

Evaluation of trace mineral source and preharvest deletion of trace minerals from finishing diets on tissue mineral status in pigs

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Objective: An experiment was conducted to evaluate dietary supplemental trace mineral source and deletion on mineral content in tissues.

Methods: Weanling crossbred pigs (n = 144; 72 barrows and 72 gilts; body weight [BW] = 7.4±1.05 kg) were used. A basal diet was prepared, and trace mineral premix containing either inorganic (ITM) or organic (OTM) trace minerals (Cu, Fe, Mn, and Zn) was added to the basal diet. Pigs were blocked by sex and BW and randomly allotted to 24 pens for a total of 6 pigs per pen, and fed a diet containing either ITM or OTM supplemented at the 1998 NRC requirement estimates for each of 5 BW phases (Phase I to V) from 7 to 120 kg. The trace mineral supplementation was deleted for 6, 4, 2, and 0 wk of Phase V; regarding nutrient adequacy during this phase, the indigenous dietary Fe and Mn was sufficient, Cu was marginal and Zn was deficient.

Results: At the end of Phase IV, Mn content (mg/kg on the dry matter basis) was greater (p<0.05) in heart (0.77 vs 0.68), kidney (6.32 vs 5.87), liver (9.46 vs 8.30), and *longissimus dorsi* (LD; 0.30 vs 0.23) of pigs fed OTM. The pigs fed OTM were greater (p<0.05) in LD Cu (2.12 vs 1.89) and Fe (21.75 vs 19.40) and metacarpal bone Zn (141.86 vs 130.05). At the end of Phase V, increased length of deletion period (from 0 to 6 wk) resulted in a decrease (linear, p<0.01) in liver Zn (196.5 to 121.8), metacarpal bone Zn (146.6 to 86.2) and an increase (linear, p<0.01) in heart Mn (0.70 to 1.08), liver Mn (7.74 to 12.96), and kidney Mn (5.58 to 7.56). The only mineral source by deletion period interaction (p<0.05) was observed in LD Zn.

Conclusion: The results demonstrated differential effects of mineral deletion on tissue mineral content depending on both mineral assessed and source of the mineral.

Keywords: Deletion; Pigs; Tissue Mineral Status; Trace Minerals

INTRODUCTION

Mineral content in various tissues has often been used as the model for bioavailability of certain minerals. For example, heart Mn concentration was used as a measure of bioavailability in sheep wherein Mn concentration increased in a linear manner when dietary Mn was added in concentrations from 500 to 4,000 mg/kg [1]. Iron concentration in the liver is generally believed to reflect the animal's overall Fe status and the trace mineral concentration in kidney typically increases with higher dietary mineral intakes [2]. Generally, muscle is not a major mineral storage tissue in the animal body but because of the large mass of the skeletal muscle, muscle mineral status might be indicative of dietary mineral requirements. Serum and metacarpal bone Zn are generally accepted as measurements of Zn status in the body. Furthermore, with some minerals (e.g., Se [3]) an organic form of the mineral has resulted in greater tissue mineral concentration than the standard inorganic form because of its direct incorporation into protein as selenomethionine.

Numerous experiments have demonstrated that the trace mineral premix can be removed from the diet up to 42 days prior to slaughter with no deleterious effects on performance, pork quality, or carcass characteristics [4-6]. Regarding tissue mineral status, Shelton et al [6] reported that

removing inorganic trace mineral premix in pig diets from 22 to 109 kg has variable effects on tissue mineral content. Therefore, an objective of this experiment was to evaluate sources (inorganic [ITM] vs organic [OTM]) of added essential trace minerals (Cu, Fe, Mn, and Zn) on heart, liver, *Longissimus dorsi* (LD) muscle, kidney, spleen, and bone mineral status when those added minerals were removed from the diet for 0 (no deletion), 2, 4, and 6 wk before harvest. The effects of these dietary treatments on growth performance, carcass characteristics, and pork quality were previously reported [7].

MATERIALS AND METHODS

The experimental use of animals and procedures followed for their management, the collection of tissues, and harvest procedures were conducted under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

Experimental design and treatments

Nursery to growing period (Phase I to IV): This experiment used a total of 144 crossbred pigs (72 barrows and 72 gilts) weaned at 21±3 days with an initial body weight (BW) of 7.4±1.05 kg. The pigs were blocked on the basis of sex, initial BW, and ancestry, and randomly assigned to 1 of 2 dietary treatments in a randomized complete block design. Each treatment consisted of 12 pens, each with 6 pigs (3 barrows and 3 gilts). The housing of the pigs was described in Ma et al [7].

Pigs were fed a common corn-soybean meal diet and nutrients were formulated based on NRC [8] requirements for 5 BW categories from 7 to 120 kg (equivalent to 14, 14, 42, 28, and 42-d periods, respectively; Tables 1, 2). The basal diet without trace mineral premix was mixed homogeneously and then trace mineral premix containing either inorganic (CuSO₄·5H₂O, FeSO₄·H₂O, MnO, and ZnO) or organic trace minerals was added to the basal diet to obtain the different treatment diets. The organic trace minerals (Bioplex; Alltech Inc., Nicholasville, KY, USA) were chelated to soy protein hydrolysate by a proprietary process. Selenium was included as Na selenite at 0.3 mg/kg and I as Ca iodate at 1.0 mg/kg in both diets. Premixes for each BW category were individually blended with ground corn based on the trace mineral requirement estimate for that category. The analyzed mineral composition of the diets for each phase is presented in Table 3 along with the NRC [8] requirement estimates for Ca, P, Cu, Fe, Mn, and Zn.

Mineral deletion of finishing period: At the end of Phase IV (BW = 82.6±5.99 kg), 2 pigs from each pen (1 barrow and 1 gilt; with the initial pig chosen randomly and then a pig of the alternate gender selected whose weight would result in a mean weight of the selected pigs closely representing the mean pen weight) were removed and harvested for tissue collection. The remaining 96 pigs (4 pigs per pen) continued for the mineral deletion period. Three random pens from each treatment were switched to a basal

Table 1. Composition of nursery pig diets (%; as-fed basis)

Item	Phase I	Phase II
	7 to 10 kg	10 to 20 kg
Ingredient		
Corn, ground	49.27	55.03
Soybean meal, 48% crude protein	26.00	22.25
Dried whey	10.00	10.00
Spray dried blood meal	-	2.00
Spray dried animal plasma ¹⁾	3.00	-
Fish meal, menhaden	3.00	2.00
Lactose	4.00	4.00
Corn oil	2.00	2.00
Dicalcium phosphate	0.61	0.80
Limestone, ground	0.85	0.65
Salt	0.40	0.40
Vitamin premix ²⁾	0.10	0.10
Trace minerals ^{3),4)}	0.50	0.50
Antibiotic ⁵⁾	0.25	0.25
Antioxidant ⁶⁾	0.02	0.02
Calculated composition ⁷⁾		
Metabolizable energy (kcal/kg)	3,280	3,390
Crude protein (%)	21.89	19.43
Lysine (%)	1.35	1.15
Calcium (%)	0.80	0.70
Phosphorus (%)	0.64	0.60
Available phosphorus (%)	0.40	0.32

¹⁾ APC-920 obtained from American Protein Corp. (Ankeny, IA, USA).

²⁾ Supplied per kg of diet: vitamin A (acetate), 6,600 IU; vitamin D₃ (cholecalciferol), 880 IU; vitamin E (DL- α tocopheryl acetate), 44 IU; vitamin K (as menadione sodium bisulfate complex), 6.4 mg; thiamin, 4.0 mg; riboflavin, 8.8 mg; pyridoxine, 4.4 mg; vitamin B₁₂, 33 μ g; folic acid, 1.3 mg; niacin, 44 mg; pantothenic acid, 22 mg; and D-biotin, 0.22 mg.

