Biol Trace Elem Res (2016) 173:316–324
DOI 10.1007/s12011-016-0658-7

Effects of Replacing of Inorganic Trace Minerals by Organically Bound Trace Minerals on Growth Performance, Tissue Mineral Status, and Fecal Mineral Excretion in Commercial Grower-Finisher Pigs

Bing Liu [1,2] · Pingwen Xiong [1,2] · Nana Chen [1,2] · Junna He [1,2] · Gang Lin [2,3] · Yan Xue [2,3] · Weifen Li [1,2] · Dongyou Yu [1,2]

Received: 23 October 2015 / Accepted: 17 February 2016 / Published online: 27 February 2016

Abstract A total of 180 crossbred pigs (Duroc × Landrace × Large White; BW = 47.1 ± 4.8 kg) were used to investigate the effects of totally replacing inorganic trace minerals (ITMs) by organically bound trace minerals (OTMs) on growth performance, tissue mineral status, liver antioxidant enzyme activities, and fecal mineral excretion in grower-finisher pigs. A randomized complete block design with three treatments and six replicates (n = 10 pigs per pen) was used in this 69-day, 2-phase feeding trial. Experimental treatments were as follows: (1) a basal diet without trace mineral supplementation, (2) basal + ITMs (Fe, Mn, and Zn from sulfates, Cu oxychloride, and sodium selenite providing commercially recommended levels in China at 125, 22.5, 117.5, 30, and 0.3 mg/kg, respectively), and 3) basal + OTMs (Fe, Mn, Zn, and Cu from Bioplex and Se as Sel-Plex (Alltech Inc., Nicholasville, KY) providing levels identical to ITMs). No significant differences ($P > 0.05$) were observed in ADG, ADFI, or G:F among the treatments during the entire grower-finisher period. Supplementation with minerals, regardless of source, increased ($P < 0.05$) the Fe, Cu, and Se levels in the plasma; Fe and Zn levels in the liver; and Se levels in heart. Furthermore, compared with ITM group, the concentration of Zn and Se in the liver and heart, and Se in plasma and longissimus muscle were greater ($P < 0.05$) in OTM group. Hepatic Cu/Zn-SOD and ALP activities were increased ($P < 0.05$) when either ITMs or OTMs were supplemented. Pigs supplemented with OTMs displayed greater activities of Cu/Zn-SOD, ALP, and GSH-Px in the liver compared to pigs supplemented with ITMs. Dietary mineral supplementation to pig diets greatly increased ($P < 0.05$) fecal mineral (Fe, Mn, Zn, Cu, and Se) excretion in both grower and finisher phases. Fecal concentrations of Zn, Cu, and Se excretion were lower ($P < 0.05$) with OTMs supplementation than that in pigs fed diets containing ITMs. These results indicate that use of organic trace minerals, as well as no trace mineral supplementation, did not influence pig growth performance. Totally replacing ITMs by equivalent levels of OTMs could improve hepatic Cu/Zn-SOD, ALP, and GSH-Px activities and reduce fecal Mn, Cu, and Se excretion for grower-finisher pigs when supplemented at commercially recommended levels.

Keywords Organically bound trace minerals · Fecal mineral excretion · Tissue mineral concentrations · Antioxidant capacity · Grower-finisher pigs

✉ Dongyou Yu
dyyu@zju.edu.cn

[1] College of Animal Science, Key Laboratory of Animal Nutrition and Feed in East China, Ministry of Agriculture, Zhejiang University, Hangzhou, China

[2] ALLTECH-ZJU Animal Nutrition and Feed Science Research Alliance, Hangzhou, China

[3] Alltech Biological Products (Beijing) Co., Ltd., Beijing, China

Abbreviations

TMs	Trace minerals
OTMs	Organically bound trace minerals
ITMs	Inorganic trace minerals
BW	Body weight
ADG	Average daily gain
ADFI	Average daily feed intake

G:F	The ratio of gain to feed intake
CAT	Catalase
Cu/Zn-SOD	Copper/zinc superoxide dismutase
Mn-SOD	Manganese superoxide dismutase
ALP	Alkaline phosphatase
GSH-Px	Glutathione peroxidase

Introduction

Trace minerals (TMs) are essential nutrients due to their vital roles in a wide variety of physiological processes, deficiency of which may result in poor health and production [1, 2]. Due to variable concentrations and unknown availabilities, the indigenous dietary microminerals are not considered in feed formulation [3]. Therefore, it is a common practice, under commercial conditions, to increase the dietary levels of supplemental minerals above NRC recommendations to meet the growth of the modern commercial pigs [4, 5]. For instance, the commercially utilized levels of Fe, Mn, Zn, Cu, and Se in the diets for grower-finisher pigs in China range from 100 to 150, 20 to 25, 110 to 125, 25 to 35, and 0.1 to 0.3 mg/kg, respectively. However, only small amount of dietary Fe, Zn, and Cu and even less Mn, under normal physiological conditions, are absorbed with the remainder are excreted into the environment [6, 7].

