

Effects of Coated Trace Minerals and the Fat Source on Growth Performance, Antioxidant Status, and Meat Quality in Broiler Chickens

Dafei Yin¹, Tiejin Tong¹, Amy F. Moss³, Ruiyang Zhang¹, Yinggu Kuang²,
Yong Zhang¹, Fangfang Li¹ and Yujing Zhu¹

¹ College of Animal Husbandry and Veterinary Medicine, Shenyang Agricultural University, 110866 Shenyang, China

² Fujian Syno Biotech Co., Ltd., 350700 Fuzhou, China

³ University of New England, Armidale, NSW 2350, Australia

Inorganic trace minerals may exacerbate lipid peroxidation, thereby impacting lipid metabolism. This study aimed to compare the effects of inorganic and coated trace minerals in diets with different fat sources, on the performance, slaughter characteristics, and antioxidant status of broiler chickens. A total of 576 21-day-old Arbor Acres broiler birds were randomly divided into four dietary treatment groups in a 2 (non-coated and coated trace minerals) × 2 (soybean oil and lard) factorial design. Each treatment was replicated 12 times (12 birds per replicate). The results showed that coated minerals significantly improved the average daily gain (ADG) in weight and the feed conversion ratio ($P < 0.01$), increased serum iron, zinc, selenium, and thyroxine contents, increased the activities of glutathione peroxidase, superoxide dismutase, total antioxidant capacity, and lipoprotein lipase ($P < 0.05$), and decreased the serum and muscle malondialdehyde (MDA) contents ($P < 0.01$). The use of soybean oil as the fat source resulted in a high ADG in weight, a low F/G ratio, reduced serum MDA content, and drip loss of breast and leg muscles ($P < 0.05$). In conclusion, the supplementation of coated trace minerals improved growth performance, antioxidant status, trace mineral retention within serum, and lipid metabolism. Additionally, soybean oil also improved the growth performance, antioxidant performance, and meat quality of broilers. The combination of coated trace minerals and soybean oil generated the best growth performance, antioxidant status, and meat quality characteristics.

Key words: antioxidant status, broiler, fat source, meat quality, performance, trace minerals

J. Poult. Sci., 59: 56–63, 2022

Introduction

Trace minerals are essential nutrients for livestock. They play critical roles in the functioning of enzymes, hormones, and cells. They are involved in several physiological, digestive, and biosynthetic processes within the body. Traditionally, inorganic trace minerals are supplemented to meet minimum mineral requirements for optimal growth (Bao *et al.*, 2007). However, inorganic trace minerals are hygroscopic and reactive, meaning they may catalyze lipid oxidation, and their bioavailability within the poultry gut is low due to dietary antagonisms (Underwood and Suttle,

1966). Trace mineral deficiency may be associated with oxidative stress and poor meat quality (Bai *et al.*, 2019; Dubey *et al.*, 2020). However, supplementation with high levels of inorganic trace minerals may be harmful to the environment, for example, phosphorus (P) pollution from pig and poultry production (Selle *et al.*, 2000). Recently, the use of coated trace mineral sources in monogastric diets has been proposed to improve the adverse effects caused by inorganic trace minerals. Differing from traditional inorganic or organic trace minerals, there is no chemical link between encapsulated material and core metal ions. Lu *et al.* (2020) reported that coating trace minerals in a matrix of carbohydrates protected the metal ions by isolating them from the environment and reduced the need for mineral feed supplementation, thereby reducing mineral excretion without jeopardizing performance. Coated zinc (Zn) oxide added to pig diets resulted in higher intestinal absorption of Zn and improved their performance compared to those offered native Zn oxide during *Escherichia coli* challenge (Kwon *et al.*, 2014; Shen *et al.*, 2014). Cheaper commercial encapsulated material is also advantageous for decreasing feed cost (Lu *et*

Received: November 4, 2020, Accepted: February 17, 2021

Released Online Advance Publication: April 25, 2021

Correspondence: Associate Professor Yujing Zhu, College of Animal Husbandry and Veterinary Medicine, Shenyang Agricultural University, China. (E-mail: zhuyujing@syau.edu.cn)

The Journal of Poultry Science is an Open Access journal distributed under the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License. To view the details of this license, please visit (<https://creativecommons.org/licenses/by-nc-sa/4.0/>).

al., 2020). To the best of our knowledge, only a few poultry studies have been conducted to determine the equivalence of inorganic and coated trace mineral sources. Thus, the ability of coated trace minerals to improve mineral bioavailability and meat quality in poultry as well as reduce environmental pollution, remains largely unexplored. We hypothesized that coated trace mineral supplementation in broiler diets may improve mineral retention and growth performance.