³⁾ The inorganic trace minerals were added at a concentration to provide 100% of the NRC [8] requirement estimates for Cu, Fe, Mn, and Zn. Supplied per kg of diet during Phase I (7 to 10 kg): Cu (sulfate), 6 mg; Fe (sulfate), 100 mg; Mn (oxide), 4.0 mg; Zn (oxide), 100 mg; I (calcium iodate), 1.0 mg; Se (sodium selenite), 0.3 mg. Supplied per kg of diet during Phase II (10 to 20 kg): Cu (sulfate), 5 mg; Fe (sulfate), 80 mg; Mn (oxide), 3.0 mg; Zn (oxide), 80 mg; I (calcium iodate), 1.0 mg, and Se (sodium selenite), 0.3 mg.

⁴⁾ Organic trace minerals were supplied in the form of Bioplex (Alltech Inc, Nicholasville, KY, USA) and provided 100% of the NRC [8] requirement estimates for Cu, Fe, Mn, and Zn. Supplied per kg of diet during Phase I (7 to 10 kg): Cu, 6 mg; Fe, 100 mg; Mn, 4 mg; Zn, 100 mg; I (calcium iodate), 1.0 mg, and Se (sodium selenite), 0.3 mg. Supplied per kg of diet during Phase II (10 to 20 kg): Cu, 5 mg; Fe, 80 mg; Mn, 3 mg; Zn, 80 mg. Supplementation was similar in all diets for I (calcium iodate), 1.0 mg; Se (sodium selenite), 0.3 mg.

⁵⁾ Mecadox-10 (Phibro Animal Health, Fairfield, NJ, USA) supplied 55 mg carbadox per kg of diet.

⁶⁾ Santoquin Mix 6 (Novus International, Inc., St. Charles, MO, USA) supplied 130 mg ethoxyquin per kg of diet.

⁷⁾ Computed from the ingredient composition taken from NRC [8] or from supplier ingredient specification sheets. The analyzed mineral content of the diets is provided in Table 3.

diet without supplemental Cu, Fe, Mn, and Zn in 2-wk intervals. This resulted in 4 groups within each mineral treatment in which these trace minerals were deleted for 6, 4, 2, or 0 wk of Phase V.

Animal harvest and tissue collection methods

On the day after the growth trial ended, 2 pigs per pen were selected (1 barrow and 1 gilt; based upon pigs near the gender

Table 2. Composition of growing-finishing pig diets (% , as-fed basis)

Item	Phase III	Phase IV	Phase V
	20 to 50 kg	50 to 80 kg	80 to 120 kg
Ingredient			
Corn, ground	72.045	78.42	84.695
Soybean meal, 48% crude protein	25.25	19.00	13.00
Dicalcium phosphate	0.675	0.675	0.425
Limestone, ground	0.90	0.775	0.75
Salt	0.40	0.40	0.40
Lysine · HCl	0.08	0.08	0.08
Vitamin premix ¹⁾	0.10	0.10	0.10
Trace minerals ^{2),3),4)}	0.50	0.50	± 0.50
Antibiotic ⁵⁾	0.05	0.05	0.05
Calculated nutrient composition ⁶⁾			
Metabolizable energy (kcal/kg)	3,337	3,350	3,332
Crude protein (%)	18.02	15.21	13.20
Lysine (%)	1.01	0.81	0.66
Calcium (%)	0.60	0.50	0.45
Phosphorus (%)	0.50	0.45	0.41
Available phosphorus (%)	0.23	0.19	0.15

¹⁾ Supplied per kg of diet: vitamin A (acetate), 6,600 IU; vitamin D₃ (cholecalciferol), 880 IU; vitamin E (DL-α tocopheryl acetate), 44 IU; vitamin K (as menadione sodium bisulfate complex), 6.4 mg; thiamin, 4.0 mg; riboflavin, 8.8 mg; pyridoxine, 4.4 mg; vitamin B₁₂, 33 µg; folic acid, 1.3 mg; niacin, 44 mg; pantothenic acid, 22 mg; and D-biotin, 0.22 mg.

²⁾ The inorganic trace minerals were provided at 100% of the NRC [8] requirement estimate for Cu, Fe, Mn, and Zn. Supplied per kg of diet during Phase III (20 to 50 kg): Cu (sulfate), 4 mg; Fe (sulfate), 60 mg; Mn (oxide), 2.0 mg; Zn (oxide), 60 mg; I (calcium iodate), 1.0 mg, and Se (sodium selenite), 0.3 mg. Supplied per kg of diet during Phase IV (50 to 80 kg): Cu (sulfate), 3.5 mg; Fe (sulfate), 50 mg; Mn (oxide), 2.0 mg; Zn (oxide), 50 mg; I (calcium iodate), 1.0 mg, and Se (sodium selenite), 0.3 mg. Supplied per kg of diet during Phase V (80 to 120 kg): Cu (sulfate), 3.0 mg; Fe (sulfate), 40 mg; Mn (oxide), 2.0 mg; Zn (oxide), 50 mg; I (calcium iodate), 1.0 mg, and Se (sodium selenite), 0.3 mg.

³⁾ Organic trace minerals were supplied in the form of Bioplex (Alltech Inc., Nicholasville, KY, USA) and provided at 100% of the NRC [8] requirement estimates for Cu, Fe, Mn, and Zn. Supplied per kg of diet during Phase III (20 to 50 kg): Cu, 4 mg; Fe, 60 mg; Mn, 2 mg; Zn, 60 mg. Supplied per kg of diet during Phase IV (50 to 80 kg): Cu, 3.5 mg; Fe, 50 mg; Mn, 2 mg; Zn, 50 mg. Supplied per kg of diet during Phase V (80 to 120 kg): Cu, 3.0 mg; Fe, 40 mg; Mn, 2.0 mg; Zn, 50 mg. Supplementation was similar in all diets for all Phases for I (calcium iodate), 1.0 mg, and Se (sodium selenite), 0.3 mg.

⁴⁾ Trace mineral supplied per kg of diet for Phase V deletion diet: I (calcium iodate), 1.0 mg, and Se (sodium selenite), 0.3 mg.

⁵⁾ Tylan-40 (Elanco Animal Health Inc., Greenfield, IN, USA) supplied 44 mg tylosin per kg of diet.

⁶⁾ Computed from the ingredient composition taken from NRC [8]. The analyzed mineral content of the diets is provided in Table 3.

mean weight within the pen with the restriction that the specific breeding of the pigs selected must be similar across pens within the original allotment blocking), transported (32 km) to the University of Kentucky Meat Science Laboratory and rested for approximately 1 h before being harvested according to humane techniques by trained personnel. Animals were electrically stunned and killed by exsanguination followed by the immediate removal of the liver, heart, kidney, spleen, and front feet. The liver, heart, kidney, and spleen were weighed and one lobe of the liver, heart, right kidney, spleen, and front feet were stored at -20°C until processing and analysis. For the LD end-of-study muscle sample, the sample was taken after a 24-h carcass chill. The left side of

Table 3. Mineral requirements and analysis of those minerals in the experimental diets (mg/kg, as-fed basis)¹⁾