Studies in swine have shown that organic Fe [8], Zn [9], Cu [10, 11], and Se [12, 13] have greater bioavailability than inorganic trace minerals (ITMs). Organically bound trace minerals (OTMs) can enhance mineral uptake, increase the mineral concentrations in circulatory system or tissue [14, 15], and reduce mineral excretion compared to that in inorganic forms [16, 17]. Therefore, formulating diets with OTMs exhibiting greater bioavailability may serve as a strategy for reducing trace mineral concentrations in waste. As reported, supplementing pigs with a combination of OTMs was superior to replacing ITMs independently as this interacts/competes less within minerals for their absorption [18]. However, most studies with OTMs in pigs have focused on either replacing one or two elements [9, 11]. Moreover, previous studies used the NRC-recommended levels of microminerals in pigs as the supplemental levels [18, 19], while few studies used levels suggested by commercial recommendations [17]. Therefore, data on totally replacing ITMs by OTMs at commercially recommended levels in diets of grower-finisher pigs are limited.

Therefore, the objective of the current experiment was to evaluate the effects of typical corn-soybean meal diets (1) without trace mineral supplementation; (2) with commercially recommended levels of inorganic Fe, Mn, Zn, Cu, and Se; or (3) with equivalent levels of Fe, Mn, Zn, Cu, and Se in organic forms on growth performance, tissue mineral concentrations, liver antioxidant enzyme activities, and fecal mineral excretion of commercial grower-finisher pigs.

Materials and Methods

Diet Composition and Treatment

According to our previous survey, the commercially utilized levels of Fe, Mn, Zn, Cu, and Se for grower-finisher pigs range from 100 to 150, 20 to 25, 110 to 125, 25 to 35, and 0.1 to 0.3 mg/kg, respectively. In this study, the mean values of each reference range (Fe 125, Mn 22.5, Zn 117.5, Cu 30, and Se 0.3 mg/kg) were defined as the commercial recommendations. The pigs were fed corn-soybean meal diets that were formulated to meet or exceed the NRC (1998) recommended nutrient requirements (Table 1) [20]. Phytase is commonly added to commercial swine diets to release P and some microminerals from feed grains and therefore [18], and all the experimental diets contained phytase (1200 phytase units/kg diet; EN Bio-tech Co., Ltd., Beijing, China). Supplemental Ca and P were provided from commonly utilized ground limestone and dicalcium phosphate. Supplemental organic forms of Fe, Cu, Mn, and Zn were metal proteinates, sequestered with enzymatically hydrolyzed soybean protein (Bioplex, Alltech Inc., Nicholasville, KY) and organic Se provided from a unique selenium yeast product (Sel-plex, Alltech Inc., Nicholasville, KY) [21]. The mineral concentrations in the diets for each phase were analyzed (Table 2).

The experiment was conducted with a randomized complete block design in six replicates and the same barrow to gilt ratio was maintained within replicate. A total of 180 crossbred pigs (Duroc × Landrace × Large White; BW = 47.1 ± 4.8 kg) were allotted (10 pigs/pen) to three dietary treatments based on BW and sex. The three treatments were: (1) NC: a basal diet (a corn-soybean meal diet without supplemental Fe, Mn, Cu, Zn, and Se), (2) ITM: a basal diet + ITMs (Fe, Mn, Zn, Cu, and Se provided at 125, 22.5, 117.5, 30, and 0.3 mg/kg, respectively), and (3) OTM: a basal diet + OTMs (provided mineral levels equivalent to ITMs).

Animal Housing and Sample Collection

A 2-phase grower-finisher feeding program was used for all pigs based on NRC (1998): grower phase and finisher phase [20]. Pigs remained on the same dietary treatments throughout both the 69-day grower and finisher phases. Pens (4.2 * 3.8 m) were separated by steel rod and composed of 50 % solid concrete floors in the front and 50 % plastic-coated expanded metal floors in the rear of the pen [18]. Pigs had ad libitum access to diets and water via a 3-hole concrete feeder or a stainless steel nipple waterer.

