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# The Effect of Increasing Dietary Manganese from an Organic Source on the Reproductive Performance of Sows

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**Abstract:** The objective of this study was to determine the effect of dietary manganese on the reproductive performance of sows. Sows ($n$ = 39; 231 ± 8 kg) were randomly assigned to one of three dietary levels of supplemented Mn (CON: 0 ppm Mn; PRO20: 20 ppm Mn; PRO40: 40 ppm Mn). The experimental treatments were initiated at breeding and continued through two parities. The sows were blocked by parity within each farrowing group. The data were analyzed as a randomized complete block design using the MIXED procedure of SAS with diet as a fixed effect and block as a random effect. The lactation feed intake increased in the PRO20 sows compared to the CON and PRO40 sows ($p < 0.05$). The PRO20 and PRO40 sows farrowed piglets with improved average daily gain from birth to weaning (CON 214 g/day; PRO20 237 g/day; 220 g/day; $p < 0.05$) compared to the CON sows. The milk fat content was lower in the PRO20 (5.5%) and PRO40 sows (6.1%; $p < 0.05$) compared to the CON sows (7.8%), possibly due to increased milk demand. Supplementary dietary Mn throughout two gestation and lactation cycles led to improved birth weights and pre-weaning growth of piglets.

**Keywords:** lactation; manganese; reproductive performance; sows



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## 1. Introduction

Reproductive efficiency is an important aspect of the swine industry. Genetics, nutrition, and the environment are contributing factors to a sow's reproductive efficiency [1], but nutrition is one of the easiest factors for producers to control. Adequate nutrition for gestating and lactating sows ensures a balance between energy expenditure and investment as her body and metabolism shift to accommodate the developing offspring and mammary tissue [1,2]. Neonates experience innate nutritional deficiencies that must be corrected via the formulation of the sow's diet because suckling piglets obtain the majority of their required nutrients from the sow via colostrum and milk until weaning [3,4]. Maximizing the performance of sows and their litters during the lactation period of reproduction is therefore a major focus for swine nutritionists [5].

Manganese (Mn) is an important inorganic dietary component found in low concentrations in most feedstuffs [6]. The basal levels of Mn in feedstuffs alone are not sufficient for optimal growth and are of unknown availability to the animal [6]. Therefore, Mn must be supplemented in the diets of pigs. It is well established that Mn plays a role in development, digestion, reproduction, antioxidant defense, and immune function in multiple species [7–12]. Leibholz et al. established that feeding piglets 0.4 mg/kg Mn is sufficient for normal growth, and no toxicity symptoms were noted in pigs supplemented with 4000 mg/kg [10]. Plumlee et al. demonstrated that feeding a Mn-deficient diet to

gilts results in bone growth abnormalities, irregular estrous cycles, fetal resorption, and the birth of small and weak neonates [13]. Supplemental Mn in the diets of swine, poultry, and other species is necessary to prevent growth abnormalities, reproductive failure, and overall negative health concerns [6,7,13]. The form in which trace minerals, such as Mn, are supplemented (organic vs. inorganic) can affect their efficacy in improving growth, utilization, and decreasing mineral excretion [14,15]. It is believed that organic mineral sources are more available to the animal because the organic mineral complex can pass through the stomach intact and arrive in the small intestine to be absorbed more readily [15]. However, the literature is varied in the supposed benefits of using organic mineral sources compared to inorganic mineral sources. The objective of this research project was to determine the effect of increasing dietary Mn from an organic source on the reproductive performance of sows and the antioxidant status of their offspring. It was hypothesized that the supplementation of Mn would improve the reproductive performance of sows and the antioxidant status of their offspring.

## 2. Materials and Methods

The animal care, handling, and processing procedures were approved by the University of Georgia Institutional Animal Care and Use Committee (AUP #A2018 11-014-R1).

