Determining the Effects of Manganese Source and Level in Diets Containing High Levels of Copper on Growth Performance of Growing-Finishing Pigs

H. R. Kerkaert  
*Kansas State University*, kerkaert@k-state.edu

J. C. Woodworth  
*Kansas State University*, jwoodworth@ksu.edu

J. M. DeRouchey  
*Kansas State University, Manhattan*, jderouch@k-state.edu

See next page for additional authors

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Authors
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Determining the Effects of Manganese Source and Level in Diets Containing High Levels of Copper on Growth Performance of Growing-Finishing Pigs¹,²

Hayden R. Kerkaert, Jason C. Woodworth, Joel M. DeRouchey, Steve S. Dritz,³ Mike D. Tokach, Robert D. Goodband, and Naiana E. Manzke⁴

Summary
A total of 1,994 pigs (PIC; 337 × 1050; initially 88.2 lb) were used to determine the effect of manganese (Mn) source and level on finishing pig growth performance. This experiment was a follow-up to an Mn source by level study conducted last year. However, unlike last year’s study, in the present study all diets contained 150 ppm added Cu from Cu hydroxychloride (IBC; Micronutrients, Indianapolis, IN). Dietary treatments were arranged in a 2 × 3 factorial with main effects of Mn source (Mn hydroxychloride, IntelliBond M, Micronutrients, Indianapolis, IN; or Mn sulfate, MnSO₄, Eurochem, Veracruz, Mexico), and increasing added Mn concentration (8, 16, and 32 ppm). The trace mineral premix was formulated without added Mn. There were 27 pigs per pen and 12 pens per treatment. Diets were corn-soybean meal-distillers dried grains with solubles-based and were fed in 4 phases. Overall, there was no (P > 0.10) Mn source × level interaction observed for average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (F/G). Pigs fed IBM had increased (P < 0.05) final body weight (BW), ADG, and ADFI compared to pigs fed MnSO₄. Pigs fed 16 ppm of Mn tended (P = 0.088) to have reduced ADFI when compared to pigs fed 8 and 32 ppm of Mn. In conclusion, there appears to be little benefit in growth performance by feeding more than 8 ppm of added Mn. However, pigs fed IBM had improved growth performance compared with those fed MnSO₄. This response is different than our previous study with identical Mn sources but without high levels of added Cu.⁵

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³ Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University.
⁴ Micronutrients, Indianapolis, IN.
⁵ Kerkaert, H.R., J.C. Woodworth, J.M. DeRouchey, S.S. Dritz, M.D. Tokach, and R.D. Goodband. 2019. Determining the effects of manganese source and level on growth performance, carcass characteristics, and economics of growing-finishing pigs. Kansas Agricultural Experiment Station Research. Reports: Vol. 5 Iss. 8. https://newprairiepress.org/kaesrr/vol5/iss8/23/.
Further research is needed to understand why we observed an Mn source difference to Mn hydroxychloride when fed in conjunction with pharmacological levels of Cu on pig growth performance.

**Introduction**

Manganese is an essential trace mineral that is a key component in carbohydrate, lipid and protein metabolism. According to the NRC, the quantitative requirement for Mn for nursery and finishing diets ranges from 2 to 4 ppm. Assuming bioavailability is not a concern, many swine diets today meet the NRC estimated requirement for Mn from the major dietary ingredients before a trace mineral premix is added to the diet. However, due to the unknown bioavailability of the innate Mn in ingredients, swine diets typically contain added Mn through a trace mineral premix. In a survey conducted by Flohr et al., swine diet Mn levels were found to be supplemented at as low as 3.3 ppm and as high as 40 ppm throughout the entire finishing period. Therefore, there is a wide discrepancy of Mn supplementation in commercial swine diets.

In a previous study, we evaluated the effects of increasing Mn from MnSO₄ or Mn hydroxychloride (IBM; Micronutrients, Indianapolis, IN). In that study there was no evidence of differences observed between the two Mn sources; however, these diets did not contain high levels of added Cu. Therefore, the objective of this study was to further determine the effects of increasing dietary levels of Mn and the source of Mn on growth performance of growing-finishing pigs fed diets with high levels of added Cu.

**Procedures**

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in this experiment.

**Animals and Diets**

This study was conducted in two barns at a commercial research-finishing site in southwest Minnesota (New Horizon Farms, Pipestone, MN). Each barn was naturally ventilated and double-curtain-sided with a slatted concrete floor and deep manure storage. Each pen (10 × 18 ft) was equipped with a 5-hole stainless steel dry self-feeder (Thorp Equipment, Thorp, WI) and a bowl waterer for *ad libitum* access to feed and water.