Pig weights and feed disappearance were measured by pens on days 1, 40, and 69 of the trial, and then average daily gain (ADG), average daily feed intake (ADFI), and the ratio of gain to feed intake (G:F) were calculated. Blood samples were

Table 1 Composition and nutrient levels of basal diets (%, as fed basis)

Item	Experimental diet, %	
	Grower phase	Finisher phase
Ingredient		
Corn, ground	60.0	60.0
Soy bean meal, 48 % CP	22.0	16.0
Wheat middling	12.0	18.0
Soya-bean oil	2.0	2.0
Choline hydrochloride	0.1	0.1
Limestone	1.0	1.0
Dicalcium phosphate	0.6	0.6
Salt	0.3	0.3
L-Lys Hcl	0.36	0.36
DL-Met	0.04	0.04
Vitamin and mineral mix[a,b]	1.6	1.6
Total	100.0	100.0
Calculated composition[c]		
DE (MJ/kg)	13.98	13.94
CP (%)	15.94	14.26
Lys (%)	1.16	1.03
Met (%)	0.28	0.26
Thr (%)	0.69	0.62
Ca (%)	0.63	0.62
Available P (%)	0.28	0.27

Fed grower phase diet from day 1 to 40 of the trial and finisher phase diet from days 41 to 69

[a] Added per kilogram of diets: 1600 IU vitamin A (acetate), 400 IU vitamin D3 (cholecalciferol), 20 IU vitamin E ($_{DL\text{-}\alpha}$tocopheryl acetate), 0.4 mg vitamin K (menadione sodium bisulfite complex), 0.5 mg vitamin B1 (thiamin), 5.6 mg riboflavin, 4.4 mg vitamin B6 (pyridoxine), 16 μg vitamin B12 (cobalamine), 5 mg niacin, 0.4 mg folic acid, 4 mg pantothenic acid, and 0.3 mg I (calcium iodate)

[b] Added 1200 phytase units/kg diet (EN Bio-tech Co., Ltd., Beijing, China)

[c] Ingredient composition taken from supplier ingredient specification sheets. The analyzed mineral content of the diets is provided in Table 2

Table 2 Mineral requirements (NRC 2012) and analysis of Fe, Cu, Mn, Zn, and Se contents in the experimental diets (mg/kg, as fed basis)

Minerals	NRC (2012)	Treatment[a]		
		NC	ITM	OTM
Grower phase (day 0 to 40)				
Fe	50	183.6 ± 22.0	295.6 ± 24.0	310.5 ± 13.9
Mn	2	46.0 ± 3.8	67.7 ± 3.6	65.67 ± 2.7
Zn	50	47.3 ± 2.9	157.0 ± 6.6	162.6 ± 8.1
Cu	3.5	16.6 ± 0.9	43.5 ± 3.3	44.4 ± 4.9
Se	0.15	0.053 ± 0.003	0.19 ± 0.012	0.16 ± 0.008
Finisher phase (days 41 to 69)				
Fe	40	178.7 ± 12.8	303.8 ± 13.5	310.8 ± 8.7
Mn	2	41.0 ± 3.3	65.1 ± 4.1	69.0 ± 3.2
Zn	50	42.7 ± 3.6	151.2 ± 8.8	156.2 ± 8.6
Cu	3	15.9 ± 0.7	48.7 ± 2.7	46.6 ± 5.2
Se	0.15	0.010 ± 0.002	0.31 ± 0.005	0.21 ± 0.008

Each value is mean ± standard deviation (SD) of 6 samples from 3 different time points of each phase

[a] NC (negative control) = basal diet, ITM = a basal diet + commercially recommended levels of inorganic trace minerals, and OTM = a basal diet + organic trace minerals with levels identical to ITM

collected on day 69 from 1 randomly selected pig per pen (about 105 kg, equal gender distribution within replicate). Pigs were bled with 10-mL samples collected via anterior cava vena into a heparinized (100 USP units of Na heparin) Vacutainer tube (Kangjie Medical Devices Co., Ltd., Jiangsu, China). Plasma was obtained by centrifugation at 2500×*g* at 4 °C for 20 min and the supernatant was collected and stored at −20 °C for micromineral analysis [17]. After fasting for 12 h, these selected pigs were slaughtered for tissue collection. The liver was immediately saved in liquid nitrogen for determination of liver enzyme activities [19]. The remaining liver, heart, kidney, and longissimus muscle tissues were removed and stored at −20 °C for micromineral analysis. Fecal samples were collected from three randomly selected pigs per pen (by entering the pen and waiting until pigs voluntarily dunged) at the last 3 days of each phase. Samples were composited across times, then dried in an oven at 65 °C for 48 h, and pooled by pen within a phase [17].