### *2.1. Sow Management*

Sows (Choice Genetics Line CG32; $n$ = 39; 231 ± 8 kg (at first breeding)) were blocked by parity (ranged 3–6 parities) within farrowing groups at breeding, with all dietary treatments being represented within each block. Sow diets were formulated with three levels of supplemental Mn (ProPath® Mn, Zinpro Corp, Eden Prairie, MN, USA): Parity 1 on treatment ($n$ = 39): Control ($n$ = 13; CON; no supplemental Mn, Table 1), 20 ppm Mn ($n$ = 13; PRO20), or 40 ppm Mn ($n$ = 13; PRO40); Parity 2 on treatment ($n$ = 35): CON ($n$ = 11), PRO20 ($n$ = 11), PRO40 ($n$ = 13). The current NRC (2012) requirement for Mn in gestating and lactating sows is 25 ppm. Sows began their respective dietary treatments on the day of breeding and remained on dietary treatment until pregnancy determination after the third breeding cycle. All the sows were on a common diet for at least 90 d prior to the start of this study. During gestation, the experimental diet was mixed to contain 320 ppm Mn and then fed at 1/16 (20 ppm) or 1/8 (40 ppm) of the daily intake with the remainder being the control diet. This blending of the experimental and control diets allowed the use of the dual hopper system in the electronic sow feeder (AP®, AGCO; Duluth, Georgia). The sows were fed to maintain a body condition score of three during gestation [16]. The low Mn gestation diet and the CON lactation diet were both 42 ppm Mn, which met the NRC requirement and was not Mn-deficient (Table 1). Although 42 ppm Mn was in excess of the NRC recommendation, basal levels of Mn in corn, soybean meal, DDGS, and other ingredients are of unknown availability to the animal. During breeding and gestation, the sows were housed in a temperature-controlled (21.1 ± 2.8 °C) barn at the University of Georgia Double Bridges Swine Unit (Oglethorpe County, Georgia).

The pregnant sows were transported to an environmentally controlled (21.1 ± 2.8 °C) farrowing room (LARU; University of Georgia, Athens, Georgia) approximately d 110 ± 1 of gestation. The sows were restrict-fed upon arrival (2.27 kg/day) until 1 d post-farrowing with ad libitum access to water. The sows consumed the lactation feed and water ad libitum after 1 d post-farrowing until weaning (Table 1). The sows remained on the same dietary treatment during the gestation and lactation phases. Diet samples were collected throughout the study and were sent to the University of Georgia Feed, Water, and Soil Laboratory (Athens, Georgia) for proximate analysis and mineral concentration via inductively coupled plasma (ICP) analysis (Table 1). The sows were weighed upon entering the farrowing unit, within 24 h of farrowing, and at weaning (Mosdal Scale Systems; Broadview, MT, USA). The weekly feed intake was recorded during the lactation period. The data were collected through two full breeding cycles and lactations. Only those sows that completed parity 1 on the treatment were included in parity 2 data collection.

**Table 1.** Dietary composition and analysis on an as-fed basis.

| Dietary Treatment | Gestation | | Lactation | | |
|---|---|---|---|---|---|
| | **Low Mn** | **High Mn** | **CON** | **PRO20** | **PRO40** |
| Ingredient, % | | | | | |
| Corn | 54.370 | 54.160 | 54.410 | 54.400 | 54.380 |
| Corn DDGS | 40.000 | 40.000 | 20.000 | 20.000 | 20.000 |
| Soybean meal, 47.5% | 1.700 | 1.700 | 21.620 | 21.620 | 21.620 |
| L-Lysine | 0.210 | 0.210 | 0.220 | 0.220 | 0.220 |
| Dicalcium phosphate | 0.870 | 0.870 | 1.260 | 1.260 | 1.260 |
| Limestone | 1.600 | 1.600 | 1.240 | 1.240 | 1.240 |
| Salt | 0.250 | 0.250 | 0.250 | 0.250 | 0.250 |
| Vitamin premix [1] | 0.250 | 0.250 | 0.250 | 0.250 | 0.250 |
| Sow Add Pack- Vit [2] | 0.250 | 0.250 | 0.250 | 0.250 | 0.250 |
| Mineral premix [3] | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 |
| ProPath® Mn [4] | 0.000 | 0.210 | 0.000 | 0.014 | 0.027 |
| Analysis [5] | | | | | |
| ME [6,7], Mcal/kg | 3303.000 | 3303.000 | 3297.000 | 3297.000 | 3297.000 |
| Crude protein, % | 18.400 | 17.600 | 20.000 | 19.900 | 19.900 |
| Lysine [6], % | 0.520 | 0.520 | 0.970 | 0.970 | 0.970 |
| Crude fat, % | 3.700 | 3.600 | 3.800 | 4.100 | 3.800 |
| Ash, % | 5.900 | 5.900 | 5.500 | 5.600 | 6.000 |
| Crude fiber, % | 5.400 | 5.100 | 3.700 | 4.000 | 3.900 |
| Phosphorus (total), % | 0.600 | 0.600 | 0.700 | 0.700 | 0.700 |
| Phosphorus (avail) [6], % | 0.400 | 0.420 | 0.390 | 0.390 | 0.390 |
| Calcium, % | 1.000 | 0.900 | 0.900 | 0.900 | 0.900 |
| Potassium, % | 0.800 | 0.740 | 0.920 | 0.940 | 0.920 |
| Magnesium, % | 0.210 | 0.180 | 0.190 | 0.210 | 0.210 |
| Sulfur, % | 0.070 | 0.080 | 0.080 | 0.080 | 0.080 |
| Manganese, ppm | 42.000 | 310.000 | 42.000 | 73.000 | 81.000 |
| Iron, ppm | 243.000 | 176.000 | 464.000 | 576.000 | 479.000 |
| Copper, ppm | 34.000 | 30.000 | 48.000 | 40.000 | 35.000 |
| Zinc, ppm | 181.000 | 113.000 | 225.000 | 255.000 | 251.000 |