Minerals	NRC [8]	Treatment		
		Inorganic ²⁾	Organic ³⁾	Deletion
Phase I (7 to 10 kg)				
Calcium	8,000	10,725 ± 645	10,358 ± 870	-
Phosphorus	6,500	7,197 ± 736	7,114 ± 682	-
Copper	6	10.8 ± 0.8	11.5 ± 0.3	-
Iron	100	167.4 ± 17.2	163.4 ± 18.9	-
Manganese	4	25.8 ± 2.0	26.1 ± 0.7	-
Zinc	100	126.0 ± 2.3	130.7 ± 4.2	-
Phase II (10 to 20 kg)				
Calcium	7,000	8,440 ± 626	8,466 ± 214	-
Phosphorus	6,000	6,995 ± 316	6,919 ± 100	-
Copper	5	9.6 ± 2.4	9.7 ± 0.2	-
Iron	80	270.0 ± 26.1	270.9 ± 23.1	-
Manganese	3	23.3 ± 0.4	23.5 ± 0.2	-
Zinc	80	113.8 ± 5.2	112.5 ± 0.3	-
Phase III (20 to 50 kg)				
Calcium	6,000	7,264 ± 209	7,332 ± 136	-
Phosphorus	5,000	5,434 ± 159	5,415 ± 133	-
Copper	4	10.0 ± 2.2	9.7 ± 1.0	-
Iron	60	204.1 ± 11.7	200.7 ± 5.7	-
Manganese	2	22.8 ± 3.9	22.4 ± 0.6	-
Zinc	60	101.5 ± 2.1	102.3 ± 0.2	-
Phase IV (50 to 80 kg)				
Calcium	5,000	6,782 ± 730	6,797 ± 211	-
Phosphorus	4,500	5,322 ± 242	5,272 ± 28	-
Copper	3.5	7.5 ± 0.7	8.0 ± 1.2	-
Iron	50	145.7 ± 1.1	144.2 ± 6.0	-
Manganese	2	19.3 ± 0.8	19.2 ± 2.4	-
Zinc	50	78.1 ± 1.9	79.8 ± 6.2	-
Phase V (80 to 120 kg)				
Calcium	4,500	5,216 ± 177	5,197 ± 157	5,179 ± 362
Phosphorus	4,000	4,600 ± 274	4,622 ± 176	4,690 ± 329
Copper	3	5.6 ± 0.7	6.7 ± 0.9	2.9 ± 0.3
Iron	40	111.7 ± 8.2	115.1 ± 2.1	73.5 ± 3.4
Manganese	2	14.9 ± 0.2	15.8 ± 1.3	12.2 ± 1.0
Zinc	50	72.0 ± 5.7	77.7 ± 2.7	19.3 ± 1.0

¹⁾ The amount of each mineral supplemented was equivalent to the NRC [8] requirement estimate. The mineral source differed only for Cu, Fe, Mn, and Zn.

²⁾ Inorganic trace mineral Cu as CuSO₄ · 5H₂O, Fe as FeSO₄ · H₂O, Mn as MnO, and Zn as ZnO and values are means ± standard deviation for 2 duplicated samples.

³⁾ Organic trace minerals as Bioplex Cu, Fe, Mn, and Zn (Alltech Inc., Nicholasville, KY, USA) and values are means ± standard deviation for 2 duplicated samples.

each carcass was divided at the 10th rib and a sample taken from the center of the muscle. For the LD muscle sample at the end of Phase IV, the sample was taken on the day of slaughter without carcass chilling from the same location.

Laboratory analytical methods

Diet trace mineral analysis: Diet samples were finely ground with a coffee grinder (Proctor Silex E160B, Hamilton Beach Brands, Inc., Washington, NC, USA). Samples (~ 1 gram) were then digested with 70% trace metal nitric acid (Fisher Scientific, Fairlawn, NJ, USA) in a pressurized microwave (MDA-2000, CEM Corpor-

ation, Matthews, NC, USA) and initially diluted by a factor of 50. The samples were analyzed in duplicate for concentrations of Cu, Fe, Mn, and Zn using inductively coupled plasma (ICP) spectrophotometry (method 985.01) [9] using a simultaneous ICP-optical emission spectrometer (Model Vista-mpx CCD; Varian, Palo Alto, CA, USA) with aqueous Cu, Fe, Mn, and Zn standards (AccuStandard, New Haven, CT, USA) and reference tissues (bovine liver; reference standard 1577c) with known mineral concentrations from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA).

Tissue mineral analysis: Liver, LD muscle, heart, kidney, and spleen were individually ground through a small food processor (HC 3000, Black & Decker Inc., Shelton, CT, USA). Sample portions were remixed and reground if needed. Subsamples were collected and weighed and placed into plastic containers, sealed to prevent moisture loss, and frozen at -20°C . Tissues were subsequently lyophilized (Botanique model 18 DX48SA, Botanique Preservation Equipment, Inc., Peoria, AZ, USA) for 12 to 14 days to a constant weight. These dried samples were then finely ground (Proctor Silex E160B, Hamilton Beach Brands, Inc., USA) and analyzed for their mineral content using ICP technology, as previously described. After mineral analysis, wet tissue mineral contents were calculated based on the percentage of dry matter in the respective tissues.

Front feet were dissected and the 3rd and 4th metacarpals of each foot were removed and cut in half to remove marrow. After drying in an oven, they were wrapped in cheesecloth and the fat extracted with fresh petroleum ether three times at 24-h intervals. Once defatted, bones were placed overnight in an exhaust hood to evaporate the ether, oven-dried overnight at 105°C , taken out of the cheese cloth, placed into pre-weighed porcelain crucibles and ashed overnight at 600°C in a muffle furnace (method 942.05) [9]. Ash weight was recorded and the ash percentage in dry, fat-free bone was determined. Copper, Zn, Mn, and Fe concentration in ash samples were assessed by flame atomic absorption spectrophotometry at proper wavelength (AAS; thermochemical, SOLAAR M5, Thermo Electron Corp., Verona, WI, USA) according to a modification of the AOAC [9] procedure (method 927.02) using a Cu, Zn, Mn, and Fe reference solution (1,000 ppm; Fisher Scientific, USA) for development of standard curves.

Statistical analysis

Data were analyzed using the general linear model procedure of SAS (SAS Inst. Inc., Cary, NC, USA). From weaning to 80 kg BW, the model included trace mineral source and block. During the trace mineral deletion period (80 to 120 kg), the model included block, mineral source, deletion length, and mineral source \times deletion interaction. Orthogonal polynomials (derived from the SAS IML procedure) were used to determine the linear and quadratic effects of the duration of trace mineral deletion. In addition, a preplanned single degree-of-freedom comparison of 0 vs 6 wk deletion was made. The pen was the experimental unit for all

analysis. When a pig was removed from the experimental analysis the pen feed intake was adjusted based on a model developed by Lindemann and Kim [10]. Significance was declared at $p \leq 0.05$ and statistical tendencies noted at $0.05 < p \leq 0.10$.

RESULTS

Diet trace mineral analysis

From Phase I to IV, analyzed trace mineral concentration in the diets were similar between the 2 treatments (ITM vs OTM; Table 3). During the mineral deletion period (Phase V), indigenous dietary Cu concentration was 2.9 mg/kg diet; relatively close to the NRC [8] requirement estimate of 3.0 mg/kg, whereas the indigenous Fe and Mn concentrations were 73.5 and 12.2 mg/kg diet, respectively, an amount exceeding the NRC [8] requirement estimates (40 and 2 mg/kg diet, respectively). The indigenous dietary Zn was 19.3 mg/kg; an amount less than the NRC [8] requirement estimate (50 mg/kg diet) for finishing pigs.