Sample Mineral Analysis

Both diet and fecal samples were finely ground with a grinder to pass a 1-mm screen to achieve homogenous samples for micromineral analysis [22]. The liver, heart, kidney, and longissimus muscle were rinsed with double-distilled water, freeze-dried, finely ground using a 1-mm screen, and subsequently extracted of fat with diethylether (method 963.15; AOAC, 2000) [23]. Plasma samples were deproteinated by 10 % (*w*/*v*) trichloracetic acid, and the resulting supernatant fraction was used to analyze mineral concentrations [24].

The Fe, Mn, Zn, and Cu concentrations in diets, fecal samples, tissues, and plasma were estimated in an air-acetylene flame on an atomic absorption spectrophotometer (model Thermo Scientific S Series, Thermo Fisher Scientific Inc., USA) after wet ashing with nitric acid and hydrogen peroxide in a microwave digester (model MDS-5, version 194A07, CEM Corp., USA; method 999.10; AOAC, 2000) [23]. Selenium concentration was analyzed after wet ashing with nitric and perchloric acids and measured by the fluorometric method (method 996.16; AOAC, 2000) [23]. Validation of the mineral analysis was conducted using bovine liver powder (GBW (E) 080193, National Institute of Standards and Technology, Beijing, China) as standard reference materials [25].

Liver Enzyme Activities Assay

Liver samples were homogenized in cold 0.1 M Tris-HCl buffer at pH 7.4 to produce a 10 % (*w*/*v*) homogenate. The homogenate was then centrifuged at 2500×*g* at 4 °C for 10 min and subsequently the resulting supernatant was collected for analysis. The catalase (CAT) activity was determined by the method of Beers and Sizer [26], based on the decomposition of H_2O_2 per minute. Total superoxide dismutase (T-SOD) activity was measured according to the xanthine oxygenation method [27]. One unit of SOD activity was defined as the amount of enzyme causing 50 % inhibition of the xanthine and xanthine oxidase system reaction in 1 ml enzyme extraction of 1 mg protein. Determination of copper/zinc superoxide dismutase (Cu/Zn-SOD) activity was accomplished by the modification of the methods of Shaw et al. [28]. Briefly, samples were diluted appropriately with the reaction buffer (50 mM Tris-HCl, 1.0 mM diethyllenetriamine pentaacetic acid, pH 8.2) and used to measure Cu/Zn-SOD activity. Manganese-superoxide dismutase (Mn-SOD) activity was calculated by subtracting the Cu/Zn-SOD value from the T-SOD value. Alkaline phosphatase (ALP) activity was measured using disodium phenyl phosphate as substrate [5]. Glutathione peroxidase (GSH-Px) activity was determined by the analysis of reduced GSH in the enzymatic reaction. The final result was defined as a reduction of 1.01 M GSH per 5 min at 37 °C after the nonenzymatic reaction is subtracted, and data were expressed as U/mg protein [5]. Protein concentrations were estimated by the method of Bradford [29].

Statistical Analysis

Data were analyzed as a randomized complete block design using the MIXED model procedure of SAS [18]. The pen averages served as the experimental unit for growth performance and fecal mineral excretion, and the individual pig (one pig per pen) served as the experimental unit for tissue mineral concentrations and liver enzyme activities. The model was as follows:

$$Y_i = \mu + r_i + \mathrm{T}_j + e_{ij},$$

in which Y_i was the continuous dependent variable, μ was the population mean, r_i was the random effect of the ith replicate (i = 1, ……, 6), T_j was the fixed effect of the jth treatment (j = 1, 2, 3), and e_{ij} was the residual error. Average daily feed intake of each collecting day was a covariate for the analyses of fecal mineral concentrations [16]. The significance of difference between means was determined by Duncan's multiple range test. Significance was declared at $P < 0.05$.

Results

Diet Trace Minerals

Mineral analyses of the two basal diets for each growth phase were presented as means (means ± SD) along with NRC (1998) micromineral requirements for the grower and finisher pig (Table 2). The indigenous Fe, Mn, Zn, Cu, and Se in the basal diet for the grower pigs were 184, 46, 47, 17, and 0.05 mg/kg, while the concentrations of Fe, Mn, Zn, Cu, and Se in the basal diet for the finisher pig were 179, 41, 43, 16, and 0.01 mg/kg, respectively. The indigenous levels of trace minerals, except for Zn and Se, exceeded the NRC (1998) recommendation. Individual treatment diets were analyzed and were within the acceptable ranges for analytical variation except for Se. However, Se content in diet of OTM group (0.21 mg/kg) was less than that in diet of ITM group (0.31 mg/kg) for finisher pigs.