1.Vitamin Premix: supplied per kg of diet: vitamin A (4,134 IU); vitamin D (1,653 IU); vitamin E (66 IU); vitamin K (3.3 mg); riboflavin (8.27 mg); niacin (49.6 mg); vitamin B12 (0.033 mg); pantothenic acid (27.6 mg); ADM Alliance Nutrition, Quincy, IL 62305. 2.Sow Add Pack: supplied per kg of diet: vitamin A (4,134 IU); vitamin E (33 IU); pyridoxine (0.992 mg); folic acid (2.205 mg); biotin (0.2205 mg); choline (551.25 mg); carnitine (49.6 mg); ADM Alliance Nutrition, Quincy, IL 62305. 3.Mineral Premix: supplied per kg of diet: Copper (10 ppm Cu as CuSO4; 10 ppm Cu as ProPath® Cu, Zinpro); Zinc (50 ppm Zn as ZnO and 50 ppm Zn as ProPath® Zn, Zinpro); Iron (100 ppm Fe as FeSO4); Iodine (1 ppm iodine as KIO3); Selenium (0.3 ppm Se as Na2SeO3). 4.Zinpro, Eden Prairie, MN. 5.Analysis performed at University of Georgia Feed, Water, and Soil Laboratory (Athens, GA). 6.Metabolizable energy. 7.Calculated value.

### *2.2. Piglet Handling and Care*

The piglets were processed and weighed (Ohaus Corporation; Parsippany, NJ, USA) within 24 h of birth and at 21 ± 3 d of age (weaning). Pre-weaning mortality, number of live piglets, number of stillborn piglets, and number of mummies were recorded. The males were castrated at 7–10 d of age. Pre-weaning survivability was calculated on a per litter basis. The number of litters on treatment from the LARU were: Parity 1 ($n$ = 39): CON ($n$ = 13), PRO20 ($n$ = 13), PRO40 ($n$ = 13); Parity 2 ($n$ = 35): CON ($n$ = 11), PRO20 ($n$ = 11), PRO40 ($n$ = 13). The piglets were not cross-fostered or allowed access to creep feed during the course of this study.

### *2.3. Blood Collection and Storage*

Sow blood samples were obtained 10 d ± 1 post-breeding and at 3 d ± 1 of lactation via syringe using the jugular vein. The blood samples were transferred into heparinized blood tubes (BD Vacutainer®, Franklin Lakes, NJ, USA), inverted several times, and placed on ice until arriving at the laboratory within an hour. The samples were centrifuged (2000 × $g$, 10 min, 4 °C) and plasma was aliquoted and stored at −80 °C for subsequent analyses.

The samples obtained 10 d post-breeding were analyzed for progesterone concentration. The samples obtained 3 d into lactation were analyzed for prolactin and immune marker concentrations.

