6[a]	54.2[a]	1.72
	60[3]	101.9[b]	56.4[b]	1.71
	120[3]	102.7[b]	57.0[b]	1.70
	180[3]	102.7[b]	57.2[b]	1.70
Pooled SE		1.17	0.678	0.008
			P-value[4]	
Source		0.538	0.732	0.230
Level		0.043	0.008	0.384
Source × level		0.979	0.946	0.854

[a,b]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Each value represents the mean of 6 replicate cages with 6 chicks per cage (n = 6).

[2]Each value represents the mean of 18 replicate cages with 6 chicks per cage (n = 18).

[3]Each value represents the mean of 24 replicate cages with 6 chicks per cage (n = 24).

[4]Probability values for main effects of ANOVA.

Results

Growth Performance

The broilers were healthy and normal throughout the experiment with a mortality of <2% that was unrelated to dietary treatment. The ADFI, ADG, and feed conversion efficiency (F/G = feed intake/body weight gain) of birds were not affected by Zn source or an interaction between Zn source and supplemental Zn level, however, supplemental Zn level affected ($P < 0.05$) ADFI and ADG, but not F/G (Table 4). Compared to the control, the additions of 60, 120, or 180mg Zn/kg increased

Table 5. Effects of dietary Zn source and level on Zn contents and antioxidant ability in liver and breast and thigh muscles of broilers at 42 days of age.

		Liver				Breast muscle			Thigh muscle		
Zn source	Added Zn level (mg/kg)	Zn content (mg/kg)	CuZnSOD (NU/mgprot)	MDA (nmol/mgprot)	MT (mmol/mgprot)	Zn content (mg/kg)	CuZnSOD (NU/mgprot)	MDA (nmol/mgprot)	Zn content (mg/kg)	CuZnSOD (NU/mgprot)	MDA (nmol/mgprot)
Control[1]	0	25.3	205	1.402	0.321	5.13	12.7	1.33	11.0	15.0	1.73
$ZnSO_4.7H_2O$[1]	60	28.7	211	1.140	0.415	5.52	13.8	1.22	13.2	15.3	1.59
	120	28.1	227	0.816	0.457	5.64	14.4	1.27	15.6	17.3	1.67
	180	27.8	218	0.865	0.328	5.58	14.7	1.33	14.1	16.6	1.60
Zn-AA W[1]	60	26.4	220	0.710	0.449	5.36	13.4	1.24	13.7	17.0	1.69
	120	29.8	209	0.860	0.630	5.55	13.6	1.12	13.8	16.2	1.45
	180	31.0	215	0.816	0.628	5.46	12.9	1.19	13.0	16.4	1.25
Zn-Pro M[1]	60	27.4	225	0.858	0.466	5.41	13.3	1.18	13.9	17.0	1.62
	120	30.3	223	0.984	0.466	5.44	14.0	1.09	13.7	16.1	1.22
	180	29.0	218	1.054	0.382	5.43	14.8	1.16	14.1	16.3	1.55
Zn-Pro S[1]	60	26.5	214	0.901	0.639	5.30	14.8	1.16	14.1	16.5	1.43
	120	26.3	212	0.813	0.472	5.50	13.9	1.08	13.2	16.2	1.42
	180	32.5	224	1.036	0.647	5.48	14.5	1.06	13.9	16.7	1.34
Pooled SE		1.330	8.96	0.099	0.117	0.119	0.750	0.111	0.692	0.869	0.160
Zn source[2]	$ZnSO_4.7H_2O$	27.5	215	1.056	0.380	5.47	13.9	1.22	13.5	16.0	1.65
	Zn-AA W	28.1	212	0.947	0.507	5.38	13.1	1.22	12.9	16.1	1.53
	Zn-Pro M	28.0	217	1.074	0.409	5.35	13.7	1.19	13.2	16.1	1.53
	Zn-Pro S	27.6	213	1.038	0.520	5.35	14.0	1.16	13.0	16.1	1.48
Pooled SE		0.666	4.49	0.050	0.059	0.060	0.375	0.056	0.346	0.434	0.080
Zn level	0[1]	25.3[a]	205	1.402[a]	0.321	5.13[a]	12.7[a]	1.33[a]	11.0[a]	15.0[a]	1.73[a]
	60[3]	27.3[b]	217	0.902[b]	0.492	5.40[b]	13.8[b]	1.20[b]	13.7[b]	16.4[b]	1.58[b]
	120[3]	28.6[b]	218	0.868[b]	0.506	5.53[b]	13.9[b]	1.14[b]	14.1[b]	16.5[b]	1.44[b]
	180[3]	30.1[b]	219	0.943[b]	0.496	5.49[b]	14.2[b]	1.12[b]	13.8[b]	16.5[b]	1.43[b]
Pooled SE		0.666	4.48	0.050	0.059	0.060	0.375	0.056	0.434	0.434	0.080
		P-value[4]									
Source		0.914	0.869	0.285	0.244	0.479	0.426	0.999	0.624	0.999	0.502
Level		0.001	0.075	0.001	0.078	0.001	0.039	0.042	0.001	0.042	0.033
Source × level		0.120	0.906	0.230	0.801	0.996	0.894	0.905	0.5604	0.905	0.711