Two groups of 972 pigs (1,944 total pigs, PIC 337 × 1050; initial BW 80.2 lb) were used in a 100-d growth trial. Pigs were housed in mixed gender pens with 27 pigs per pen and 12 pens per treatment. Daily feed additions to each pen were achieved by using a robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) able to record feed amounts for individual pens. The treatments were structured as a randomized complete block design and arranged in a 2 × 3 factorial with main effects of Mn source (MnSO₄, Eurochem, Veracruz, Mexico; or Mn hydroxychloride, IBM; IntelliBond M, Micronutrients USA, LLC, Indianapolis, IN) and increasing Mn (8, 16, or 32 ppm). All treatment diets were manufactured at the New Horizon Farms Feed Mill in Pipestone,

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6 NRC. 2012. Nutrient Requirements of Swine. 11th rev. ed. National Academy Press, Washington, DC.
7 Flohr, J. R., J.M. DeRouchey, J.C. Woodworth, M.D. Tokach, R.D. Goodband, and S.S. Dritz. 2016. A survey of current feeding regimens for vitamins and trace minerals in the US swine industry. J. Swine Health Prod. 24:290-303.
MN, and were formulated to meet or exceed NRC\textsuperscript{6} requirement estimates for growing-finishing pigs for their respective weight ranges (Table 1). Diets were fed in meal form and in 4 dietary phases within each experiment.

Experimental diets were corn-soybean meal-DDGS-based and were formulated with premix without Mn, Cu, and Zn. Manganese, Cu, and Zn were added to the diet by hand-made premixes, which were added in place of corn in the diet. Each hand-made addition contributed the desired source of Mn, MnSO\textsubscript{4} or IBM, and Mn level, 8, 16, or 32 ppm to the appropriate treatment, along with hand additions to provide 150 ppm of Cu from IntelliBond C (Micronutrients, Indianapolis, IN) and 80 ppm of Zn from IntelliBond Z (Micronutrients, Indianapolis, IN).

Pigs were weighed approximately every 14 days to determine ADG, ADFI, and F/G. Because of the ongoing outbreak of COVID-19, only final weights were obtained, and no carcass data were collected.

**Chemical Analysis**

Representative diet samples were obtained from all feeders of each treatment and delivered to the Kansas State University Swine Laboratory, Manhattan, KS, and stored at -20°C until analysis. Samples of the diets were combined within dietary treatment, and two composite samples from each treatment were analyzed in duplicate (Cumberland Valley Analytical Services, Hagerstown, MD). Samples were analyzed for Mn, Cu, and Zn content (Method 985.01; AOAC Int., 2000).\textsuperscript{8}

**Statistical Analysis**

Data were analyzed as a randomized complete block design for one-way ANOVA using the lmer function from the lme4 package in R (version 3.5.1 (2018-07-02), R Foundation for Statistical Computing, Vienna, Austria) with pen considered the experimental unit, BW as blocking factor, and treatment as fixed effect. Predetermined orthogonal contrasts were used to evaluate the interactive effects of Mn source and level. Interactions (\(P \leq 0.10\)) were evaluated linearly or quadratically within the source. All results were considered significant at \(P \leq 0.05\) and marginally significant between \(P > 0.05\) and \(P \leq 0.10\).

**Results**

The analyzed dietary Mn, Cu, and Zn were consistent with calculated values used in diet formulation and followed the intended Mn titration additions (Table 2).

High levels of Cu were included in all diets and in the grower period, there was an Mn source × level interaction (linear, \(P = 0.029\)) observed for F/G, with F/G improving as Mn increased from IBM, but decreasing with increased Mn from MnSO\textsubscript{4}. There was no evidence (\(P > 0.10\)) for Mn source or Mn level effect on ADG, but ADFI was greater (\(P = 0.034\)) when Mn was provided by IBM. Pigs fed 16 ppm Mn tended (\(P = 0.052\)) to have decreased ADFI when compared to those fed 8 or 32 ppm, regardless of source.

\textsuperscript{8} AOAC International. 2000. Official methods of analysis of AOAC International. 17th ed. AOAC Int., Gaithersburg, MD.
In the finisher period, there was an Mn source × level interaction (linear, $P = 0.039$) for ADG, with ADG improving as supplemental Mn was increased for MnSO$_4$ but decreasing when Mn was increased for pigs fed IBM. There was no evidence ($P > 0.10$) of difference for Mn level to influence ADFI, but ADFI was greater ($P = 0.049$) for pigs fed Mn from IBM compared to pigs fed Mn from MnSO$_4$. There was no evidence of difference ($P > 0.10$) for an Mn source or level effect on F/G in the finisher period.