Apart from trace mineral bioavailability, the bioavailability of lipids is also important for the metabolism and physiology of animals. Fats provide a concentrated source of energy, which is useful in the formulation of modern energy-dense broiler diets. However, the dietary addition of fats, in combination with inorganic minerals, may lead to lipid peroxidation and poor meat quality (Chan, 1987). Manganese (Mn), Zn, and copper (Cu) are essential micronutrients incorporated into the regulatory pathways of oxidative stress. Thus, there may be a relationship between these trace elements and dietary lipid content that could manipulate the level of oxidative stress. A diverse array of fats and oils, including vegetable oils (e.g., soybean, corn, and palm oils) and rendering by-products (e.g., lard, tallow, mutton fat, and poultry fat), are available for use in poultry feed manufacturing. However, the impact of the fat source on energy utilization in broilers requires further study, as fats and oils have considerable variation in their bioavailability and metabolizable energy (Blanch *et al.*, 1996).

Therefore, the present study investigated the effects of coated trace minerals (Cu, iron (Fe), Zn, Mn, and selenium (Se)) and two fat sources (soybean oil and lard) on the growth performance, antioxidant capacity, lipid metabolism, and meat quality of broilers.

Materials and Methods

Ethics

All procedures complied with the Liaoning Province Regulations of Laboratory Animals, and the study was approved by the Laboratory Animal Ethical Committee of Shenyang Agricultural University (permit number SYXK 20021001).

Experimental Design

The experimental design consisted of a 2×2 factorial arrangement with two types of trace minerals (non-coated and coated) and two sources of fat (soybean oil and lard). The coated trace minerals were commercially produced and provided by Fujian Syno Biotech Co., Ltd. The surfaces of the trace minerals were coated with carboxymethylcellulose (CMC), which is a low-cost commercial water-soluble cellulose with non-toxic and biodegradable properties. The coating process was as follows: after the preparation of inorganic trace mineral premix, metal ions and basic coating material were mixed in the solid phase. The constituents of the commercial coated trace minerals were dicopper chloride trihydroxide (20 mg), Fe [II] sulfate monohydrate (100 mg), manganous sulfate monohydrate (50 mg), Zn sulfate monohydrate (100 mg), sodium selenite (0.6 mg), calcium iodate anhydrous (3.0 mg), calcium carbonate (102.5 mg), and CMC

(25 mg). Dietary supplementation of trace minerals was undertaken with a premix that comprised the following minerals per 1000 mg/kg: 10 mg/kg Cu, 140 mg/kg Fe, 120 mg/kg Mn, 120 mg/kg Zn, and 0.8 mg/kg Se.

A total of 576 21-day-old Arbor Acres male broilers were randomly divided into four treatment groups, each consisting of 12 replicates with 12 chicks per replicate cage. Proprietary diets were provided at 21 d post-hatch. Dietary treatments were applied 21–42 d post-hatch. The composition and nutrient levels of the experimental diets are shown in Table 1. The nutrient values met or exceeded the recommendations of NY/T33-2004. The birds were kept under a 23:1 h light:dark cycle with free access to mash feed and water. Total feed consumption and body weight for each replicate cage of birds were recorded at 21 and 42 d post-hatch. Average daily feed intake (ADFI), average daily gain (ADG) in weight, and feed conversion ratios (FCRs) were determined over the experimental period.

Sample Collection

On day 42, one bird per replicate cage (12 birds per treatment) was selected and blood samples were drawn from the brachial vein. Serum samples were stored at −20°C after centrifugation for further analysis. Then, the selected birds were euthanized by intracardial administration of sodium pentobarbital (50 mg/kg of body weight) into the wing vein. The pectoralis major and leg muscles of each bird were removed and stored at either 4°C or −20°C for the assessment of meat quality and antioxidant index, respectively.

Meat Quality Assessment

pH value Meat pH_{45min} of the breast and leg was measured at 45 min post-mortem, using a portable pH meter (ST5000/F, Ohaus, Parsippany, NJ, USA). Muscle samples were stored at 4°C for 23 h to obtain pH_{24h} values via the same methodology.

Shear force A cube was cut (2×2×2 cm) from the breast and leg muscles at the same position on each carcass and stored at 4°C for 48 h. Each cube was then subjected to measurement of shear force using a tenderometer (MODEL 2000D, GR Manufacturing Manhattan, KS, USA) (Yoon, 2002).

Drip loss The breast and leg muscle drip loss was measured following the method described by Downs *et al.* (2000). Drip loss percentage was determined as follows: [(initial weight−24 h weight)/initial weight]×100.

Antioxidant capacity The muscle malondialdehyde (MDA) content was determined with thiobarbituric acid reactive substances according to the manufacturer's instructions of the test kit provided by Jiangsu Baolai Biotechnology Co. Ltd.

Determination of Serum Biomarkers

Trace mineral contents The Fe, Mn, Cu, Zn, and Se contents in serum were determined using a flame atomic absorption spectrometer (PinAAcle900, Perkin Elmer, Waltham, MA, USA).