Organ weight

At the end of Phase IV, 2 pigs (1 barrow and 1 gilt) from each pen were selected and slaughtered. In general, the source of trace mineral supplementation did not change the absolute and relative weights of tissues (Table 4). However, the relative liver weight of pigs fed ITM diets tended to be greater ($p = 0.07$) than those from pigs fed OTM diets. No differences were observed in dry matter content of the tissues between the 2 sources of trace mineral

Table 4. Effects of dietary trace mineral source (inorganic or organic) on tissue weight of 80 kg pigs¹⁾

Item	Mineral source		SEM	p-value
	Inorganic	Organic		
Slaughter body weight (kg)	82.3	83.1	0.79	0.47
Tissue absolute weight ²⁾ (g)				
Heart	304	313	7.3	0.41
Liver	1,241	1,218	18.3	0.39
Kidney	140	144	2.2	0.16
Spleen	118	117	2.5	0.73
Tissue relative weight ³⁾ (%)				
Heart	0.37	0.38	0.01	0.58
Liver	1.51	1.47	0.02	0.07
Kidney	0.17	0.17	0.01	0.24
Spleen	0.14	0.14	0.01	0.61
Dry matter concentration (%)				
Heart	24.1	24.7	0.51	0.45
Liver	19.8	19.6	0.37	0.85
Kidney	29.4	29.4	0.27	0.91
Spleen	22.0	21.4	0.40	0.32
<i>Longissimus dorsi</i> muscle	27.7	28.2	0.35	0.31

SEM, standard error of the mean.

¹⁾ Each least squares mean represents 12 pens of 2 pigs per pen.

²⁾ Fresh whole organ weights except for kidney, which was only from the right side of animal.

³⁾ As a percentage of body weight.

supplementation.

At the end of Phase V, 2 pigs from each pen were again selected and slaughtered. Generally speaking, the source of trace mineral supplementation, again, and the duration of trace mineral deletion did not change the absolute and relative weights of tissues or the percentage of tissue dry matter content (Table 5).

Tissue mineral status of 80 kg pigs

Manganese concentrations from pigs fed OTM were greater ($p < 0.05$) than those fed ITM for heart, kidney, liver, and LD muscle (Table 6). Organic Cu resulted in more Cu ($p < 0.05$) deposited in LD muscle than inorganic Cu and tended ($p = 0.06$) to result in more Cu in kidney than inorganic Cu. More Fe was deposited ($p < 0.05$) in LD muscle with organic Fe than inorganic Fe; alternately, more Fe ($p < 0.05$) was deposited in liver with inorganic Fe. Organic Zn increased metacarpal bone Zn ($p < 0.05$) and tended to result in more Zn ($p = 0.08$) deposit in kidney than inorganic Zn.

Total tissue Mn contents from pigs fed the OTM diets were greater ($p < 0.05$) than those fed the ITM diets for heart, kidney, and liver (Table 7). Organic Cu tended ($p = 0.10$) to result in more Cu deposited in kidney than inorganic Cu. Inorganic Fe resulted in more Fe ($p < 0.05$) deposited in liver than organic Fe.

Tissue mineral status following the mineral deletion period

Manganese concentrations increased linearly ($p < 0.01$) with increasing duration of trace mineral deletion for heart, kidney, and liver and tended to increase linearly ($p = 0.07$) in LD muscle and decrease quadratically ($p = 0.06$) in metacarpal bone (Table 8).

Manganese deposition in heart for pigs fed OTM was greater ($p < 0.05$) than those fed ITM. A trend for an interaction ($p = 0.08$) between the source and the duration of trace mineral deletion for Mn concentrations was observed in spleen where increasing deletion time was related to more Mn in ITM-fed pigs but not in OTM-fed pigs.

Copper concentrations in kidney decreased in linear ($p < 0.01$) and quadratic ($p < 0.01$) manners and in metacarpal bone decreased linearly ($p = 0.07$) with increasing time of mineral deletion; inorganic Cu tended ($p = 0.06$) to result in more Cu in bone. For Zn, similar to Cu, the deposition in tissues decreased with increasing duration of trace mineral deletion for kidney ($p < 0.01$; linear and quadratic), liver ($p < 0.01$; linear and quadratic), spleen ($p < 0.05$; linear), and metacarpal bone ($p < 0.01$). The only mineral source by deletion period interaction ($p < 0.05$) was observed in LD muscle where increasing deletion was associated with less Zn in ITM-fed pigs but not in OTM-fed pigs. Iron in metacarpal bone decreased linearly ($p < 0.05$) whereas in kidney showed a tendency for linear increase ($p = 0.06$) with increasing duration of trace mineral deletion and spleen Fe concentration from the pigs from the 6-wk deletion period tended to be lower ($p = 0.08$) than the control (non-deletion) treatment.

Total Mn content in tissues (Table 9) with increasing mineral deletion time showed a linear pattern for heart ($p < 0.01$), kidney ($p < 0.01$), liver ($p < 0.01$), and spleen ($p = 0.05$). Organic Mn deposited more Mn ($p < 0.05$) in heart than the ITM-fed treatment. An interaction ($p < 0.05$) between the source and the duration of trace mineral deletion was observed in spleen Mn content with various differences from the sources at different durations of trace

Table 5. Effects of dietary trace mineral source (inorganic or organic) and duration of deletion on tissue weight at the end of the study¹⁾

Item	Inorganic (wk)				Organic (wk)				SEM
	0	2	4	6	0	2	4	6	
Slaughter body weight (kg)	132.4	131.5	131.7	130.9	130.0	134.3	131.5	134.3	2.47
Tissue absolute weight ²⁾ (g)									
Heart	436	448	437	427	443	435	434	464	11.4
Liver	1,788	1,815	1,783	1,770	1,742	1,742	1,687	1,887	65
Kidney	173	189	176	173	160	194	166	185	8.4
Spleen	180	178	203	176	189	191	169	186	7.5
Tissue relative weight ³⁾ (%)									
Heart	0.33	0.34	0.33	0.33	0.34	0.32	0.33	0.35	0.01
Liver	1.35	1.38	1.36	1.35	1.34	1.30	1.28	1.41	0.04
Kidney	0.13	0.14	0.13	0.13	0.12	0.14	0.13	0.14	0.01
Spleen	0.14	0.14	0.15	0.14	0.15	0.14	0.13	0.14	0.01
Dry matter concentration (%)									
Heart	24.7	24.1	24.1	24.3	25.5	24.8	25.4	23.5	0.57
Liver	29.9	30.0	28.4	29.3	29.2	29.5	29.3	29.1	0.59
Kidney	20.5	20.5	20.8	20.5	19.7	21.3	20.4	20.2	0.62
Spleen	22.7	22.6	21.7	23.1	22.4	22.4	22.9	23.9	0.51
Longissimus dorsi muscle	30.6	31.8	30.5	30.6	30.2	31.5	29.3	30.8	0.89

SEM, standard error of the mean.

¹⁾ Each least squares mean represents 3 pens of 2 pigs per pen. There were no treatment effects at $p \leq 0.10$.

²⁾ Fresh whole organ weights except for kidney, which was only from right side of kidney.

³⁾ As a percentage of body weight.