Overall, there was no evidence ($P > 0.10$) for an Mn source × level interaction for final body weight or any observed growth responses. Pigs fed Mn provided by IBM had greater ($P < 0.05$) ADG and ADFI and heavier ($P < 0.05$) final BW than pigs fed Mn from MnSO$_4$. Regardless of the source, pigs fed 16 ppm of Mn tended (quadratic, $P = 0.088$) to have lower overall ADFI than pigs fed 8 or 32 ppm of Mn. There was no evidence of difference ($P > 0.10$) for an Mn source or level effect on F/G.

**Discussion**

According to the NRC, the Mn requirement estimate for growing-finishing pigs is 2 to 4 ppm of the diet. However, most of the research for determining the Mn requirement was conducted more than 50 years ago. Due to the unknown bioavailability of Mn from ingredients commonly used in diets, Mn is usually added to swine diets through a trace mineral premix, frequently as MnSO$_4$. Manganese hydroxychloride is another source of Mn that can be added to trace mineral premixes for swine diets. Hydroxychloride-based minerals are manufactured through the reaction of hydrochloric acid, high purity forms of metal, and water. The products of this reaction are hydroxychloride crystals that contain the desired metal covalently bonded to chloride and hydroxyl groups. The covalent bonds processed by hydroxychloride minerals reduce the ability for the minerals to react with other components of the diet and potentially improve bioavailability.

In a previous study, there was no main effect of Mn source on any influence growth performance or carcass characteristics; however, in the present study pigs fed supplemental Mn from IBM had increased ADG and ADFI and heavier final BW when compared to pigs fed MnSO$_4$. The reason for these inconsistent effects of IBM on growth performance was not clear, but could have been a result of the higher levels of dietary Cu in the present experiment. Pharmacological levels of Cu have been shown to improve growth performance in growing-finishing pigs. Copper is excreted from the body through bile. However, when dietary Mn was increased to 200 ppm in rats, Mercandante et al. observed a reduction in Cu levels in the bile, signifying a hepatobiliary metabolism relationship between Mn and Cu. With the potential of improved bioavailability of the Mn hydroxychloride, less Cu may have been excreted, allowing for potentially greater utilization of Cu in pigs being fed Mn hydroxychloride. However, this theory warrants further investigation.

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Mercandante, C. J., C. Herrera, M. A. Pettiglio, M. L. Foster, L. C. Johnson, D. C. Dorman, and T. B. Bartnikas. 2016. The effect of high dose oral manganese exposure on copper, iron, and zinc levels in rats. Biometals. 29:417-422. doi:10.1007/s10534-016-9924-6.
In summary, our results suggest that supplementing growing-finishing diets with greater than 8 ppm of Mn did not lead to any improvements in growth performance. Pigs fed Mn provided by IBM had greater ADG, ADFI, and heavier final BW than pigs fed Mn from MnSO$_4$. More research is needed to further understand the potential benefits of Mn hydroxychloride fed in conjunction with high levels of Cu on pig growth performance.

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Table 1. Composition of basal diet (as-fed basis)\(^1\)

| Ingredients, % | Phase 1 | Phase 2 | Phase 3 | Phase 4 |
|----------------|---------|---------|---------|---------|
| Corn           | 58.80   | 66.88   | 72.51   | 80.66   |
| Soybean meal (46.5% CP) | 26.60   | 18.77   | 13.29   | 15.35   |
| DDGS\(^2\)     | 10.00   | 10.00   | 10.00   | ---     |
| Beef tallow    | 1.50    | 1.50    | 1.50    | 1.50    |
| Limestone, ground | 1.08    | 1.00    | 0.95    | 0.73    |
| Monocalcium phosphate (21% P) | 0.90    | 0.75    | 0.65    | 0.75    |
| Salt           | 0.35    | 0.35    | 0.35    | 0.35    |
| L-Lysine-HCl   | 0.37    | 0.39    | 0.39    | 0.30    |
| DL-Methionine  | 0.06    | 0.03    | 0.01    | 0.02    |
| L-Threonine    | 0.09    | 0.09    | 0.10    | 0.10    |
| L-Tryptophan   | 0.02    | 0.03    | 0.03    | 0.03    |
| Phytase\(^3\)  | 0.04    | 0.04    | 0.04    | 0.04    |
| Vitamin-trace mineral premix\(^4\) | 0.15    | 0.15    | 0.15    | 0.15    |
| Mn source\(^5\) | +/-     | +/-     | +/-     | +/-     |