[a,b]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Each value represents the mean of 6 replicate cages with 6 chicks per cage (n = 6).

[2]Each value represents the mean of 18 replicate cages with 6 chicks per cage (n = 18).

[3]Each value represents the mean of 24 replicate cages with 6 chicks per cage (n = 24).

[4]Probability values for main effects of ANOVA.

Table 6. Effects of dietary Zn source and level on *CuZnSOD* and *MT* mRNA levels in liver and breast and thigh muscles of broilers at 42 days of age.

Zn source	Added Zn level(mg/kg)	Liver		Breast muscle		Thigh muscle	
		CuZnSOD (RQ)[5]	*MT* (RQ)[5]	*CuZnSOD* (RQ)[5]	*MT* (RQ)[5]	*CuZnSOD* (RQ)[5]	*MT* (RQ)[5]
Control[1]	0	1.17	0.920	1.02	0.993	1.04	1.11
$ZnSO_4.7H_2O$[1]	60	1..89	1.643	1.18	1.012	1.18	1.07
	120	1.86	1.946	1.39	1.650	1.1	1.25
	180	1.67	1.820	1.17	1.323	1.39	1.21
Zn-AA W[1]	60	2.02	1.465	1.17	1.465	1.07	1.10
	120	1.58	1.508	1.64	1.208	1.47	1.49
	180	1.86	1.576	1.27	1.257	1.40	1.57
Zn-Pro M[1]	60	1.59	1.772	1.06	1.135	1.45	1.31
	120	2.10	1.763	1.16	1.427	1.37	1.44
	180	1.72	1.483	1.17	1.032	1.24	1.13
Zn-Pro S[1]	60	1.72	1.823	1.24	1.232	1.44	1.36
	120	1.90	1.500	1.02	0.927	1.51	1.52
	180	1.84	1.467	1.18	1.147	1.12	1.39
Pooled SE		0.182	0.220	0.141	0.172	0.151	0.156
Zn source[2]	$ZnSO_4.7H_2O$	1.64	1.58	1.19	1.240	1.18	1.16
	Zn-AA W	1.66	1.37	1.27	1.230	1.25	1.32
	Zn-Pro M	1.64	1.48	1.10	1.150	1.27	1.25
	Zn-Pro S	1.66	1.43	1.11	1.070	1.28	1.35
Pooled SE		0.091	0.111	0.071	0.086	0.076	0.078
Zn level	0[1]	1.17[a]	0.920[a]	1.02	0.993	1.04[a]	1.11[a]
	60[3]	1.81[b]	1.675[b]	1.16	1.211	1.28[b]	1.21[b]
	120[3]	1.86[b]	1.679[b]	1.30	1.303	1.36[b]	1.42[b]
	180[3]	1.77[b]	1.587[b]	1.20	1.190	1.29[b]	1.33[b]
Pooled SE		0.091	0.111	0.071	0.086	0.076	0.078
				P-value[4]			
Source		0.999	0.513	0.294	0.471	0.756	0.346
Level		0.001	0.001	0.051	0.084	0.023	0.035
Source × level		0.514	0.910	0.512	0.206	0.338	0.749

[a,b]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Each value represents the mean of 6 replicate cages with 6 chicks per cage (n = 6).

[2]Each value represents the mean of 18 replicate cages with 6 chicks per cage (n = 18).