Table 6. Effects of dietary trace mineral source (inorganic or organic) on tissue mineral concentration (mg/kg, dry matter basis) of 80 kg pigs¹⁾

Tissue	Mineral source		SEM	p-value
	Inorganic	Organic		
Heart				
Copper	15.03	15.15	0.25	0.72
Iron	141.3	140.6	4.56	0.92
Manganese	0.68	0.77	0.03	0.02
Zinc	67.79	67.31	0.51	0.52
Kidney				
Copper	24.65	27.97	1.10	0.06
Iron	214.1	204.5	8.47	0.44
Manganese	5.87	6.32	0.13	0.03
Zinc	103.6	109.2	2.06	0.08
Liver				
Copper	36.90	35.84	3.43	0.83
Iron	564.4	466.1	29.26	0.04
Manganese	8.30	9.46	0.23	0.01
Zinc	177.5	189.9	7.88	0.29
Spleen				
Copper	4.27	4.19	0.21	0.78
Iron	637.6	606.9	37.41	0.57
Manganese	0.89	0.96	0.03	0.13
Zinc	106.5	110.0	3.09	0.45
Longissimus dorsi muscle				
Copper	1.89	2.12	0.06	0.02
Iron	19.40	21.75	0.61	0.02
Manganese	0.23	0.30	0.01	0.01
Zinc	53.29	51.75	1.33	0.43
Metacarpal bone				
Copper	8.28	8.56	0.31	0.54
Iron	72.83	71.60	1.87	0.65
Manganese	3.16	3.21	0.03	0.22
Zinc	130.05	141.86	2.79	0.01

SEM, standard error of the mean.

¹⁾ Each least square mean represents 12 pens of 2 pigs per pen.

mineral deletion.

Total Zn content decreased in multiple tissues during mineral deletion, including heart ($p = 0.05$, linear), kidney ($p < 0.01$, linear), and liver ($p < 0.01$, linear and quadratic). The source of trace mineral affected heart Zn content (OTM vs ITM, 6.88 vs 6.55 mg; $p = 0.06$). Kidney Cu content decreased ($p < 0.01$, linear and quadratic) and Fe content increased ($p = 0.05$, linear), irrespective of trace mineral source. A trend for an interaction ($p = 0.08$) between the source and the duration of trace mineral deletion occurred for heart Fe content where increasing time of deletion resulted in less heart Fe in ITM-fed pigs but more heart Fe in OTM-fed pigs.

DISCUSSION

National Research Council nutrient requirement estimates are considered as the minimum requirements for pigs. In practice, trace minerals are generally supplemented at NRC requirement

Table 7. Effects of dietary trace mineral source (inorganic or organic) on quantitative mineral content (mg/tissue) of 80 kg pigs tissues¹⁾

Tissue	Mineral source		SEM	p-value
	Inorganic	Organic		
Heart				
Copper	1.11	1.18	0.04	0.19
Iron	10.35	10.96	0.41	0.31
Manganese	0.049	0.061	0.001	0.01
Zinc	5.00	5.25	0.18	0.35
Kidney				
Copper	0.68	0.79	0.04	0.10
Iron	5.95	5.80	0.28	0.70
Manganese	0.16	0.19	0.01	0.03
Zinc	2.86	3.09	0.11	0.16
Liver				
Copper	13.51	12.85	1.27	0.72
Iron	203.7	166.3	10.90	0.03
Manganese	3.03	3.36	0.07	0.01
Zinc	64.54	67.84	3.12	0.47
Spleen				
Copper	0.11	0.10	0.004	0.17
Iron	16.26	15.10	1.23	0.52
Manganese	0.023	0.024	0.001	0.44
Zinc	2.74	2.70	0.07	0.63

SEM, standard error of the mean.

¹⁾ Each least square mean represents 12 pens of 2 pigs per pen.

estimates or greater without considering the indigenous trace minerals in the feedstuffs. Because of this, the actual concentrations of trace minerals in the diet are generally greater than the requirement estimate of trace minerals. In the current study, dietary trace mineral analysis indicated that the concentrations of Cu, Fe, and Mn in the basal diets without trace mineral premix were adequate to meet the NRC [8] requirement estimate for pig growth. The Mn concentration in the basal diet exceeded the requirement estimate by at least 3-fold and only the concentration of Zn in the basal diet was generally deficient across all dietary phases. After removing the trace mineral premix in Phase V, the indigenous Mn and Fe concentrations were still greater than the actual requirements estimates while Cu was marginal and Zn was potentially deficient in comparison to the NRC [8] nutrient requirement estimate. Therefore, the deletion diet in Phase V may actually have been only a Zn-deficient diet.

In the current study, the source of trace minerals had no impact on absolute or relative organ weights during the growing and developing periods, with the exception of relative liver weight. This is in agreement with Gheisari et al [11] who suggested that organic chelates of Zn, Mn, and Cu had no effects on the absolute and relative weights of spleen and liver in birds. A similar result was observed by Peters et al [12], where sow liver weights were not affected by the source of trace minerals (organic vs inorganic) and the level of trace mineral supplementation over six parities. However, in the current study, the relative weight of the liver from pigs fed ITM diets tended to be heavier than those fed OTM diets.

Table 8. Effects of dietary trace mineral source (inorganic or organic) and duration of deletion on tissue mineral concentration (mg/kg, dry matter basis) of the finishing pigs¹⁾

Tissue	Inorganic (wk)				Organic (wk)				SEM
	0	2	4	6	0	2	4	6	
Heart									
Copper	15.07	14.79	14.19	14.35	13.95	14.47	14.27	14.96	0.42
Iron	150.7	162.2	140.3	147.6	137.4	142.1	135.5	160.4	7.35
Manganese ^{2),3)}	0.64	0.69	0.94	1.03	0.76	0.83	0.91	1.12	0.05
Zinc	63.2	62.6	61.5	60.2	64.5	62.7	61.8	63.0	1.75
Kidney									
Copper ⁴⁾	22.79	15.93	14.44	16.95	26.58	16.44	16.42	15.41	1.76
Iron ⁵⁾	190.6	236.7	214.4	215.9	199.0	216.9	200.2	283.1	22.3
Manganese ³⁾	5.03	6.19	6.67	7.81	6.14	6.08	6.63	7.30	0.36
Zinc ⁴⁾	95.8	84.6	80.0	82.3	101.0	80.7	86.8	80.6	3.61
Liver									
Copper	42.64	53.79	52.04	86.00	56.39	53.22	54.91	43.48	13.73
Iron	543.2	570.9	478.0	520.0	488.0	514.2	490.0	509.6	35.5
Manganese ³⁾	7.27	9.19	12.09	13.23	8.21	9.80	10.72	12.68	0.59
Zinc ⁴⁾	183.8	116.0	115.3	124.8	209.2	113.7	119.9	118.8	15.2
Spleen									
Copper	4.09	3.95	4.29	4.09	4.69	4.40	4.14	4.39	0.36
Iron	844.5	750.2	918.6	822.6	1,055	899.7	915.6	797.4	73.7
Manganese ⁶⁾	0.84	0.91	1.08	1.04	1.04	1.04	0.96	1.02	0.06
Zinc ⁷⁾	114.4	106.6	111.7	108.7	126.8	112.2	105.3	105.0	5.63
<i>Longissimus dorsi</i> muscle									
Copper	2.06	1.85	1.98	1.93	1.87	1.82	1.75	2.04	0.53
Iron	20.28	18.64	19.27	18.62	21.28	19.07	18.24	18.75	1.45
Manganese ⁵⁾	0.20	0.21	0.26	0.27	0.26	0.26	0.26	0.28	0.03
Zinc ⁸⁾	66.98	55.67	56.82	49.82	56.44	59.53	56.74	62.04	2.97
Metacarpal bone									
Copper ^{5),9)}	6.01	5.52	5.35	5.37	5.34	5.33	5.10	5.15	0.23
Fe ⁷⁾	45.71	37.60	39.06	36.61	36.60	39.45	36.20	36.20	35.24
Manganese ¹⁰⁾	2.83	2.59	2.61	2.67	2.69	2.64	2.43	2.63	0.10
Zinc ³⁾	140.90	122.22	98.49	84.34	152.22	123.40	101.47	87.97	4.88

SEM, standard error of the mean.