Calculated analysis

Standardized ileal digestible amino acids, %

| Amino Acids                      | Phase 1 | Phase 2 | Phase 3 | Phase 4 |
|----------------------------------|---------|---------|---------|---------|
| Lysine                           | 1.15    | 0.97    | 0.84    | 0.79    |
| Isoleucine:lysine                | 63      | 61      | 59      | 60      |
| Leucine:lysine                   | 140     | 147     | 155     | 147     |
| Methionine:lysine                | 31      | 30      | 29      | 29      |
| Methionine and cysteine:lysine   | 55      | 55      | 56      | 56      |
| Threonine:lysine                 | 62      | 62      | 64      | 65      |
| Tryptophan:lysine                | 19      | 19      | 19      | 20      |
| Valine:lysine                    | 70      | 70      | 70      | 70      |
| Lysine:net energy, g/Mcal        | 4.62    | 3.82    | 3.26    | 3.05    |
| Net energy, kcal/kg              | 2,486   | 2,539   | 2,574   | 2,594   |
| Crude protein, %                 | 20.8    | 17.8    | 15.6    | 14.4    |
| Calcium, %                       | 0.73    | 0.63    | 0.57    | 0.52    |
| Standardized total tract digestible phosphorus, % | 0.52 | 0.47 | 0.41 | 0.39 |

\(^1\)In Exp. 1, phases 1, 2, 3, and 4 were fed from 76.1 to 125.0 lb, 125.0 to 160.1 lb, 160.1 to 220.0 lb, and 220.0 lb to market, respectively. In Exp. 2, phases 1, 2, 3, and 4 were fed from 88.2 to 108.0 lb, 108.0 to 169.8 lb, 169.8 to 229.9 lb, and 229.9 lb to market, respectively.

\(^2\)DDGS = dried distillers grains with solubles.

\(^3\)Optiphos 2000 (Huvepharma Inc. Peachtree City, GA) provided 858.7 units of phytase FTU/kg of diet with an assumed release of 0.12 available P.

\(^4\)Tribasic copper chloride (IntelliBond C, Micronutrients, Indianapolis, IN) provided 150 ppm of Cu.

\(^5\)Mn hydroxychloride (IntelliBond M, Micronutrients, Indianapolis, IN); or Mn sulfate (MnSO\(_4\), Eurochem, Veracruz, Mexico).
### Table 2. Chemical analysis of diets (as-fed basis)¹

| Mineral, ppm | MnSO₄, ppm | IBM, ppm |
|--------------|------------|----------|
|              | 8          | 16       | 32       | 8          | 16       | 32       |
| Cu           | 217        | 207      | 194      | 197        | 199      | 206      |
| Mn           | 34         | 35       | 50       | 33         | 42       | 52       |
| Zn           | 126        | 132      | 130      | 126        | 131      | 125      |

¹Values represent means from 16 composite samples (4 per phase). For each treatment, samples were collected from multiple feeders, blended, subsampled, ground, and analyzed (Cumberland Valley Analytical Services, Hagerstown, MD). IntelliBond M (IBM, Micronutrients, Indianapolis, IN).

### Table 3. Interactive effects of Mn source and level on grow-finish pig growth performance¹

| Item²³ | MnSO₄, ppm | IBM, ppm | Source × level, P = |
|--------|------------|----------|---------------------|
|        | 8          | 16       | 32                  | SEM | Linear | Quadratic |
| BW, lb | 88.2       | 88.2     | 88.2                | 88.0 | 0.77   | 0.821     | 0.650 |
| Grower | 167.3      | 165.6    | 164.9               | 167.1 | 1.20  | 0.468     | 0.699 |
| Final  | 286.4      | 286.2    | 286.6               | 289.2 | 1.32  | 0.391     | 0.207 |
| Grower | 1.96       | 1.94     | 1.92                | 1.96  | 0.013 | 0.330     | 0.615 |
| ADFI, lb| 4.25       | 4.17     | 4.23                | 4.30  | 0.034 | 0.578     | 0.879 |
| F/G    | 2.160      | 2.155    | 2.212               | 2.179 | 0.0051| 0.029     | 0.652 |
| Finisher | 2.09      | 2.12     | 2.14                | 2.14  | 0.011 | 0.039     | 0.258 |
| ADFI, lb| 6.19       | 6.15     | 6.24                | 6.28  | 0.030 | 0.272     | 0.782 |
| F/G    | 2.941      | 2.907    | 2.915               | 2.941 | 0.0041| 0.267     | 0.402 |
| Overall | 2.05       | 2.05     | 2.05                | 2.07  | 0.007 | 0.284     | 0.213 |
| ADFI, lb| 5.36       | 5.31     | 5.40                | 5.42  | 0.029 | 0.236     | 0.856 |
| F/G    | 2.625      | 2.604    | 2.639               | 2.632 | 0.0038| 0.801     | 0.332 |