[3]Each value represents the mean of 24 replicate cages with 6 chicks per cage (n = 24).

[4]Probability values for main effects of ANOVA.

[5]The mRNA abundances of enzymes were calculated as the relative quantities (RQ) of enzyme mRNA to β-actin mRNA; $RQ = 2^{-\Delta\Delta Ct}$ (Ct = threshold cycle).

the ADFI ($P < 0.05$) and ADG ($P < 0.01$) of broilers. However, no differences were detected among all supplemental Zn levels.

Zn Contents

Dietary Zn level affected ($P < 0.01$) Zn contents in the liver, breast and thigh muscles of broilers, while no effects of dietary Zn source and an interaction between Zn source and level were observed (Table 5). Compared to the control, dietary supplemental Zn increased ($P < 0.01$) the Zn contents in liver and breast and thigh muscles with no difference among supplemental Zn levels. Liver Zn content was about twice of that in the thigh muscle, which was about twice of that in the breast muscle.

Antioxidant Ability

The data are presented in Tables 5 and 6. Dietary Zn level affected the CuZnSOD activities in the breast and thigh muscles ($P < 0.05$) and MDA contents in the liver ($P < 0.01$) and the breast and thigh muscles ($P < 0.05$), and had no effect on CuZnSOD activity and MT content in the liver, while dietary Zn source and an interaction between Zn source and level had no effects on the above indices (Table 5). Compared to the control, dietary supplemental Zn increased ($P < 0.05$) CuZnSOD activities in breast and thigh muscles, and decreased MDA contents in the liver ($P < 0.01$) and the breast and thigh muscles ($P < 0.05$). No differences in the above parameters were observed among supplemental Zn levels.

Similarly, dietary Zn level had an effect on the mRNA levels of CuZnSOD and MT in the liver ($P < 0.01$) and thigh muscle ($P < 0.05$), and had no effect on the mRNA levels of CuZnSOD and MT in the breast muscle of broilers, while dietary Zn source and an interaction between Zn source and level did not affect the above indices (Table 6). Compared to the control, dietary supplemental Zn up-regulated the mRNA levels of *CuZnSOD* and *MT* in the liver ($P < 0.01$) and thigh muscle ($P < 0.05$) with no differences among supplemental Zn levels.

Table 7. Effects of dietary Zn source and level on some fat metabolism-related enzyme activities in liver and abdominal fat of broilers at 42 days of age.

Zn source	Added Zn level (mg/kg)	Liver			Abdominal fat		
		FAS (U/mgprot)	MDH (U/mgprot)	LPL (U/mgprot)	MDH (U/mgprot)	LPL (U/mgprot)	HSL (U/mgprot)
Control[1]	0	0.317	5.75	0.517	2.83	3.11	1.105
$ZnSO_4.7H_2O$[1]	60	0.469	6.25	0.616	3.28	3.85	1.284
	120	0.503	5.75	0.592	3.94	3.45	1.114
	180	0.549	5.35	0.605	3.23	3.45	1.122
Zn-AA W[1]	60	0.455	5.82	0.784	3.52	3.38	0.944
	120	0.523	5.26	0.611	3.05	3.62	0.939
	180	0.418	5.76	0.651	3.04	3.60	1.302
Zn-Pro M[1]	60	0.459	6.52	0.744	3.05	3.39	1.005
	120	0.473	6.24	0.613	3.63	4.02	1.495
	180	0.497	6.16	0.654	3.73	3.54	0.901
Zn-Pro S[1]	60	0.508	6.25	0.598	3.83	3.61	1.113
	120	0.550	5.80	0.607	3.76	3.73	1.470
	180	0.453	6.17	0.652	3.78	3.68	1.341
Pooled SE		0.0411	0.325	0.0539	0.352	0.332	0.265
Zn source[2]	$ZnSO_4.7H_2O$	0.460	5.77	0.58	3.32	3.47	1.16
	Zn-AA W	0.428	5.65	0.64	3.11	3.43	1.07
	Zn-Pro M	0.437	6.17	0.63	3.31	3.51	1.13
	Zn-Pro S	0.457	5.99	0.59	3.55	3.53	1.26
Pooled SE		0.0206	0.163	0.0269	0.176	0.166	0.133
Zn level	0[1]	0.317[a]	5.75	0.517[a]	2.83[a]	3.11[a]	1.110
	60[3]	0.473[b]	6.21	0.686[b]	3.42[b]	3.56[b]	1.090
	120[3]	0.512[b]	5.76	0.606[b]	3.60[b]	3.70[b]	1.250
	180[3]	0.479[b]	5.86	0.640[b]	3.45[b]	3.57[b]	1.170
Pooled SE		0.492	0.347	0.0269	0.176	0.166	0.133
				P-value[4]			
Source		0.643	0.117	0.348	0.376	0.471	0.797
Level		0.001	0.160	0.001	0.015	0.014	0.808
Source × level		0.646	0.802	0.724	0.696	0.975	0.830