Antioxidant The total antioxidant capacity (T-AOC), Cu/Zn superoxide dismutase (Cu/Zn-SOD) activity, glutathione peroxidase (GSH-Px) activity, and MDA content in

Table 1. Feed ingredients and nutrient composition of experiment diets

Ingredients	Uncoated		Coated	
	Soy oil	Lard	Soy oil	Lard
Corn	61.36	61.36	61.36	61.36
Soybean meal	26.50	26.50	26.50	26.50
Soy oil	5.00	—	5.00	—
Lard	—	5.00	—	5.00
Protein powder, 60%	1.50	1.50	1.50	1.50
Glutamic acid residue, 68%	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.00	1.00	1.00	1.00
Limestone	1.20	1.20	1.20	1.20
NaHCO ₃	0.10	0.10	0.10	0.10
NaCl	0.28	0.28	0.28	0.28
Phytase	0.01	0.01	0.01	0.01
Premix ¹⁾	1.05	1.05	1.05	1.05
Nutrient levels ²⁾				
ME, MJ/kg	13.33	13.34	13.33	13.34
Crude protein (g)	20.00	20.00	20.00	20.00
Calcium (g)	0.79	0.79	0.79	0.79
Available phosphorous (g)	0.30	0.30	0.30	0.30
Digestible lysine (g)	1.25	1.25	1.25	1.25
Digestible methionine (g)	0.58	0.58	0.58	0.58
Digestible threonine (g)	0.82	0.82	0.82	0.82

¹⁾The premix provides the following nutrients per kg of diet: VA 9 000 IU, VE 0.81 mg, VD₃ 2 700 IU, VK₃ 0.78 mg, VB₁ 0.58 mg, VB₂ 21.8 mg, VB₆ 0.74 mg, VB₁₂ 0.06 mg, biotin 5.7 mg, pantothenic acid 5.76 mg, folic acid 0.57 mg, nicotinamide 40 mg, lysine 450 mg, methionine 250 mg, threonine 100 mg, choline chloride 100 mg, Fe 140 mg, Mn 120 mg, Cu 10 mg, Zn 120 mg, and Se 0.8 mg.

²⁾Nutritional levels are calculated values.

serum were determined using the light amide method, colorimetric method, TBA method, and an r-911 automatic radioactivity meter (7160, Hitachi, Tokyo, Japan), respectively, following the manufacturer's instructions (Jiangsu Baolai Biotechnology Co. Ltd, Nanjing, China).

Serum biochemical indicators Total cholesterol (TC), triglyceride (TG), lipoprotein lipase (LPL), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) serum contents were determined using the assay kits from the Jiangsu Baolai Biotechnology Institute. Growth-related hormone levels in serum, such as those of insulin (INS), glucagon (GLU), and thyroxine (T₄), were determined with a radioimmunoassay (Multiskan FC, Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

Data derived from broilers in 12 replicate cages per treatment were analyzed as a 2×2 factorial arrangement using IBM SPSS Statistics 20.0 (IBM Corporation, Somers, NY, USA). A probability level of less than 5%, determined with a two-tailed test, was considered significant.

Results

Growth Performance

The effects of dietary treatments on growth performance are shown in Table 2. Trace minerals significantly affected the FCR ($P<0.05$), wherein the coated trace minerals (1.86) had a more pronounced effect than the uncoated ones (1.96).

The fat source also significantly impacted the FCR ($P<0.05$), wherein the soy oil (1.86) had a greater effect than the lard (1.94). Birds fed coated trace minerals showed higher ADG ($P<0.001$) in weight than those fed inorganic minerals. Soy oil significantly increased the ADG in weight compared to lard ($P<0.05$). Dietary treatments had no significant effect on the ADFI.

Serum Trace Mineral Contents

The effects of dietary treatments on serum trace mineral contents are shown in Table 3. There were no significant interactions between trace minerals and the fat source in serum. Trace minerals had significant effect on the Fe, Zn, and Se contents ($P<0.05$). The birds fed coated trace mineral diets had higher serum Fe, Zn, and Se levels than those fed uncoated mineral diets. The dietary fat source had no significant influence on serum trace mineral contents.

Antioxidant Performance

The effects of dietary trace minerals and the fat source on serum and muscle antioxidant levels are shown in Table 4. Trace minerals had a significant effect on the T-AOC, GSH-Px, SOD, and MDA contents in the serum and muscle. The use of coated trace minerals in the diet resulted in higher T-AOC, GSH-Px, and SOD activities in the serum of broilers ($P<0.05$) and decreased the MDA contents in both the serum and muscle ($P<0.05$). The dietary fat source tended to affect the T-AOC and SOD activities in serum and MDA content in the muscle ($P<0.1$). The supplementation of lard

Table 2. Effects of dietary trace mineral forms and fat source on the growth performance of broilers