Piglets were chosen for blood sampling by selecting an average-sized piglet from each litter based on the average weaning weight for that litter. The piglet blood samples were obtained 5 d ± 1 post-weaning via the orbital sinus [17] and collected into heparinized tubes (BD Vacutainer®, Franklin Lakes, NJ, USA). The samples were inverted several times after collection and placed on ice until arriving at the laboratory within an hour. The samples were then centrifuged (2000 × *g*, 10 min, 4 °C), and plasma was aliquoted and stored at −80 °C for subsequent analysis of immune marker concentrations. All the piglets were on a common phase 1 nursery diet containing 12 ppm Mn (supplemented) post-weaning.

### *2.4. Prolactin and Progesterone Assays*

A previously described radioimmunoassay (RIA) was used to determine the concentration of prolactin [18] with the modification that 100 µL of plasma sample was used. The radio-inert prolactin and the first antibody to porcine prolactin were purchased from A.F. Parlow (U.S. National Hormone and Peptide Program, Harbor UCLA Medical Centre, Torrance, CA, USA). The parallelism of a pooled sample from lactating sows was 98.4%. The average recovery calculated by the addition of various doses of the radio-inert prolactin to 50 µL of a pooled sample was 96.3%. The sensitivity of the assay was 1.5 ng/mL. The intra- and inter-assay CV were 1.57% and 3.16%, respectively. Progesterone was measured with a RIA commercial kit (Progesterone CT, ICN Pharmaceuticals Inc., Costa Mesa, CA, USA). The validation showed a parallelism of 105.3% and an average recovery of 94.4%. Intra- and inter-assay CV were 2.96% and 0.65%, respectively.

### *2.5. Cytokine Analyses*

Cytokines were measured using Luminex xMAP technology for multiplexed quantification of 13 porcine cytokines, chemokines, and growth factors. The multiplexing analysis was performed using the Luminex™ 200 system (Luminex, Austin, TX, USA) by Eve Technologies Corp. (Calgary, Alberta). Thirteen markers were measured simultaneously using Eve Technologies' Porcine Cytokine 13-Plex Discovery Assay® (MilliporeSigma, Burlington, MA, USA) according to the manufacturer's protocol. The 13-plex consisted of GM-CSF (granulocyte-macrophage colony-stimulating factor), IFNγ, IL-1α, IL-1ra, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and TNF-α. The assay sensitivities of these markers ranged from 5 to 42 pg/mL for the 13-plex. The individual analyte sensitivity values are available in the MilliporeSigma MILLIPLEX® MAP protocol.

### *2.6. Tissue Collection and Storage*

Piglet tissue samples were collected at weaning (21 ± 3 d of age) during the second parity. An average-sized female piglet was chosen at weaning for tissue sampling based on the average weaning weight of each litter. The piglets were euthanized with $CO_2$ and the ileum, heart, and liver were removed. The ileum was flushed with 1X phosphate buffered saline solution, and the heart and liver were rinsed with the same solution. Approximately 45–65 cm of the ileum was removed immediately prior to the ileocecal junction. The left ventricle of the heart and the right medial lobe of the liver were removed. The gall bladder was removed from the liver before tissue was homogenized and frozen. The tissues (1.0 ± 0.05 g) were homogenized in 4 mL of homogenizing buffer according to Marklund and Marklund [19]. The resulting tissue homogenates were frozen at −80 °C for subsequent analysis of MnSOD activity. Additional ileum, heart, and liver tissues (5.0 ± 0.1 g) were sent to the University of Georgia Feed, Water, and Soil Laboratory (Athens, Georgia) to determine the tissue mineral concentrations via ICP analysis. The samples were kept at −20 °C until analysis.

### *2.7. MnSOD Analysis*

The tissue MnSOD specific activity (EC 1.15.1.1) was determined according to the protocol outlined by Marklund and Marklund [19] with slight modifications. The tissue samples were collected and homogenized as previously stated. The tissue homogenates were then kept at −80 °C until analysis, according to the cited protocol.