¹⁾ Each least square mean represents 3 pens of 2 pigs per pen. ²⁾ Main effect of mineral source, $p < 0.05$.

³⁾ Main effect of mineral deletion, $p < 0.01$ linear. ⁴⁾ Main effect of mineral deletion, $p < 0.01$ linear and quadratic.

⁵⁾ Main effect of mineral deletion, $p < 0.10$ linear. ⁶⁾ Mineral source \times deletion interaction, $p < 0.10$.

⁷⁾ Main effect of mineral deletion, $p < 0.05$ linear. ⁸⁾ Mineral source \times deletion interaction, $p < 0.05$.

⁹⁾ Main effect of mineral source, $p = 0.06$. ¹⁰⁾ Main effect of mineral deletion, $p < 0.10$ quadratic.

In agreement, Martin et al [13] evaluated OTM vs ITM supplementation in starter pigs and demonstrated that pigs fed ITM providing 100% of the NRC [8] requirement estimates had greater liver weight when expressed as percentage of BW than pigs fed the OTM diets. Martin et al [13] stated the effect of this greater liver weight in pigs fed inorganic trace minerals is unknown. The liver is known as the major tissue for trace mineral storage and body homeostasis. Trace minerals in liver are stored in different protein-binding complexes. The current study showed that more Fe from the ITM treatment was deposited in the liver than from the OTM treatment; therefore, the differences of trace mineral content or concentration and corresponding protein-binding complex in the liver from the different sources may contribute to the relative liver weight difference.

Shelton et al [6,14] reported that removing the trace mineral premix (Cu, Fe, Mn, Zn, I, and Se) in pigs during the whole grow-

ing and finishing period resulted in an increase in liver weight of pigs, but phytase reversed the response; other tissues (e.g., kidney) were not affected; in another study, Shelton et al [15] reported a decreased liver weight in growing pigs fed diets with phytase. However, Deyhim and Teeter [16] reported that the liver and spleen weights and the relative weights of the same tissues were not affected when chicks were fed diets without trace minerals (Cu, Fe, Mn, Zn, I, and Se) from 28 to 49 days. In the current study, heart, liver, kidney, and spleen weights were not affected by the trace mineral deletion. The different results from that of Shelton et al [6,14] may be related to fewer days of removal of the trace mineral premix from the diet in the current experiment or to the fact that I and Se remained in the deletion diet in the current study.

For all minerals, changes in the tissue content and concentration varied by tissue. There were no patterns that were distinct

Table 9. Effects of dietary trace mineral source (inorganic vs organic) and deletion on the quantitative mineral contents (mg) of 120 kg pig tissues¹⁾

Tissue	Inorganic (wk)				Organic (wk)				SEM
	0	2	4	6	0	2	4	6	
Heart									
Copper	1.62	1.60	1.50	1.49	1.56	1.55	1.57	1.62	0.07
Iron ²⁾	16.07	17.35	14.76	15.19	15.43	15.34	14.65	17.42	0.75
Manganese ^{3),4)}	0.067	0.075	0.098	0.104	0.084	0.089	0.099	0.122	0.006
Zinc ^{5),6)}	6.76	6.72	6.49	6.23	7.18	6.74	6.75	6.85	0.22
Kidney									
Copper ⁷⁾	0.80	0.61	0.52	0.60	0.85	0.67	0.55	0.57	0.06
Iron ⁶⁾	6.73	9.05	7.78	7.58	6.27	9.00	6.72	10.89	0.95
Manganese ⁴⁾	0.18	0.23	0.24	0.28	0.19	0.25	0.22	0.27	0.01
Zinc ⁴⁾	3.39	3.23	2.91	2.90	3.37	3.31	2.92	2.97	0.12
Liver									
Copper	22.29	27.80	27.15	44.12	29.56	27.71	26.31	24.38	7.30
Iron	283.2	300.0	247.6	273.5	247.5	268.2	241.6	284.3	21.47
Manganese ⁴⁾	3.79	4.84	6.23	6.92	4.19	5.14	5.28	6.99	0.36
Zinc ^{7),8)}	95.8	61.1	59.7	65.2	106.1	59.1	59.0	65.8	7.14
Spleen									
Copper	0.17	0.16	0.19	0.17	0.20	0.18	0.16	0.19	0.02
Iron	33.96	30.21	39.74	33.07	43.41	39.29	34.49	34.55	3.77
Manganese ^{6),8)}	0.034	0.037	0.047	0.043	0.043	0.044	0.037	0.045	0.003
Zinc	4.67	4.27	4.90	4.46	5.29	4.75	4.10	4.61	0.29

SEM, standard error of the mean.

¹⁾ Each least square mean represents 3 pens of 2 pigs per pen. ²⁾ Mineral source × deletion interaction, $p < 0.10$.

³⁾ Main effect of mineral source, $p < 0.01$. ⁴⁾ Main effect of mineral deletion, $p < 0.01$ linear.

⁵⁾ Main effect of mineral source, $p < 0.10$. ⁶⁾ Main effect of mineral deletion, $p = 0.05$ linear.

⁷⁾ Main effect of mineral deletion, $p < 0.01$ linear and quadratic. ⁸⁾ Mineral source × deletion interaction, $p < 0.05$.

for all tissues. The lack of patterns may be related to mineral form but also to the relative priority of different tissues.

In the current study, the different sources of trace mineral premix were supplemented in the diet beginning at weaning until 80 kg BW, which was a period of about 98 d. Heart Mn concentration has been used to measure the bioavailability of Mn in sheep because the response best fit a linear model when dietary Mn was added at 500 to 4,000 mg/kg [1]. In the current study, heart Mn content or concentration responded positively to the organic source of Mn as did other tissues consistently. However, Li et al [17], in a study with chicks, indicated that heart Mn content or concentration failed to show the difference among the sources of Mn, including Mn methionine complexes, Mn proteinates, and Mn amino acids. Another possibility for the different response is potential interactions between Mn and other trace minerals. Manganese is believed to share the same transporter (i.e., divalent metal transporter) with Cu and Fe and previous evidence has also shown that supplementing pig diets with Zn reduced Mn absorption and retention [6,18]. In the current study, four trace minerals, namely Cu, Fe, Mn, and Zn, were supplemented as an organic form and the uptake mechanism is believed to be different from metal transporters, such that the interactions between Mn and other trace minerals might be eliminated or reduced. A tissue increase was evidenced not only in heart Mn, but also kidney, liver, and muscle Mn were elevated from the

organic source. However, the mechanism behind those results needs to be further investigated.

With regard to Fe, Yu et al [19] demonstrated that organic Fe (an iron amino acid complex) increased the total Fe concentration in liver, spleen, and muscle (ham) more than ferrous sulfate. However, in the current study, tissue Fe from the different sources of Fe showed differences in liver and LD muscle but inconsistently between the two tissues. Organic Fe deposited more Fe in muscle but inorganic Fe deposited more Fe in liver. Iron is known to be stored in liver as ferritin and hemosiderin. Iron in muscle, on the other hand, is stored mainly as myoglobin (heme Fe). However, the stored Fe only accounts for 1/3 of total Fe in the body, the remaining 2/3 Fe is in the circulation, the majority as hemoglobin (heme Fe) in blood [2]. Liver Fe status is generally believed to reflect the animal's Fe status. In a recent sow study, Peters et al [12] fed OTM (Bioplex Cu, Fe, Mn, Zn; Alltech Inc., USA) to reproducing sows across 6 parities and demonstrated that the liver Fe was not affected by the source of trace minerals (OTM vs ITM: 133.9 vs 144.6 mg/kg; $p = 0.39$). Even though the result was not significant, the similar trend, where inorganic trace mineral deposition was greater than organic trace mineral deposition, was shown in the current study. In agreement, Martin et al [13] evaluated the OTM and ITM supplementation from weaning to 35 d postweaning and demonstrated that iron sulfate deposited more Fe in liver than organic (chelated) source of Fe but not in

kidney. A recent trial conducted by Thomaz et al [20] indicated that iron sulfate fed pigs have more liver Fe than Fe proteinate fed pigs for an inclusion rate at 100% of requirement but similar liver Fe at 50% requirements for nursery (postweaning to 42 d) pigs. In an early study by Standish and Ammerman [21], excess Fe as ferrous sulfate or ferric citrate fed to sheep increased liver and spleen Fe dramatically, but the muscle Fe was not affected. However, the current finding indicated the muscle Fe can be changed with organic sources of Fe and there may not be adequate Fe for liver from the organic source. This is consistent with the suggestion that the organic source of Fe is absorbed via alternative pathways, possibly amino acid or peptide transporters, and stored in different tissues.