Treatment		Final weight, kg	ADFI, g/d	ADG, g/d	FCR
Trace minerals	Fat				
Uncoated	Soy oil	2.22±0.70	102.32±3.57	52.52±2.21	1.93±0.13
	Lard	2.20±0.86	101.32±5.39	51.62±2.81	1.99±0.14
Coated	Soy oil	2.33±0.48	101.99±4.67	56.30±1.62	1.82±0.07
	Lard	2.26±0.45	102.33±3.06	53.93±1.64	1.89±0.07
Main effect: Trace minerals					
Uncoated		2.21±0.77	101.82±4.50	52.01±2.52	1.96±0.14
Coated		2.29±0.60	102.16±3.87	55.12±2.00	1.86±0.08
Fat					
Soy oil		2.28±0.82	102.16±4.07	54.41±2.71	1.86±0.12
Lard		2.23±0.73	101.82±4.32	52.77±2.54	1.94±0.12
Significance (<i>P</i> -value)					
Trace minerals		<0.001	0.783	<0.001	0.002
Fat		0.014	0.788	0.011	0.046
Minerals×Fat		0.136	0.587	0.235	0.789

Table 3. Effects of dietary trace mineral forms and fat source on mineral retention in the serum of broiler chickens

Treatment		Fe, $\mu\text{mol/L}$	Mn, nmol/L	Cu, $\mu\text{mol/L}$	Zn, $\mu\text{mol/L}$	Se, $\mu\text{g/L}$
Trace minerals	Fat					
Uncoated	Soy oil	19.10±0.78	10.09±0.55	11.40±0.59	49.27±1.35	1.01±0.06
	Lard	18.89±0.12	10.38±0.62	11.42±1.39	52.72±4.62	1.00±0.02
Coated	Soy oil	23.39±0.56	10.08±1.02	12.58±1.23	57.11±1.21	1.13±0.08
	Lard	22.14±0.21	10.50±0.99	12.36±1.43	56.52±0.81	1.10±0.05
Main effect: Trace minerals						
Uncoated		18.99±0.53	10.24±0.58	11.41±0.99	51.00±3.65	1.00±0.04
Coated		22.77±1.29	10.29±0.95	12.47±1.24	56.81±1.00	1.11±0.07
Fat						
Soy oil		21.25±2.38	10.09±0.76	11.99±1.09	53.19±4.35	1.07±0.09
Lard		20.52±2.03	10.44±0.77	11.89±1.40	54.62±3.68	1.05±0.06
Significance (<i>P</i> -value)						
Trace minerals		<0.001	0.898	0.105	0.001	0.003
Fat		0.144	0.407	0.867	0.278	0.514
Minerals×Fat		0.283	0.884	0.844	0.134	0.711

in diets resulted in lower activity of T-AOC and SOD in serum and higher MDA content in the muscle. Soy oil significantly decreased the MDA content in serum ($P<0.05$).

Serum Lipoprotein Cholesterol Contents

The effects of dietary treatments on serum biomarkers are shown in Table 5. The trace minerals significantly affected LPL ($P<0.05$), wherein the effect of coated trace minerals was more pronounced than that of uncoated treatments. Meanwhile, coated trace minerals tended to increase the TC, TG, and HDL-C contents in serum and decrease LDL-C content ($P<0.1$). The dietary fat source significantly affected serum LPL activity ($P<0.05$), wherein lard was superior to soy oil.

There was no significant interaction between the trace minerals and fat source ($P>0.05$). Compared with uncoated treatments, coated trace minerals significantly reduced the serum T₄ content ($P<0.01$) and increased the serum INS

content in broilers ($P=0.052$). Compared to lard, soybean oil tended to increase the serum T₄ content in broilers ($P=0.054$).

Meat Quality

As shown in Table 6, there was no significant interaction between trace minerals and fat source ($P>0.05$) that affected the meat quality of broilers. Dietary trace minerals had no significant effect on meat quality ($P>0.05$); however, the drip loss rates and shear force of the meat from broilers fed the coated mineral diets were lower. Soybean oil diets significantly decreased the drip loss rate of chicken meat compared to lard diets ($P<0.05$).

Discussion

The diets containing coated trace minerals yielded superior ADG in weight and higher FCRs compared to those containing uncoated trace minerals. As feed intake was not

Table 4. Effects of dietary trace mineral forms and fat source on the serum and muscle antioxidant status of broilers

Treatment		T-AOC U/mL	GSH-Px U/L	SOD U/L	MDA, nmol/L	
Trace minerals	Fat				serum	muscle
Uncoated	Soy oil	16.02±0.51	101.09±2.96	1175.14±87.73	6.48±0.54	7.55±0.32
	Lard	15.00±0.49	98.41±5.10	1083.84±71.00	6.60±0.23	7.79±0.31
Coated	Soy oil	17.16±0.78	113.00±2.59	1271.58±32.98	4.89±0.31	5.87±0.41
	Lard	16.33±1.38	111.82±7.27	1218.53±65.15	5.70±0.39	6.03±0.31
Main effect: Trace minerals						
Uncoated		15.51±0.71	99.75±4.12	1129.49±88.55	6.54±0.39	7.67±0.33
Coated		16.75±1.13	112.41±5.09	1245.06±55.58	5.30±0.54	5.95±0.37
Fat						
Soy oil		16.59±0.86	107.05±6.87	1223.36±80.14	5.69±0.94	6.71±0.93
Lard		15.67±1.20	105.12±9.23	1151.19±95.72	6.15±0.57	6.91±0.95
Significance (<i>P</i> -value)						
Trace minerals		0.015	<0.001	0.005	<0.001	<0.001
Fat		0.055	0.442	0.053	0.033	0.053
Minerals×Fat		0.837	0.762	0.580	0.101	0.704