Growth Performance

During day 0 to 40, days 41 to 69, and overall 69-day period, there were no significant effects ($P > 0.05$) by adding essential minerals (Fe, Mn, Zn, Cu, and Se), regardless of the sources, on ADG, ADFI, or G:F (Table 3). Similarly, no significant differences ($P > 0.05$) were observed in ADG, ADFI, or G:F during the entire period between ITM and OTM treatments.

Plasma and Tissue Trace Mineral Status

The concentrations of Zn, Cu, and Se increased significantly ($P < 0.05$) in the plasma (Table 4) in TM-fed pigs, regardless of the sources, compared to pigs fed the basal diets. The Fe and Zn levels increased ($P < 0.05$), while Cu decreased ($P < 0.05$) in the liver, in TM-fed pigs compared to that in pigs fed NC diets (Table 5). Pigs fed TM diets had higher Se tissue levels in heart and kidney compared to that fed NC diets ($P < 0.05$). Compared to ITM group, Se levels in the plasma and longissimus muscle (Table 6) and Zn and Se levels in the liver and heart of pigs fed diets containing OTMs were increased significantly ($P < 0.05$).

Liver Enzyme Activities

The activities of Cu/Zn-SOD and ALP were increased significantly ($P < 0.05$) in pigs fed TM diets compared to that fed NC diets (Table 7). There were no treatment effects on CAT or Mn-SOD activities ($P > 0.05$). Pigs fed diets containing OTMs showed increased ($P < 0.05$) activities of Cu/Zn-SOD, ALP, and GSH-Px in comparison to pigs with ITMs supplementation ($P < 0.05$).

Table 3 Effects of dietary trace mineral sources (inorganic and organic) on grower and finisher pig performance

Item	Treatment[a]			SEM	*P* value
	NC	ITM	OTM		
Body weight					
Day 1, kg	47.0	47.1	47.0	2.1	1.00
Day 40, kg	82.3	84.1	84.8	3.0	0.85
Day 69, kg	103.3	105.4	106.2	2.9	0.79
Grower phase (day 0 to 40)					
ADG, g	881	925	945	32	0.38
ADFI, g	2330	2422	2345	105	0.82
G:F	0.38	0.39	0.40	0.01	0.45
Finisher phase (days 41 to 69)					
ADG, g	724	735	739	36	0.59
ADFI, g	2671	2805	2755	85	0.55
G:F	0.27	0.26	0.28	0.01	0.32
Overall phase (day 1 to 69)					
ADG, g	816	845	858	27	0.46
ADFI, g	2519	2657	2535	113	0.67
G:F	0.32	0.32	0.34	0.01	0.32

Values are presented as means; each mean represents 6 pens of 10 pigs/pen

[a] NC (negative control) = basal diet, ITM = a basal diet + commercially recommended of inorganic trace minerals, and OTM = a basal diet + organic trace minerals with levels identical to ITM

Fecal Trace Mineral Concentration

Pigs fed the basal diet without TM supplementation had less ($P < 0.05$) fecal micromineral (Fe, Mn, Zn, Cu, and Se) excretion than those supplemented with ITMs or OTMs in both grower and finisher phases (Table 8). The excretion of Zn, Cu, and Se decreased ($P < 0.05$) in grower phase and Fe, Zn, Cu, and Se concentrations in feces decreased in finisher phase from OTMs- versus ITMs-fed pigs. However, no significant differences ($P > 0.05$) were observed in the excretion of Mn between OTM and ITM groups.

Table 4 Effects of trace mineral sources (inorganic and organic) on plasma micromineral levels of grower-finisher pigs (μg/mL)

Item	Treatment[a]			SEM	*P* value
	NC	ITM	OTM		
Fe	3.15	3.36	3.44	0.14	0.31
Mn	0.31	0.35	0.36	0.03	0.45
Zn	1.42b	1.86a	1.97a	0.11	<0.01
Cu	1.62b	1.80a	1.73a	0.04	<0.01
Se	0.08c	0.15b	0.18a	0.01	<0.01

Values reported as means ($n = 6$). In the same line, values with different letters mean significant difference ($P < 0.05$)

[a] NC (negative control) = basal diet, ITM = a basal diet + commercially recommended levels of inorganic trace minerals, and OTM = a basal diet + organic trace minerals with levels identical to ITM

Table 5 Effects of trace mineral sources (inorganic and organic) on tissue (liver, heart, and kidney) micromineral concentrations of grower-finisher pigs (mg/kg, as wet-tissue basis)