Copper, after being absorbed in the intestine, rapidly enters blood circulation and is quickly deposited mainly into the liver [22]. Transcuprein is involved in the initial distribution of incoming dietary Cu to liver and kidney [23]. The trace mineral concentration in kidney typically increases with higher dietary mineral intakes. In the current study, organic Cu deposited more Cu in LD muscle and in kidney than inorganic Cu but the liver Cu was similar between the two treatments. Guo et al [24], in a chick study, reported that Cu proteinates deposited more Cu in liver compared with Cu sulfate and Hansen et al [25] reported that feeding copper glycinate in calves resulted in greater liver Cu, but those two studies did not report other tissue Cu concentrations. However, Miles et al [26] in chicks and Mondal et al [27] in kids failed to show any sustained advantage for organic Cu in liver. In a rat study, Rojas et al [28] reported that muscle and kidney Cu concentrations from copper sulfate were higher than copper lysine. In another study, Engle et al [29] failed to detect the effect of organic Cu (proteinates) on liver and *longissimus* muscle Cu concentration in growing and finishing steers. There are inconsistent results from a variety of sources of Cu. In the current study, the organic Cu did elevate Cu in LD muscle and kidney. High Cu in kidney, which is also an excretory route for many minerals may indicate that the organic Cu provides more available Cu than the pigs need; as a result, animals have to excrete them instead.

Organic Zn has received much attention in pig research. In the current study, only metacarpal bone Zn was elevated by the organic source of Zn. Kidney Zn showed some tendency to increase with organic Zn. In agreement, Cheng et al [30] reported zinc lysine and ZnSO₄ have equal effects on liver and kidney Zn concentrations in young pigs. A similar result reported by van Heugten et al [31] demonstrated piglets fed basal diets with an addition of 80 ppm Zn from zinc methionine and zinc lysine did not affect Zn concentrations in liver, pancreas, and spleen. In another pig study, Case and Carlson [32] supplemented 500 ppm Zn as a Zn-amino acid complex or ZnO in piglets and showed a similar Zn concentration in liver and kidney. A recent study done by Thomaz et al [20] reported that Zn proteinate resulted in similar metacarpal bone Zn content compared with ZnSO₄,

but both treatments were greater than control (no Zn supplementation) for postweaning to 42 d trial. Clearly, bone Zn was elevated with Zn supplementation but there was no source difference. But in the current trial, bone Zn was elevated with organic Zn, the disparity might be due to the duration of the trial (42 d vs 98 d) or level of supplementation. The current result might indicate that the NRC [8] level of Zn is adequate for normal animal growth.

Tissue mineral concentration may be one of the indicators of mineral metabolism in the body and to some extent, might be one of the measurements of the mineral bioavailability. Generally, the current results indicate that the organic source of the trace minerals Cu, Fe, Mn, and Zn might have higher bioavailability based on greater mineral being deposition into the various tissues, especially for Mn and Cu. The exception may be that the current study showed that the organic Fe had a negative impact on liver Fe concentration. Research needs to be conducted on more indicators (e.g., plasma Fe status or hemoglobin status) to give the full picture of Fe status in the body.

In the current study, the trace mineral premix (Cu, Fe, Mn, and Zn) was removed from the study prior to slaughter for 6, 4, 2, and 0 (no removal) weeks. The results did show trace mineral status in the various tissues was changed with the duration of trace mineral deletion. One remarkable response was evidenced by the change of Mn concentration during the deletion period. In almost all the collected tissues (heart, kidney, liver, and LD muscle), Mn concentrations increased with increasing duration of trace mineral deletion. Kidney Fe showed a similar trend as Mn in visceral tissues. As expected, Zn concentrations in kidney, liver, spleen, and metacarpal bone decreased as the time of trace mineral deletion increased as did kidney Cu.

Shelton et al [6] removed the trace mineral premix (Cu, Fe, Mn, Zn, I, and Se) in growing and finishing pigs (22 to 109 kg) and demonstrated that liver Zn content or concentration was decreased whereas Mn was increased; liver Fe and Cu content or concentration were not affected by the trace mineral removal; metacarpal bone Zn was decreased as well as muscle Zn tended to be decreased by the trace mineral premix removal, which agrees with the present results of Zn status in liver, muscle, and bone. In addition, Adeola et al [18] indicated that supplementing pig diets with Zn decreased Mg and Mn absorption and retention, which indicated that there might be an interaction between Zn and Mn. In the current study, even though Mn and Zn concentrations in heart, spleen, and LD muscle were not shown as consistent as liver and kidney, Mn and Zn concentrations in those tissues of ITM-fed pigs clearly provided evidence of the interaction between Mn and Zn. However, Shelton et al [14] in a nursery pig study, and Shelton and Southern [33] in a chick study, did not observe the Zn×Mn interaction, which indicates that the degree of this interaction may differ in different physiological stages and among species.

In the current study, liver and LD muscle Cu and Fe content or concentration were not affected by the trace mineral deletion,

which is consistent with Edmonds and Arentson [4], Shaw et al [5], and Shelton et al [6]. However, the current research did observe that kidney Cu content or concentration was decreased and Fe content or concentration tended to be increased with increasing duration of trace mineral deletion. The decreasing kidney Cu content or concentration was probably due to the decreasing dietary Cu level. No explanation is made for a kidney Fe increase with decreasing dietary Fe level.

Metacarpal bone mineral content has often been assessed for Ca and P, but less so for trace minerals. In the current trial, with increasing duration of mineral deletion, almost all of the trace minerals analyzed in bone were decreased, especially for Zn. In agreement, Thomaz et al [20] reported that metacarpal bone Zn was elevated dramatically with mineral supplementation compared with control (no mineral supplementation) during a 42-d nursery pig study. Interestingly, bone Cu and Mn were decreased with additional mineral supplementation, which may suggest a certain level of mineral-mineral interactions. In addition, Shelton et al [6] reported that bone Zn and Mn were decreased when mineral premix was removed from the grow-finish diets, but for Cu and Fe there was no consistent effect on the mineral content in bone.

Interactions between the source and the duration of trace mineral deletion were observed in the current study. With increasing time of trace mineral deletion, organic Zn deposited more in LD muscle whereas inorganic Zn deposited less in muscle. A similar observation occurred in spleen Mn content or concentration, namely, ITM deletion caused spleen Mn content or concentration to increase, but, in the OTM treatment, spleen Mn content or concentration remained constant during the deletion, indicating that animals can mobilize body reserve Mn to compensate for the lower Mn level in the diet.