Table 5. Effects of dietary trace mineral forms and fat source on the serum biomarkers of broilers

Treatment		TC mmol/L	TG mmol/L	LPL U/L	HDL-C mmol/L	LDL-C mmol/L	INS mU/L	GLU pg/mL	T ₄ µg/L
Trace minerals	Fat								
Uncoated	Soy oil	3.88±0.06	1.20±0.11	122.74±1.13	1.77±0.02	0.91±0.05	13.03±0.63	42.82±0.70	87.45±1.39
	Lard	3.91±0.05	1.16±0.05	124.78±1.78	1.79±0.03	0.95±0.09	12.35±0.19	42.29±1.31	85.73±0.68
Coated	Soy oil	4.03±0.17	1.30±0.04	125.66±1.25	1.80±0.07	0.81±0.12	13.71±1.08	43.25±1.01	75.12±0.79
	Lard	4.07±0.23	1.22±0.10	127.77±2.72	1.86±0.04	0.84±0.11	13.07±0.32	42.79±1.05	74.44±1.42
Main effect: Trace minerals									
Uncoated		3.90±0.05	1.18±0.08	123.76±1.76	1.78±0.02	0.93±0.07	12.69±0.57	42.55±1.02	86.593±1.37
Coated		4.05±0.19	1.26±0.08	126.72±2.26	1.83±0.06	0.82±0.11	13.39±0.82	43.02±0.98	74.779±1.12
Fat									
Soy oil		3.95±0.14	1.25±0.10	124.20±1.91	1.79±0.05	0.86±0.10	13.37±0.90	43.03±0.83	81.28±6.68
Lard		3.99±0.17	1.19±0.08	126.28±2.66	1.82±0.05	0.89±0.11	12.71±0.46	42.54±1.13	80.09±6.62
Significance (<i>P</i> -value)									
Trace minerals		0.058	0.077	0.007	0.053	0.055	0.052	0.391	<0.001
Fat		0.605	0.174	0.043	0.122	0.460	0.064	0.359	0.054
Minerals×Fat		0.935	0.641	0.972	0.379	0.925	0.944	0.941	0.368

TC, Total cholesterol; TG, Triglyceride; LPL, Lipoprotein lipase; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; INS, Insulin; GLU, Glucagon; T₄, Thyroxine

influenced by mineral source, the improvement in FCR was driven by the improvement in weight gain. Previous studies suggested that controlling the release of minerals within the gastrointestinal tract may improve nutrient digestibility and the epithelial structure of the intestines (Bao *et al.*, 2007), thereby improving mineral metabolism (Mondal *et al.*, 2010). Accordingly, the results presented in this study indicate that supplementation of coated trace minerals may improve broiler chicken weight gain by improving either digestibility or mineral metabolism and antioxidant capacity.

Dietary lipids are important energy sources within poultry diets. Additionally, the dietary inclusion of fats provides essential fatty acids (FAs) and may influence the FA composition of membranes, thereby impacting cell functionality (Watkins *et al.*, 2000). Vegetable oils, such as soybean oil, are rich in polyunsaturated FAs (PUFAs). In contrast, ani-

mal fats, such as lard, are rich in saturated FAs (SFAs). The results of this study demonstrated that soybean oil inclusion significantly improved the growth performance of broilers compared to lard. This is in agreement with the results of Watkins *et al.* (2000), albeit in rats. Thus, it appears that the higher proportion of PUFAs in soybean oil may have contributed to the improved performance via preferable cell membrane composition (Watkins *et al.*, 2000).

Serum mineral concentration can be used as an indicator of the mineral status of animals. The present study demonstrated that coated trace minerals significantly increase the serum concentrations of Fe, Se, and Zn in broiler chickens, which is consistent with the results of a previous study (Shen *et al.*, 2014). Coated trace minerals improve the intestinal absorption of Se, leading to Se further entering the cells via the clathrin-mediated endocytosis pathway (Chithrani and

Table 6. Effects of dietary trace mineral forms and fat source on the meat quality of broilers