In order to inactivate the Cu/Zn-dependent SOD, 1 mmol potassium cyanide (KCN) was added to the reaction buffer (50 mM Tris-HCl, 1.0 mM diethylenetriamine pentaacetic acid, pH 8.2) and was used to measure the relative MnSOD activity [19]. The tissue homogenate (0.5 mL) was added to 1.1 mL of reaction buffer and centrifuged (2000× *g*, 15 min, 4 °C). The resulting supernatant was diluted in a reaction buffer (1:10). An assay control with no added sample (900 µL reaction buffer + 50 µL of 10 mM sodium azide ($NaN_3$)), 200 µL (of diluted homogenate + 700 µL reaction buffer + 50 µL $NaN_3$), and 400 µL (of diluted homogenate + 500 µL reaction buffer + 50 µL $NaN_3$) were plated in triplicate on a 12-well microcuvette plate (VWR® Tissue Culture Plates, Radnor, PA, USA), with each well having a volume of 950 µL. The reaction was initiated when 50 µL of 4 mM pyrogallol in 10 mM HCl was added to each well and the plate was quickly mixed. The reaction was monitored at 320 nm for 3 min using the kinetic reading program of a spectrophotometer (Biotek® µQuant, 2006). Deionized water (1.0 mL) was used to blank the spectrophotometer. The amount of supernatant that resulted in the 50% inhibition of the autooxidation of pyrogallol was the equivalent of one unit of MnSOD activity (IU). The Lowry protein determination [20] was performed on the tissue homogenate to determine the milligram of soluble protein. Specific activity was defined by the internationally recognized unit, IU mg/soluble protein.

### *2.8. Milk Collection and Component Analysis*

Milk samples were obtained from sows on d 1, 7, and 14 of their individual lactation. The success of adequate milk collection was dependent on sow temperament and safety of collection. The piglets were removed 45 min to 1 hour prior to milking. The sow's udder was manually stimulated in order to induce milk letdown. In cases where milk letdown would not occur after manual stimulation, an injection of 0.25 mL of oxytocin (20 USP oxytocin/mL) was administered to accelerate milk letdown. Several minutes post-injection, milk was collected from the sow into labelled, 15-mL conical tubes. Milk was collected from as many of the sow's functional teats (those teats that expressed milk) as possible to ensure a representative sample. The samples were frozen at −20 °C until milk component analysis.

Protein concentration of colostrum and milk was determined with the Lowry method [20]. The total fat percent of colostrum and milk was determined using a modification of the method described by Folch et al. [21]. The milk samples were thawed, mixed thoroughly, and one mL of sample was placed in a 25 mL glass screw top tube. Ten milliliters of chloroform and methanol (1:2) were added to the tube, which was then capped and vortexed, and left at room temperature for at least 10 min. Then, 5 mL of chloroform and 5 mL of saline (9.0 g/L NaCl) were added, the tubes were capped, vortexed, and the phases were allowed to separate overnight. The next morning, samples were centrifuged (800 × *g*; 37 °C; 10 min) to further separate the phases. The upper methanol and water layer was aspirated, and the protein precipitate at the interface was discarded. Aluminum weigh pans were accurately weighed using gloves and forceps, and 6 mL of chloroform were then transferred from the tube to its corresponding weigh pan. The weigh pans with chloroform were left under the fume hood to allow the chloroform to evaporate (1 h minimum) and were placed in a 100 °C drying oven for an hour to allow any additional water to evaporate. The pans with dried lipid were weighed.

The milk samples were submitted to the University of Georgia Feed, Water, and Soil Laboratory (Athens, Georgia) for determination of the mineral concentration via the method of ICP analysis.

### 2.9. Statistical Analysis

All the analyses were performed using sow or litter as the experimental unit and a farrowing group based on farrowing dates as the block. There were five groups of 8, 8, 8, 8, and 7 sows, respectively. The sow performance data were analyzed as a randomized complete block design. The dietary treatment served as a fixed effect, and the block served as a random effect. All the models were analyzed using the MIXED procedure of SAS 9.4 (SAS Enterprise, Cary, NC, USA). The dietary treatment within the study parity (first or second) was included to detect the differences between the diets within each parity. The lactation length was utilized as a covariate for sow feed intake, total number weaned, and survivability. The piglet birthweights, weaning weights, and average daily gains were analyzed on an individual piglet basis. For the total litter weaning weight, the of number piglets weaned was used as a covariate in addition to the lactation length. The cytokine data was not normally distributed, and thus a $\log_{10}$ transformation of the cytokine concentrations was analyzed with the MIXED procedure of SAS as described above. Pairwise comparisons between the least squares means of the Mn level comparisons were computed using the PDIFF option of the LSMEANS statement. Statistical significance was declared at $p < 0.05$ and tendencies were considered at $0.05 \leq p < 0.10$.