[a, b]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Each value represents the mean of 6 replicate cages with 6 chicks per cage (n = 6).

[2]Each value represents the mean of 18 replicate cages with 6 chicks per cage (n = 18).

[3]Each value represents the mean of 24 replicate cages with 6 chicks per cage (n = 24).

[4]Probability values for main effects of ANOVA.

Fat Metabolism-Related Enzymes

The data are presented in Tables 7 and 8. Dietary Zn level influenced FAS and LPL activities in the liver ($P < 0.01$) and MDH and LPL activities in the abdominal fat ($P < 0.05$) of birds, and had no effect on MDH activity in the liver and HSL activity in the abdominal fat, while no effects of dietary Zn source and an interaction between Zn source and level were observed (Table 7). Compared with the control, dietary supplemental Zn increased FAS and LPL activities in the liver ($P < 0.01$) and MDH and LPL activities in the abdominal fat ($P < 0.05$), with no differences among supplemental Zn levels.

Similarly, dietary Zn level had an effect on the mRNA abundances of *FAS* and *LPL* in the liver ($P < 0.01$) and *MDH* and *LPL* in abdominal fat ($P < 0.01$), while dietary Zn source and an interaction between Zn source and level did not affect the above indices (Table 8). Dietary supplemental Zn increased ($P < 0.01$) the mRNA abundances of *FAS* and *LPL* in liver, and *MDH* and *LPL* in abdominal fat compared with the control. No differences in the above parameters were observed among supplemental Zn levels.

DISCUSSION

Zn is essential for organisms and participates in several of the pathways of metabolism. Tissue mineral concentrations are indicators of body mineral storage and status, and have been used in studies of mineral requirements and bioavailabilities (Wedekind et al., 1992; Yan and Waldroup, 2006). Our data indicated that dietary Zn supplementation increased the Zn contents in the liver and breast and thigh muscles, which contributed to the improvement of the Zn status of broilers.

MT is an important maintainer of the Zn pool of the organisms, and plays a protective role in antioxidant responses by scavenging free radicals, particularly the hydroxyl radical (Ruttkay-Nedecky et al., 2013). Zn is necessary for the structure and function of CuZnSOD, which comprised 90% of the total SOD and protects tissues from oxidative damage (Noor et al., 2002). Dietary Zn was reported to increase liver Zn, MT content, and CuZnSOD activity in piglets (Wang et al., 2012). Tissue concentration, especially in bone, increased with dietary Zn content (Henry et al., 1987, Huang et al., 2007). In our study,

Table 8. Effects of dietary Zn source and level on gene expressions of some fat metabolism-related enzymes in liver and abdominal fat of broilers at 42 days of age.