¹A total of 1,944 pigs (initial BW of 88.2 lb) were used in two groups with 27 pigs per pen and 12 replicates per treatment. Mn sources were Mn sulfate (MnSO₄, Erachem, Veracruz, Mexico) or IntelliBond M (IBM, Micronutrients, Indianapolis, IN).

²BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. F/G = feed efficiency. HCW = hot carcass weight.

³The grower period was from d 0 to d 42 in group 1 and from d 0 to 38 in group 2. The finisher period was from d 42 to 97 in group 1 and from d 38 to 100 in group 2.
Table 4. Main effects of Mn source and level on finishing pig growth performance

| Item | Source | Level, mg/lb | Probability, P | Probability, P | SEM | Linear | Quadratic |
|------|--------|--------------|----------------|----------------|-----|---------|-----------|
| | Source | MnSO₄ | IBM | SEM | Probability, P | 8 | 16 | 32 | SEM | Linear | Quadratic |
| BW, lb | | | | | | | | | | | |
| Initial | | 88.2 | 88.2 | 0.76 | 0.683 | 88.2 | 88.2 | 88.2 | 0.76 | 0.977 | 0.920 |
| Grower | | 166.0 | 167.1 | 1.12 | 0.202 | 167.8 | 165.8 | 166.0 | 1.14 | 0.153 | 0.182 |
| Final | | 286.4 | 289.7 | 1.18 | 0.011 | 289.2 | 286.8 | 287.7 | 1.21 | 0.391 | 0.201 |
| Grower | | | | | | | | | | | |
| ADG, lb | | 1.94 | 1.96 | 0.0095 | 0.169 | 1.97 | 1.94 | 1.94 | 0.0104 | 0.273 | 0.221 |
| ADFI, lb | | 4.22 | 4.30 | 0.0287 | 0.034 | 4.31 | 4.21 | 4.26 | 0.0301 | 0.478 | 0.052 |
| F/G | | 2.174 | 2.188 | 0.0041 | 0.407 | 2.179 | 2.169 | 2.193 | 0.0043 | 0.340 | 0.319 |
| Finisher | | | | | | | | | | | |
| ADG, lb | | 2.12 | 2.15 | 0.0069 | 0.049 | 2.15 | 2.13 | 2.13 | 0.0079 | 0.650 | 0.352 |
| ADFI, lb | | 6.20 | 6.29 | 0.0213 | 0.049 | 6.27 | 6.19 | 6.26 | 0.0240 | 0.930 | 0.140 |
| F/G | | 2.924 | 2.915 | 0.0032 | 0.846 | 2.915 | 2.907 | 2.933 | 0.0035 | 0.612 | 0.671 |
| Overall | | | | | | | | | | | |
| ADG, lb | | 2.04 | 2.07 | 0.0042 | 0.009 | 2.07 | 2.05 | 2.06 | 0.0048 | 0.309 | 0.103 |
| ADFI, lb | | 5.36 | 5.42 | 0.0238 | 0.023 | 5.44 | 5.36 | 5.42 | 0.0254 | 0.912 | 0.088 |
| F/G | | 2.625 | 2.625 | 0.0032 | 0.865 | 2.618 | 2.618 | 2.639 | 0.0034 | 0.809 | 0.332 |

1A total of 1,944 pigs (initial BW of 88.2 lb) were used in two groups with 27 pigs per pen and 12 replicates per treatment. Mn sources were Mn sulfate (MnSO₄, Eurochem, Veracruz, Mexico) or IntelliBond M (IBM, Micronutrients, Indianapolis, IN).
2BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. F/G = feed efficiency. HCW = hot carcass weight.
3The grower period was from d 0 to d 42 in group 1 and from d 0 to 38 in group 2. The finisher period was from d 42 to 97 in group 1 and from d 38 to 100 in group 2.