Treatment		pH _{45min}		pH _{24h}		Drip loss, %		Shear force, N/cm ²	
Trace minerals	Fat	Pectoralis major	Leg muscle	Pectoralis major	Leg muscle	Pectoralis major	Leg muscle	Pectoralis major	Leg muscle
Uncoated	Soy oil	6.34±0.31	6.41±0.19	5.98±0.18	6.26±0.20	2.81±0.50	2.67±0.41	9.28±2.46	34.33±8.52
	Lard	6.29±0.26	6.38±0.11	5.93±0.15	6.11±0.31	3.06±0.48	2.94±0.48	9.81±1.59	39.40±9.40
Coated	Soy oil	6.38±0.22	6.45±0.12	6.04±0.21	6.23±0.14	2.57±0.35	2.49±0.32	8.73±2.56	33.29±8.68
	Lard	6.30±0.21	6.41±0.10	5.97±0.19	6.21±0.84	2.96±0.59	2.70±0.33	9.80±2.58	34.38±9.53
Main effect: Trace minerals									
Uncoated		6.31±0.28	6.40±0.15	5.95±0.16	6.19±0.27	2.93±0.50	2.80±0.46	9.54±2.04	36.87±9.15
Coated		6.34±0.21	6.43±0.11	6.00±0.21	6.22±0.11	2.76±0.52	2.59±0.33	9.27±2.57	33.84±8.93
Fat									
Soy oil		6.36±0.26	6.43±0.16	6.01±0.21	6.25±0.17	2.69±0.44	2.58±0.37	9.01±2.47	33.81±8.43
Lard		6.29±0.23	6.40±0.10	5.95±0.17	6.16±0.23	3.01±0.53	2.82±0.42	9.80±2.10	36.89±9.60
Significance (<i>P</i> -value)									
Trace minerals		0.715	0.393	0.374	0.587	0.229	0.066	0.682	0.245
Fat		0.351	0.417	0.287	0.149	0.028	0.036	0.243	0.252
Minerals×Fat		0.802	0.857	0.887	0.286	0.609	0.793	0.692	0.451

Chan, 2007). Zn plays an important role in the antioxidant defense system of the body and protects cell membranes from oxidative damage. This may occur through Zn scavenging free radicals via increased synthesis of metallothionein (Oteiza *et al.*, 1996). Zn may also mediate metallothionein and act as a P53 co-transcription factor to repair DNA damage caused by oxidative stress (Troy and Shelanski, 1994). This may explain the improved growth performance observed in broilers offered diets containing coated trace minerals.

Maintenance of the redox balance within the broiler gut is important to prevent lipid peroxidation (Zhang *et al.*, 2008). In the present study, lipid peroxidation status within the chicken gut was assessed. Supplementation of diets with coated trace minerals decreased lipid peroxidation. This likely occurred via the reduction in serum and muscle MDA levels, which is consistent with the results of Aksu *et al.* (2010). The reduction in MDA concentration may be linked to the enhanced capacity of the gut to scavenge free radicals and decrease the tissue damage of cells (Uchida, 2000). Higher serum Zn concentrations generated by coated mineral supplementation may increase antioxidant enzyme activity, resulting in more efficient removal of MDA. A previous study (Petrovič *et al.*, 2009) demonstrated that inorganic minerals significantly increase peroxide and MDA levels in breast muscle, likely due to negative interactions between sodium selenite and transition metal ions supplanted in the form of inorganic salts.

In the present study, coated trace minerals significantly increased the serum activities of T-AOC, SOD, and GSH-Px by 7.99%, 10.23%, and 12.69% in broilers, respectively. Previously, Sun *et al.* (2012) reported that high T-AOC levels is an indicator of strong antioxidant defense, and that high levels of T-AOC may decrease oxidative damage. Cu/Zn-SOD and GSH-Px are two important enzymes that aid in antioxidant defense by clearance of reactive oxygen species. The protective effects of cellular SOD against oxida-

tive tissue damage can mainly be attributed to their breakdown of superoxide radicals into hydrogen peroxide and oxygen, thereby decreasing hydroxy radical formation, which is the prime initiator of lipid peroxidation (Balevska *et al.*, 1981). This is of high relevance, as Zn and Se are two key components of the antioxidant enzymes Cu/Zn-SOD and GSH-Px, which were observed in elevated concentrations within broilers fed diets with coated trace minerals.

Different dietary fat sources have previously been suggested to influence the antioxidant enzyme activity. The present study demonstrated that soybean oil significantly reduced the serum and muscle MDA levels, and increased the serum T-AOC (5.87%) and SOD (6.27%) activities. High PUFA concentrations within soybean oil resulted in high levels of SOD, which is consistent with the results of Chen *et al.* (2012), wherein SOD was reported to work in conjunction with reduced GSH to scavenge free radicals, such as H₂O₂, and lipid peroxides, thereby decreasing MDA levels. Thus, the enhancement of antioxidant enzyme function with soybean oil may be attributable to the PUFA concentration within the oil that is associated with the regulation of antioxidant enzymes.

In the present study, supplementation of coated trace minerals in diets for broiler chickens increased LPL levels. LPL plays an important role in lipid metabolism by hydrolyzing the core TGs from circulating chylomicrons and very low-density lipoproteins (Beisiegel, 1996). Increased LPL activity leads to increased levels of HDL-C, which is an important cholesterol and TG transporter in poultry (Elkin *et al.*, 1993). HDL may clear cholesterol from plasma via promoting cholesterol delivery from the periphery to the liver, where it may be excreted in bile. Increased HDL levels in broilers fed diets containing coated trace minerals may also affect the alteration of TC and TG in serum. Grummer *et al.* (1988) reported that high LPL gene expression may improve meat tenderness, which is in accordance with the meat quality results reported in the present study. In comparison

with lard, soybean oil significantly increased the LPL activity, which is potentially related to SFA content (Sato *et al.*, 1999).