## 3. Results

### 3.1. Sow Performance

The sow body weights during lactation were not affected by treatment ($p > 0.10$; Table 2). There was a dietary treatment within parity effect on relative weight change between d 110 of gestation and d 1 of lactation ($p = 0.024$). The PRO20 sows lost less weight from d 110 until d 1 of lactation in the second parity compared to their first ($p < 0.05$). There were no differences between treatments in sow body weight loss during lactation or from d 110 until weaning ($p > 0.10$). The gestation length was not affected by dietary treatment ($p > 0.10$). The weekly sow feed intake was affected by dietary treatment during all three weeks of lactation ($p < 0.05$). In either parity, feed intake during week 1 of lactation was higher for the PRO20 sows than the CON sows ($p < 0.05$) and did not differ from that of the PRO40 sows ($p > 0.10$). The feed intake during week 2 of the second parity was increased by 0.5–1.0 kg when compared to the first parity across all dietary treatments ($p < 0.01$). In either parity, the feed intake during week 3 of lactation was greater for the PRO20 sows than the CON sows ($p < 0.05$) and did not differ from that of the PRO40 sows ($p > 0.10$). Overall, average daily feed intake was affected by dietary treatment. In the first parity, the PRO20 sows ate significantly more feed ($p < 0.05$) when compared to either the CON or PRO40 sows. During parity 2, the PRO20 sows ate significantly more feed than the PRO40 sows ($p < 0.05$), while feed intake did not differ from the CON ($p > 0.10$).

**Table 2.** The effect of supplemental dietary Mn (0, 20, 40 ppm) on the reproductive performance of sows over two parities.

| | Parity 1 [1] | | | Parity 2 [1] | | | | *p*–Values [2] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Dietary Treatment** | **CON** | **PRO20** | **PRO40** | **CON** | **PRO20** | **PRO40** | **SEM** | **Mn** | **Lin Mn** | **Mn (Parity)** |
| Sow body weight, kg (*N*) | 13 | 13 | 13 | 11 | 11 | 13 | | | | |
| d 110 ± 1 Gestation | 233.0 | 226.4 | 233.1 | 222.0 | 225.4 | 233.5 | 8.0 | 0.588 | 0.450 | 0.807 |
| d 1 ± 1 Lactation | 224.6 | 217.7 | 224.4 | 216.9 | 221.9 | 222.9 | 10.9 | 0.879 | 0.707 | 0.907 |
| d 21 ± 1 Lactation | 233.4 | 223.6 | 230.6 | 230.2 | 228.8 | 236.5 | 9.3 | 0.612 | 0.822 | 0.918 |
| Relative weight change, kg | | | | | | | | | | |
| d 110–d 1 Lactation | −15.3 $^{a}$ | −11.0 $^{a}$ | −8.9 $^{ab}$ | −7.2 $^{ab}$ | −3.6 $^{b}$ | −16.2 $^{a}$ | 3.6 | 0.118 | 0.689 | 0.024 |
| d 1 Lactation–d 21 Lact | 11.2 | 7.0 | 6.3 | 16.8 | 6.6 | 15.6 | 3.9 | 0.148 | 0.376 | 0.260 |
| d 110–d 21 Lact | −4.2 | −0.3 | 0.3 | 8.7 | 2.2 | 1.6 | 4.5 | 0.932 | 0.748 | 0.273 |
| Gestation length, d | 114.7 | 115.2 | 114.8 | 115.3 | 115.1 | 115.0 | 0.5 | 0.878 | 0.899 | 0.695 |
| Feed intake, kg/sow/day (*N*) | 13 | 13 | 13 | 11 | 11 | 13 | | | | |
| Week 1 | 5.15 $^{b}$ | 6.26$^{a}$ | 5.72 $^{ab}$ | 5.63 $^{b}$ | 6.68 $^{a}$ | 6.20 $^{ab}$ | 0.43 | 0.012 | 0.128 | 0.726 |
| Week 2 | 6.38 $^{c}$ | 7.73$^{a}$ | 6.38 $^{c}$ | 7.87 $^{ab}$ | 8.01 $^{ab}$ | 7.50 $^{ab}$ | 0.38 | 0.039 | 0.621 | 0.003 |
| Week 3 | 6.36 $^{bc}$ | 6.58$^{bc}$ | 6.04 $^{cd}$ | 7.63 $^{a}$ | 8.16 $^{a}$ | 6.41 $^{b}$ | 0.56 | 0.025 | 0.060 | 0.131 |