Item	Treatment[a]			SEM	*P* value
	NC	ITM	OTM		
Liver					
Fe	76.53b	119.65a	137.51a	11.76	<0.01
Mn	2.76	2.52	2.83	0.18	0.52
Zn	66.10c	82.14b	91.47a	4.11	<0.01
Cu	7.05a	5.20b	6.36a	0.51	0.03
Se	0.45b	0.46b	0.51a	0.02	<0.01
Heart					
Fe	38.39	42.95	44.46	2.27	0.11
Mn	2.26	2.41	2.50	0.10	0.23
Zn	14.30b	14.59b	16.54a	0.57	<0.01
Cu	2.77	2.90	3.08	0.10	0.11
Se	0.27b	0.28b	0.30a	0.01	0.05
Kidney					
Fe	45.60	48.44	48.51	1.07	0.08
Mn	1.78	1.96	1.71	0.10	0.18
Zn	24.01	25.65	25.26	1.56	0.76
Cu	5.00	5.50	5.56	0.31	0.39
Se	2.48b	2.79a	2.95a	0.11	<0.01

Values reported as means ($n = 6$). In the same line, values with different letters mean significant difference ($P < 0.05$)

[a] NC (negative control) = basal diet, ITM = a basal diet + commercially recommended levels of inorganic trace minerals, OTM = a basal diet + organic trace minerals with levels identical to ITM

Table 6 Effects of trace mineral sources (inorganic and organic) on the longissimus muscle micromineral concentrations of grower-finisher pigs (mg/kg, as wet-tissue basis)

Item	Treatment[a]			SEM	*P* value
	NC	ITM	OTM		
Fe	11.07	12.13	12.90	0.80	0.29
Mn	0.94	0.95	1.09	0.06	0.13
Zn	16.05	17.35	16.67	0.63	0.36
Cu	2.70	2.71	2.78	0.07	0.78
Se	0.13b	0.14b	0.16a	0.005	0.01

Values reported as means ($n = 6$). In the same line, values with different letters mean significant difference ($P < 0.05$)

[a] NC (negative control) = basal diet, ITM = a basal diet + commercially recommended levels of inorganic trace minerals, and OTM = a basal diet + organic trace minerals with levels identical to ITM

Table 7 Effects of trace mineral sources (inorganic and organic) on hepatic antioxidant enzyme activities of grower-finisher pigs

Item	Treatment[a]			SEM	*P* value
	NC	ITM	OTM		
Enzyme activities, units/mg protein					
CAT	86.8	88.6	97.5	3.7	0.09
Cu/Zn-SOD	112.6c	138.9b	173.2a	9.9	0.01
Mn-SOD	47.6	52.5	59.2	5.4	0.33
ALP	72.3c	125.9b	166.5a	19.6	0.01
GSH-Px	86.2b	95.3b	112.1a	6.5	0.01

Data are expressed as means ($n = 6$). In the same line, values with different letters mean significant difference ($P < 0.05$)

[a] NC (negative control) = basal diet, ITM = a basal diet + commercially recommended of inorganic trace minerals, and OTM = a basal diet + organic trace minerals with levels identical to ITM

CAT catalase, *Cu/Zn-SOD* copper/zinc superoxide dismutase, *Mn-SOD* manganese superoxide dismutase, *ALP* alkaline phosphatase, *GSH-Px* glutathione peroxidase

Discussion

Few studies have specifically focused on the response of tissue mineral concentrations, hepatic antioxidant enzyme activities, and fecal mineral excretion to a combination of OTMs-treated grower and finisher pigs. The main findings of this study were that there may be sufficient amount of endogenous microminerals in a typical corn-soybean diet fortified with limestone, dicalcium phosphate, and phytase to meet the grower-finisher pig's requirement for growth with lower fecal mineral excretion. Additionally, totally replacing ITMs by equivalent levels of OTMs could improve hepatic Cu/Zn-SOD, ALP and GSH-Px activities, and reduce fecal Mn, Cu, and Se excretion in grower-finisher pig.

Since we want to evaluate the effects of totally replacing ITMs by OTMs in grower-finisher pig under commercial conditions, most commonly utilized feedstuffs (i.e., limestone, dicalcium phosphate) and phytase were added to our experimental basal diet. The feedstuff limestone and dicalcium phosphate are not only good sources of Ca or P but also a source of Fe and Mn (NRC 1998) [20]. As reported, commercial dicalcium phosphate contains approximately 10,000 mg/kg Fe [30]. Phytase is commonly added to commercial swine diets to hydrolyze P from the phytate molecule and enhance micromineral availability in feed grains [4, 31]. In general, the Fe concentration analyzed in the basal diet (184 and 179 mg/kg for grower and finisher phase, respectively) exceed NRC (1998) recommendations (50 and 40 mg/kg, respectively) by at least twofold.