Trace minerals play an essential role in thyroid metabolism. Bik (2003) reported that the supplementation of Se could increase T₃ and decrease T₄ levels in sheep, which is important, as the serum T₄ levels may alter fat metabolism through regulating the synthesis and degradation of cholesterol. The present study reported that coated trace minerals significantly reduced the serum T₄ content. The enhanced utilization of Se by the supplementation of diets with coated trace minerals may stimulate 5'-deiodinase activity and increase the conversion of T₃ to T₄ (Tapiero *et al.*, 2003). The form of supplementary dietary trace minerals had no effect on the serum INS and GLU levels; however, coated trace mineral supplementation increased INS concentration within the serum. INS is a key hormone that regulates various metabolic pathways. Dietary trace minerals, especially Zn, can stabilize the INS protein and activate carboxypeptidase B to promote INS conversion (Mantzoros *et al.*, 1998). Therefore, coated trace mineral inclusion may have also improved performance via the upregulation of INS within the serum of broiler chickens.

In the present study, drip loss of chicken meat was reduced in broilers fed diets containing coated trace minerals or those containing soybean oil. The mechanisms for the reduction of drip loss with coated trace mineral inclusion are currently unclear. However, soybean oil contains a greater concentration of PUFAs (Ayed *et al.*, 2015), which can maintain impermeability and improve water-holding capacity. This may explain why the meat of broilers offered soybean oil had less drip loss compared to that of broilers offered lard.

Supplementation of broiler diets with coated trace minerals considerably improved growth performance and antioxidant status compared to the diets with inorganic minerals. Trace mineral retention in serum and lipid metabolism were also improved in the broilers fed diets containing coated trace minerals. Importantly, supplementation of broiler diets with coated trace minerals also impacted the regulatory pathways for INS, GLU, and T₄, which may have contributed to the improved performance. Additionally, soybean oil improved the growth performance, antioxidant performance, and meat quality of broilers, compared to lard. Although no significant interaction was detected between the two factors, the combination of coated trace minerals and soybean oil improved the growth performance, antioxidant capacity, and meat quality in broilers.

Acknowledgments

We thank Yinggu Kuang (Institute Fujian Syno Biotech Co., Ltd.) for providing coated trace mineral samples.

Conflicts of Interest

The authors declare no conflicts of interest.

References

Aksu DS, Aksu T, Ozsoy B and Baytok E. The effects of replacing

inorganic with a lower level of organically complexed minerals (Cu, Zn and Mn) in broiler diets on lipid peroxidation and antioxidant defense systems. *Asian-Australasian Journal of Animal Sciences*, 23: 1066-1072. 2010.

Ayed HB, Attia H and Ennouri M. Effect of oil supplemented diet on growth performance and meat quality of broiler chickens. *Advanced Techniques in Biology & Medicine*, 4: 2379-1764. 2015.

Bai MM, Xu K, Zhang XF, Deng BC, Tan CQ, Deng JP, Bing PP and Yin YL. Compensation effects of coated cysteamine on meat quality, amino acid composition, fatty acid composition, mineral content in dorsal muscle and serum biochemical indices in finishing pigs offered reduced trace minerals diet. *Science China*, 62: 1550-1553. 2019.

Balevska PS, Russanov EM and Kassabova TA. Studies on lipid peroxidation in rat liver by copper deficiency. *International Journal of Biochemistry and Cell Biology*, 13: 489-493. 1981.

Bao YM, Choct M, Iji PA and Bruerton K. Effect of Organically Complexed Copper, Iron, Manganese, and Zinc on Broiler Performance, Mineral Excretion, and Accumulation in Tissues. *Journal of Applied Poultry Research*, 16: 448-455. 2007.

Beisiegel U. New aspects on the role of plasma lipases in lipoprotein catabolism and atherosclerosis. *Atherosclerosis*, 124: 1-8. 1996.

Bik DE. Influence of selenium and iodine supplementation on thyroid hormone concentrations in the blood serum of sheep. *Medycyna Weterynaryjna*, 59: 1126-1129. 2003.

Blanch A, Barroeta AC, Baucells MD, Serrano X and Puchal F. Utilization of different fats and oils by adult chickens as a source of energy, lipid and fatty acids. *Animal Feed Science & Technology*, 61: 335-342. 1996.

Chan HS. The mechanism of autoxidation. *Autoxidation of unsaturated lipids*, 1987.

Chen W, Wang J and Huang Y. Effects of dietary n-6: n-3 polyunsaturated fatty acid ratio on cardiac antioxidative status, T-cell and cytokine mRNA expression in the thymus, and blood T lymphocyte subsets of broilers. *Livestock science*, 150: 114-120. 2012.