IMPLICATIONS

The current research demonstrated that the organic source of the trace minerals Cu, Fe, Mn, and Zn that was used may have better bioavailability than the traditional inorganic source of trace minerals, evidenced by higher deposition in visceral organs although the effects differed by organ, mineral, and body weight of the pigs. Trace mineral concentrations in various tissues during trace mineral deletion indicated that trace mineral concentrations were affected by the trace mineral deletion and that there might be an Zn×Mn interaction, suggesting potential inhibition of Mn absorption might be occurring based on current trace mineral supplementation and caution should be considered when removing trace minerals from the diet during the finishing period.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial

organization regarding the material discussed in the manuscript.

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REFERENCES

1. Black JR, Ammerman CB, Henry PR. Effects of high dietary manganese as manganese oxide or manganese carbonate in sheep. *J Anim Sci* 1985;60:861-6.
2. Beard J, Dawson H. Iron. New York, USA: Marcel Dekker, Inc; 1997.
3. Mahan DC, Parrett NA. Evaluating the efficacy of selenium-enriched yeast and sodium selenite on tissue selenium retention and serum glutathione peroxidase activity in grower and finisher swine. *J Anim Sci* 1996;74:2967-74.
4. Edmonds MS, Arentson BE. Effect of supplemental vitamins and trace minerals on performance and carcass quality in finishing pigs. *J Anim Sci* 2001;79:141-7.
5. Shaw DT, Rozeboom DW, Hill GM, Booren AM, Link JE. Impact of vitamin and mineral supplement withdrawal and wheat middling inclusion on finishing pig growth performance, fecal mineral concentration, carcass characteristics, and the nutrient content and oxidative stability of pork. *J Anim Sci* 2002;80:2920-30.
6. Shelton JL, Southern LL, LeMieux FM, Bidner TD, Page TG. Effects of microbial phytase, low calcium and phosphorus, and removing the dietary trace mineral premix on carcass traits, pork quality, plasma metabolites, and tissue mineral content in growing-finishing pigs. *J Anim Sci* 2004;82:2630-9.
7. Ma YL, Lindemann MD, Cromwell GL, et al. Evaluation of trace mineral source and preharvest deletion of trace minerals from finishing diets for pigs on growth performance, carcass characteristics, and pork quality. *J Anim Sci* 2012;90:3833-41.
8. Committee on Nutrient Requirements of Swine, National Research Council. Nutrient requirements of swine. 11th ed ed. Washington, DC, USA: National Academy Press; 1998.
9. Cunniff P. Official Methods of Analysis of AOAC International, 17th edn, 2nd revision. Gaithersburg, MD, USA: AOAC International; 2003.
10. Lindemann MD, Kim BG. Technical note: a model to estimate individual feed intake of swine in group feeding. *J Anim Sci* 2007;85:972-5.
11. Gheisari AA, Rahimi-fathkoobi A, Toghiani M, Gheisari M. Effects

- of organic chelates of zinc, manganese and copper in comparison to their inorganic sources on performance of broiler chickens. *J Anim Plant Sci* 2010;6:630-6.
12. Peters JC, Mahan DC, Wiseman TG, Fastinger ND. Effect of dietary organic and inorganic micromineral source and level on sow body, liver, colostrum, mature milk, and progeny mineral compositions over six parities. *J Anim Sci* 2010;88:626-37.
 13. Martin RE, Mahan DC, Hill GM, Link JE, Jolliff JS. Effect of dietary organic microminerals on starter pig performance, tissue mineral concentrations, and liver and plasma enzyme activities. *J Anim Sci* 2011;89:1042-55.
 14. Shelton JL, LeMieux FM, Southern LL, Bidner TD. Effect of microbial phytase addition with or without the trace mineral premix in nursery, growing, and finishing pig diets. *J Anim Sci* 2005;83:376-85.
 15. Shelton JL, Southern LL, Bidner TD, et al. Effect of microbial phytase on energy availability, and lipid and protein deposition in growing swine. *J Anim Sci* 2003;81:2053-62.
 16. Deyhim F, Teeter RG. Dietary vitamin and/or trace mineral premix effects on performance, humoral mediated immunity, and carcass composition of broilers during thermoneutral and high ambient temperature distress. *J Appl Poult Res* 1993;2:347-55.
 17. Li S, Luo X, Liu B, et al. Use of chemical characteristics to predict the relative bioavailability of supplemental organic manganese sources for broilers. *J Anim Sci* 2004;82:2352-63.
 18. Adeola O, Lawrence BV, Sutton AL, Cline TR. Phytase-induced changes in mineral utilization in zinc-supplemented diets for pigs. *J Anim Sci* 1995;73:3384-91.
 19. Yu B, Huang WJ, Chiou PWS. Bioavailability of iron from amino acid complex in weanling pigs. *Anim Feed Sci Technol* 2000;86:39-52.
 20. Thomaz MC, Watanabe PH, Pascoal LA, et al. Inorganic and organic trace mineral supplementation in weanling pig diets. *An Acad Bras Cienc* 2015;87:1071-81.
 21. Standish JF, Ammerman CB. Effect of excess dietary iron as ferrous sulfate and ferric citrate on tissue mineral composition of sheep. *J Anim Sci* 1971;33:481-4.
 22. Deshpande SS. *Handbook of food toxicology*. New York, USA: Marcel Dekker; 2002.
 23. Liu N, Lo L, Tran L, Jones L, Linder MC. Identity and regulation of the copper transport protein. In: Roussel AM, Anderson RA, Favier AE, editors. *Trace Elements in Man and Animals 10*. New York, USA: Kluwer Academic/Plenum Publishers; 2000. p. 955-6.
 24. Guo R, Henry PR, Holwerda RA, et al. Chemical characteristics and relative bioavailability of supplemental organic copper sources for poultry. *J Anim Sci* 2001;79:1132-41.
 25. Hansen SL, Schlegel P, Legleiter LR, Lloyd KE, Spears JW. Bioavailability of copper from copper glycinate in steers fed high dietary sulfur and molybdenum. *J Anim Sci* 2008;86:173-9.
 26. Miles RD, Henry PR, Sampath VC, Shivazad M, Comer CW. Relative bioavailability of novel amino acid chelates of manganese and copper for chicks. *J Appl Poult Res* 2003;12:417-23.
 27. Mondal MK, Biswas P, Roy B, Mazumdar D. Effect of copper sources and levels on serum lipid profiles in Black Bengal (*Capra hircus*) kids. *Small Rumin Res* 2007;67:28-35.
 28. Rojas LX, McDowell LR, Cousins RJ, et al. Interaction of different organic and inorganic zinc and copper sources fed to rats. *J Trace Elem Med Biol* 1996;10:139-44.
 29. Engle T, Spears J, Armstrong T, Wright C, Odle J. Effects of dietary copper source and concentration on carcass characteristics and lipid and cholesterol metabolism in growing and finishing steers. *J Anim Sci* 2000;78:1053-9.
 30. Cheng J, Kornegay ET, Schell T. Influence of dietary lysine on the utilization of zinc from zinc sulfate and a zinc-lysine complex by young pigs. *J Anim Sci* 1998;76:1064-74.
 31. van Heugten E, Spears JW, Kegley EB, Ward JD, Qureshi MA. Effects of organic forms of zinc on growth performance, tissue zinc distribution, and immune response of weanling pigs. *J Anim Sci* 2003;81:2063-71.
 32. Case CL, Carlson MS. Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. *J Anim Sci* 2002;80:1917-24.
 33. Shelton JL, Southern LL. Effects of phytase addition with or without a trace mineral premix on growth performance, bone response variables, and tissue mineral concentrations in commercial broilers. *J Appl Poult Res* 2006;15:94-102.