In the present study, no adverse effects were observed on performance of grower-finisher pigs from 47 to 105 kg BW even when fed diets without micromineral supplementations. Earlier work by Shaw et al., Mavromichalis et al., and McGlone in which minerals were not supplemented also revealed that no negative effects were observed in ADG or G:F [28, 32, 33]. Sources of these essential microminerals for grower-finisher pigs originated from the earlier storage in the postweaning period, and the indigenous and supplemental microminerals in the diets. Dietary trace mineral analysis indicated that the basal diet without trace mineral supplementations was sufficient in Fe, Mn, and Cu, marginal in Zn, and deficient in Se to meet the NRC (1998) requirement estimates for grower-finisher pig's growth. Hence, this result was dependent on the concentrations and bioavailabilities in grains or other feedstuffs, limestone, and dicalcium phosphate supplementations or the earlier dietary history of these pigs [18].

Studies supplied pigs with different sources of Fe, Mn, Zn, Cu, and Se have reported variable results. For example, Martin et al. reported supplementing with NRC-recommended levels of OTMs (Fe, Mn, Zn, Cu, and Se) improved G:F in starter pigs compared to that supplemented with equivalent levels of ITMs [19]. Similar results were reported by Zhou et al. in weaned pigs [34] and by Peters et al. in sows [8]. While others fed different sources (sulfate vs. proteinates) of trace minerals at NRC (1998) recommended levels, no improvement was observed from OTM supplementation compared with equivalent amount of ITM supplementation [35, 36]. Additional studies where the concentrations of trace mineral supplementation were at 20 to 50 % of NRC (1998) recommendations to pigs, similar growth performance was

Table 8 Effects of trace mineral sources (inorganic and organic) on fecal micromineral excretion of grower and finisher pigs (mg/kg, as air-dry basis)

Item	Treatment[a]			SEM	*P* value
	NC	ITM	OTM		
Grower phase (day 1 to 40)					
Fe	832b	1345a	1279a	98	<0.01
Cu	218c	256a	242b	7	<0.01
Mn	411b	549a	556a	30	<0.01
Zn	539c	1157a	1078b	116	<0.01
Se	0.62c	1.38a	1.27b	0.05	<0.01
Finisher phase (days 41 to 69)					
Fe	834c	1398a	1279b	105	<0.01
Cu	176c	310a	285b	25	<0.01
Mn	478b	566a	584a	23	<0.01
Zn	475b	1056a	1000a	111	<0.01
Se	0.45c	1.25a	1.15b	0.03	<0.01

Values are presented as means, each mean represents 6 pens of 3 pigs/pen. In the same line, values with different letters mean significant difference ($P < 0.05$)

[a] NC (negative control) = basal diet, ITM = a basal diet + commercially recommended of inorganic trace minerals, and OTM = a basal diet + organic trace minerals with levels identical to ITM

observed [14, 17]. Previous studies used the NRC recommendations as the supplemental levels in pig, while few studies used levels suggested by commercial recommendations. In the current study, when the trace minerals were supplemented at commercial recommended levels, totally replacing ITMs by OTMs did not affect ADG, ADFI, or G:F in grower-finisher pigs.

Trace mineral concentrations in plasma and tissue are often used to indicate the mineral status of animals [19]. In the present study, the plasma and different functional tissues (e.g., liver, heart, kidney, and longissimus muscle) were chosen to monitor the mineral status. The concentrations of microminerals in plasma reflect the balance among minerals absorbed, those being transported to or released from tissues, and those bound to circulating proteins [18]. In the present study, supplementing minerals to diets of pigs, regardless of source, increased the circulating Fe, Cu, and Se in plasma. The liver is considered a storage organ for many minerals, serving as a reliable source of minerals for metabolism. The concentrations of hepatic Fe, Zn, and Se increased in OTM or ITM group compared with those in the NC group, whereas the hepatic Cu concentration decreased, especially in the ITM group. This finding was possible due to interaction between Fe and Cu as previous studies reported that the Cu absorption may be prevented by the increasing Zn and Fe [37–39]. Zinc was not added at pharmacological concentrations in our experiment, whereas the Fe concentrations were high (ITM 295.6 and 303.8 mg/kg; OTM 310.5 and 310.8 mg/kg, respectively). Hepatic Fe concentration was increased by high dietary Fe inclusion and thus may interact with and decrease the storage of hepatic Cu. The results were consistent with the results of Carlson et al. and Kim et al. [40, 41]. Furthermore, compared with the ITM group, the concentrations of Zn in liver and heart increased significantly in the OTM group. It was also of interest that Se increased in the plasma and tissue (liver, heart, and longissimus muscle) when the organic Se was provided at 0.3 mg/kg. These results demonstrate that two sources of Se (sodium selenite and Sel-plex Se) are retained differently in tissues, which are consistent with the data of Martin et al. [19].