**Table 2.** *Cont.*

| Dietary Treatment | Parity 1 [1] | | | Parity 2 [1] | | | SEM | *p*–Values [2] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CON | PRO20 | PRO40 | CON | PRO20 | PRO40 | | Mn | Lin Mn | Mn (Parity) |
| ADFI | 5.92 $^{d}$ | 7.05$^{a}$ | 6.04 $^{bd}$ | 6.96 $^{abc}$ | 7.52 $^{a}$ | 6.73 $^{bc}$ | 0.35 | 0.006 | 0.848 | 0.150 |
| Lactation length, d | 18.5 | 16.1 | 18.8 | 17.9 | 17.9 | 18.8 | 3.0 | | | |
| Litter performance (*N*) | 13 | 13 | 13 | 11 | 11 | 13 | | | | |
| Total number born | 16.2 $^{a}$ | 12.9 $^{b}$ | 15.2 $^{ab}$ | 15.2 $^{ab}$ | 13.3$^{b}$ | 14.6 $^{ab}$ | 0.9 | 0.021 | 0.396 | 0.817 |
| Total live born | 13.6 $^{a}$ | 12.0 $^{ab}$ | 13.2 $^{a}$ | 12.6 $^{ab}$ | 10.6 $^{b}$ | 12.4 $^{ab}$ | 0.7 | 0.035 | 0.713 | 0.524 |
| Stillborn | 1.7 | 0.5 | 1.3 | 2.3 | 2.3 | 2.1 | 0.6 | 0.541 | 0.594 | 0.083 |
| Mummies | 0.9 | 0.6 | 0.6 | 0.2 | 0.4 | 0.1 | 0.3 | 0.750 | 0.466 | 0.348 |
| Total number weaned | 10.7 | 10.0 | 10.5 | 9.8 | 9.2 | 9.3 | 0.7 | 0.686 | 0.652 | 0.355 |
| Survival, % | 79.8 | 82.3 | 82.3 | 78.9 | 88.4 | 77.0 | 4.3 | 0.282 | 0.937 | 0.592 |
| Avg piglet birthweight, kg[3] | 1.23 $^{c}$ | 1.59 $^{a}$ | 1.35$^{b}$ | 1.22 $^{c}$ | 1.55 $^{a}$ | 1.45 $^{b}$ | 0.04 | 0.001 | 0.001 | 0.150 |
| Avg piglet weaning wt, kg[3] | 5.18 $^{b}$ | 5.44 $^{ab}$ | 5.74$^{a}$ | 5.11 $^{b}$ | 5.82 $^{a}$ | 5.24 $^{b}$ | 0.26 | 0.001 | 0.010 | 0.023 |
| Avg litter weaning wt, kg | 52.58 $^{ab}$ | 59.08 $^{a}$ | 58.09$^{ab}$ | 51.66 $^{ab}$ | 58.54 $^{ab}$ | 51.40 $^{b}$ | 2.7 | 0.049 | 0.310 | 0.318 |
| ADG, g/pig/day[3] | 216 $^{b}$ | 232 $^{ab}$ | 236$^{a}$ | 211 $^{b}$ | 241 $^{a}$ | 204$^{b}$ | 11 | 0.001 | 0.298 | 0.014 |

a−dLS Means within a row that do not share a letter superscript differ significantly ($p < 0.05$). 1. This refers to Parity 1 or 2 on treatment; this applies throughout the text and in tables. 2. *p*-values reported are for the main effect of manganese (Mn), the preplanned linear orthogonal contrast (Lin Mn), and the manganese treatment within parity (Mn(Parity)). 3. Variables with a quadratic *p*-value of $p < 0.01$ as a result of a quadratic orthogonal contrast statement.