Zn source	Added Zn level (mg/kg)	Liver		Abdominal fat	
		FAS mRNA (RQ)[5]	LPL mRNA (RQ)[5]	MDH mRNA (RQ)[5]	LPL mRNA (RQ)[5]
Control[1]	0	0.840	1.12	0.967	1.04
$ZnSO_4.7H_2O$[1]	60	1.242	1.80	1.310	1.29
	120	1.358	1.61	1.328	1.38
	180	1.410	1.33	1.385	1.29
Zn-AA W[1]	60	1.093	1.58	1.090	1.44
	120	1.148	1.62	1.210	1.27
	180	1.258	1.28	1.153	1.38
Zn-Pro M[1]	60	1.235	1.76	1.252	1.40
	120	1.437	1.93	1.223	1.31
	180	1.353	1.73	1.190	1.11
Zn-Pro S[1]	60	1.278	1.47	1.253	1.15
	120	1.505	1.86	1.333	1.34
	180	1.340	1.49	1.085	1.34
Pooled SE		0.168	0.193	0.119	0.121
Zn source[2]	$ZnSO_4.7H_2O$	1.21	1.46	1.25	1.25
	Zn-AA W	1.09	1.40	1.11	1.28
	Zn-Pro M	1.22	1.64	1.16	1.22
	Zn-Pro S	1.24	1.49	1.16	1.22
Pooled SE		0.0843	0.0965	0.0595	0.0607
Zn level	0[1]	0.840[a]	1.12[a]	0.967[a]	1.04[a]
	60[3]	1.212[b]	1.65[b]	1.226[b]	1.32[b]
	120[3]	1.362[b]	1.76[b]	1.274[b]	1.32[b]
	180[3]	1.340[b]	1.46[b]	1.203[b]	1.28[b]
Pooled SE		0.0843	0.0965	0.0595	0.0607
			P-value[4]		
Source		0.559	0.370	0.404	0.839
Level		0.001	0.001	0.002	0.003
Source × level		0.996	0.886	0.943	0.745

[a,b]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Each value represents the mean of 6 replicate cages with 6 chicks per cage (n = 6).

[2]Each value represents the mean of 18 replicate cages with 6 chicks per cage (n = 18).

[3]Each value represents the mean of 24 replicate cages with 6 chicks per cage (n = 24).

[4]Probability values for main effects of ANOVA.

[5]The mRNA abundances of enzymes were calculated as the relative quantities (RQ) of enzyme mRNA to β-actin mRNA; $RQ = 2^{-\Delta\Delta Ct}$ (Ct = threshold cycle).

the linear relationship between dietary Zn level and tissue Zn contents has not been observed, which might be attributed to higher levels of dietary Zn supplementation. Consistently, in the present study, supplemental Zn increased the MT contents and CuZnSOD activities in the breast and thigh muscles of broilers as well as up-regulated the mRNA expressions of *MT* and *CuZnSOD* in the liver and thigh muscle. It was suggested that Zn additions to the corn-soybean meal diets could increase the antioxidant ability of broilers. This could explain why dietary Zn supplementation decreased drip loss in the breast and thigh muscles of broilers, which was shown in our previous study (Liu et al., 2011).

Lipid oxidation is a major cause of quality deterioration in meat and gives rise to rancidity and the formation of undesirable odors and flavors (Gray et al., 1996). MDA is a soluble degraded product of lipids and widely used to reflect the extent of lipid oxidation of meat (Raharjo and Sofos, 1993). We could speculate that dietary Zn might strengthen the oxidative defenses and decrease the MDA content of muscles, which would contribute to higher quality and longer shelf life of meat.

FAS, LPL, MDH, and HSL are the key enzymes involved in fat metabolism (Belfrage et al., 1984; Shen et al., 1991; Mersmann, 1998; Sato et al., 1999). Retrospective studies suggested that Zn supplementation results in an improvement in the yield grade, marbling score, and internal fat of steers (Greene et al., 1988; Malcolm et al., 2000; Spears et al., 2002). Supplemental Zn increased IMF accumulation in pigs by up-regulating intramuscular lipogenic and fatty acid transport genes (*ACC*, *FAS*, *SCD-1*, and *SREBP-1*) expression and enzyme activities while down-regulating lipolytic genes (*HSL* and *CPT-1*) expression and enzyme activities (Zhang et al., 2014). Our previous study showed that supplemental Zn increased IMF content in the breast muscle (Liu et al., 2011). In the present study, supplemental Zn in the diets elevated the activities of FAS, LPL, and MDH, and up-regulated the expressions of these lipogenic enzymes. It was suggested that supplemental Zn has a powerful impact on the activities and gene expressions of the above enzymes

involved in fat metabolism, and increases the IMF content in the breast muscles of broilers, as shown in our previous study (Liu et al., 2011).

CONCLUSIONS

Our results indicate that dietary Zn supplementation improved Zn status, antioxidant ability, and fat metabolism-related enzymes of broilers. However, there were no significant differences among either the 4 Zn sources or supplemental Zn levels. Supplemental 60 mg Zn/kg in the corn-soybean meal basal diet (a total dietary Zn of about 90 mg/kg) was adequate for improving the above aspects of broilers.

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