Chithrani BD and Chan WC. Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. *Nano letters*, 7: 1542-1550. 2007.

Downs KM, Hess JB and Bilgili SF. Selenium Source Effect on Broiler Carcass Characteristics, Meat Quality and Drip Loss. *Journal of Applied Animal Research*, 18: 61-71. 2000.

Dubey P, Thakur V and Chattopadhyay M. Role of Minerals and Trace Elements in Diabetes and Insulin Resistance. *Nutrients*, 12: 1864-1881. 2020.

Elkin RG, Freed M, Watkins B, Srebnik M, Kieft K and Newton R. Evaluation of two novel biochemicals on plasma and egg yolk lipid composition and laying hen performance. *Poultry Science*, 72: 513-520. 1993.

Grummer R and Carroll D. A review of lipoprotein cholesterol metabolism: importance to ovarian function. *Journal of Animal Science*, 66: 3160-3173. 1988.

Kwon CH, Lee CY, Han SJ, Kim SJ, Park BC, Jang I and Han JH. Effects of dietary supplementation of lipid-encapsulated zinc oxide on colibacillosis, growth and intestinal morphology in weaned piglets challenged with enterotoxigenic *E. coli*. *Animal Science Journal*, 85: 805-813. 2014.

Lu WB, Kuang YG, Ma ZY and Liu YC. The effect of feeding broiler with inorganic, organic, and coated trace minerals on performance, economics, and retention of copper and zinc. *Journal of Applied Poultry Research*, 29: 1084-1090. 2020.

- Mantzoros CS, Prasad AS, Beck FW, Grabowski S, Kaplan J, Adair C and Brewer GJ. Zinc may regulate serum leptin concentrations in humans. *Journal of the American College of Nutrition*, 17: 270–275. 1998.
- Mondal S, Haldar S, Saha P and Ghosh TK. Metabolism and tissue distribution of trace elements in broiler chickens' fed diets containing deficient and plethoric levels of copper, manganese, and zinc. *Biological Trace Element Research*, 137: 190–205. 2010.
- Oteiza PL, Olin KL, Fraga CG & Keen CL. Oxidant defense systems in testes from zinc-deficient rats. *Proceedings of the Society for Experimental Biology and Medicine*, 213: 85–91. 1996.
- Petrovič V, Marcinčák S and Popelka P. Effect of dietary supplementation of trace elements on the lipid peroxidation in broiler meat assessed after a refrigerated and frozen storage. *Journal of Animal & Feed Sciences*, 18: 499–507. 2009.
- Sato K, Takahashi T, Takahashi Y, Shiono H, Katoh N and Akiba Y. Preparation of chylomicrons and VLDL with monoacid-rich triacylglycerol and characterization of kinetic parameters in lipoprotein lipase-mediated hydrolysis in chickens. *Journal of Nutrition*, 129: 126–131. 1999.
- Selle PH, Ravindran V, Caldwell A and Bryden WL. Phytate and phytase: consequences for protein utilisation. *Nutrition Research Reviews*, 13: 255–278. 2000.
- Shen J, Chen Y, Wang Z, Zhou A, He M, Mao L, Zou H, Peng Q, Xue B and Wang L. Coated zinc oxide improves intestinal immunity function and regulates microbiota composition in weaned piglets. *British Journal of Nutrition*, 111: 2123–2134. 2014.
- Sun Q, Guo Y, Ma S, Yuan J, An S and Li J. Dietary mineral sources altered lipid and antioxidant profiles in broiler breeders and posthatch growth of their offsprings. *Biological Trace Element Research*, 145: 318–324. 2012.
- Tapiero H, Townsend D and Tew K. The antioxidant role of selenium and seleno-compounds. *Biomedicine and Pharmacotherapy*, 57: 134–144. 2003.
- Troy CM and Shelanski ML. Down-regulation of copper/zinc superoxide dismutase causes apoptotic death in PC12 neuronal cells. *Proceedings of the National Academy of Sciences*, 91: 6384–6387. 1994.
- Uchida K. Role of reactive aldehyde in cardiovascular diseases. *Free Radical Biology and Medicine*, 28: 1685–1696. 2000.
- Underwood EJ and Suttle NF. *The mineral nutrition of livestock*. 1966.
- Watkins BA, Li Y, Allen KG, Hoffmann WE & Seifert MF. Dietary ratio of (n-6)/(n-3) polyunsaturated fatty acids alters the fatty acid composition of bone compartments and biomarkers of bone formation in rats. *Journal of Nutrition*, 130: 2274–2284. 2000.
- Yoon K. Texture and microstructure properties of frozen chicken breasts pretreated with salt and phosphate solutions. *Poultry Science*, 81: 1910–1915. 2002.
- Zhang HJ, Tian YD, Guo YM and Yuan JM. Dietary conjugated linoleic acid improves antioxidant capacity in broiler chicks. *British Poultry Science*, 49: 213–221. 2008.