Although the mineral levels in plasma or tissue necessary to maximize mineral dependent functions has not been defined [19], adequate trace mineral intake and storage is required for varieties of metabolic functions including antioxidant capacity against oxidative stress and immune response to pathogenic challenge. The activities of hepatic antioxidant enzymes containing trace minerals are considered to be sensitive criterion for evaluating the mineral requirements of pigs. The present study showed that the activities of hepatic Cu/Zn-SOD and ALP increased when pigs were supplied diets containing microminerals compared to those in pigs fed the basal diet, demonstrating that activities of Cu/Zn-SOD and ALP were sensitive to dietary mineral (Zn or Cu) supplementations. Gowanlock et al. also reported the Mn-SOD activity was greater in the liver of pigs fed diets with mineral supplementations [21], but in this study, activity of Mn-SOD was not affected. The activities of Cu/Zn-SOD, ALP, and GSH-Px were higher in the liver of pigs fed diet supplemented with OTM than that with ITM. These results demonstrated that OTMs had greater abilities to stimulate the activities of Cu/Zn-SOD, ALP, and GSH-Px enzyme.

When minerals are supplemented in excess of the animal's requirement, more is excreted due to the decreased efficiency of utilization for that mineral [30]. In this study, average daily feed intake of each collecting day was a covariate for the analyses of fecal mineral concentrations. Fecal mineral excretion in pigs fed the basal diet was considerably lower than that in pigs supplemented minerals at commercially recommended levels. The mineral totals in feces of the NC group were 39.5 and 41.0 % less, respectively, in grower and finisher phase compared to the ITM group. In agreement with these results, Creech et al. reported that Zn and Cu excretion could be reduced by at least 40 % by reducing micromineral supplementation from levels commercially utilized (Cu 25 and Zn 150 mg/kg, respectively) to that recommended by NRC (1998) [17]. Despite ITM and OTM groups received the same levels of minerals, the Fe, Zn, Cu, and Se concentrations were significantly lower in feces of pigs fed organically bound minerals. In general, these findings are in agreement with those observed in previous studies conducted in weaned pigs with a decrease of fecal Zn and Cu concentrations when dietary supplementation with OTMs [17] and reduced concentrations of Fe, Zn, and Cu in feces of grower and finisher pigs when equivalent inclusion levels of organic Zn and Cu were fed [16]. However, the results were in disagreement with studies conducted by Pastorelli et al., focused on replacing one mineral source [42]. As it mentioned before, the basal diet without trace mineral supplementations in this study was sufficient in Fe, Mn, and Cu, marginal in Zn and deficient in Se to meet the NRC (1998) recommendations. These decreases in NC and OTM groups are noteworthy when considering that the market pig consumes most of its total lifetime feed intake during grower and finisher phases, thus producing most of its total fecal excretion. Therefore, reducing dietary micromineral concentrations closer to pig's requirement was an effective strategy to reduce fecal mineral excretion [9, 17], and diets formulated with inclusion of microminerals that meet, but not exceed, requirements may result in reduced mineral excretion from pigs. Additionally, replacing ITMs by OTMs in combination in diet could also reduce fecal Fe, Mn, and Cu excretion.

Conclusions

In a typical corn-soybean diet fortified with limestone, dicalcium phosphate, and phytase, the use of organic trace minerals, as well as no trace mineral supplementation, did not influence pig growth performance. However, some minerals (e.g., Fe or Se) concentrations increased in tissue and the hepatic antioxidant capacity was improved with trace minerals supplementation. Under commercial conditions in China, we advise using organic bound trace minerals totally replacing inorganic trace minerals widely used currently, as totally replacing ITMs by equivalent levels of OTMs could improve hepatic antioxidant capacity and reduce fecal Mn, Cu, and Se excretion for grower-finisher pigs. Further studies need to be carried out to evaluate the effects of replacing ITMs by low levels (below or closed to NRC recommendations) of OTMs in combination for commercial grower-finisher pigs.

Acknowledgments This work was supported by Alltech Inc. (Nicholasville, KY) for the Alltech-Zhejiang University Animal Nutrition and Feed Science Research Alliance (No. 14-AACZ-10137).

Compliance with ethical standards The experimental use of animals and procedures for their management and the collections of blood and tissues were performed in accordance with the Chinese Guidelines for Animal Welfare and approved by the Institutional Animal Care and Use Committee of Zhejiang University (Hangzhou, China).

Conflict of Interest The authors declare that they have no conflict of interest.

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