### 3.2. Litter Performance

The total number of piglets born, the total number of piglets born alive, the number of stillborn piglets, and the number of mummies were not affected by dietary treatment ($p > 0.10$; Table 2). There was a significant effect of Mn on the total number of piglets born ($p < 0.05$) and the total number of piglets born alive ($p < 0.05$). The numbers of total piglets and total live piglets were greater for the CON sows compared to the PRO20 or PRO40 sows, while values were lowest for the PRO20 sows. The PRO40 sows were intermediate between the CON and PRO20 sows. The number of stillborn piglets between Mn treatments within parity tended to be different ($p = 0.08$). The PRO20 sows tended to have lower numbers of stillborn piglets in the first parity. There was no effect of Mn and parity on the previously listed variables: the total number of piglets, the total born alive, the stillborn, and the mummies. The total number of piglets weaned and litter survivability were not affected by dietary treatment ($p > 0.23$). The average piglet birthweight increased linearly in response to increasing the concentration of dietary Mn ($p < 0.01$). The PRO20 piglets weighed more at birth (1.57 kg; $p < 0.05$) than the CON piglets (1.23 kg) and the PRO40 piglets (1.40 kg), and the PRO40 piglets weighed more at birth than the CON piglets ($p < 0.05$). The weaning weights differed in response to dietary treatment ($p < 0.01$). The PRO20 and PRO40 piglets weighed more at weaning than the CON piglets ($p < 0.05$), the PRO20 piglets (5.6 kg) had similar weights to the PRO40 piglets (5.5 kg) at weaning ($p > 0.10$). The dietary treatment did have a significant effect on litter weaning weight ($p < 0.05$; Table 2). Looking at parity 1 and parity 2 separately, there were no significant differences in litter weaning weight ($p > 0.10$). However, there was a significant difference ($p < 0.05$) in the weaning weights of the PRO20 litters from parity 1 (59.08 kg) and the PRO40 litters from parity 2 (51.40 kg). It does not appear that litter weaning weights followed any particular biological pattern in response to the maternal dietary treatment. When averaged across parity, the piglet average daily gain (ADG) was affected by dietary treatment ($p < 0.01$). The PRO20 piglets gained, on average across both parities, 23 g more per day (237 g/pig/day) than the CON piglets (214 g/pig/day; $p < 0.05$), while it was similar for the PRO20 and the PRO40 (220 g/pig/day; $p > 0.10$). In the second parity, the PRO40 piglets had decreased ADG compared to that in the first parity.

### 3.3. Sow Immune Marker and Plasma Hormone Concentrations

In sows, plasma concentrations (log transformed) of GM-CSF, IFN-γ, IL-1β, IL-1rα, IL-6, IL-8, and TNF-α did not differ in response to increasing the dietary Mn concentration (Table 3) or effect of the dietary treatment within parity ($p > 0.10$). There was an effect of the dietary treatment on the log concentrations of IL-1α, IL-2, IL-4, IL-10, IL-12, and IL-18 ($p < 0.05$; Table 3), as well as a linear effect on the log concentrations of these same

markers ($p < 0.05$). There was a linear effect of Mn on IL-1β and IL-6 ($p < 0.05$), even though the Mn effect was not significant itself. Nevertheless, mean separations for these two markers are presented in Table 3. In the first parity, concentrations of IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, and IL-18 were not significantly different from one another in response to the dietary treatment. During the second parity, IL-1α, IL-1β, IL-4, IL-6, IL-10, and IL-12 had similar patterns of log concentrations. The PRO40 sows had significantly decreased log concentrations compared to the CON sows ($p < 0.05$), while the PRO20 sows had an intermediate log concentration that did not differ from that of the CON or PRO40 sows ($p > 0.10$). During the second parity, the CON sow log concentrations of IL-2 were significantly increased compared to the log concentrations in the PRO20 and PRO40 sows ($p < 0.05$). In addition, the PRO20 sows had a significantly increased log concentration of IL-2 compared to the PRO40 sows ($p < 0.05$). During the second parity, the CON and PRO20 sow log concentrations of IL-18 were significantly increased compared to the PRO40 sows ($p < 0.05$).

**Table 3.** The effect of supplemental dietary Mn (0, 20, 40 ppm) on the log concentration of plasma immune markers in sows 3 d into lactation over two parities.

| | Parity 1 | | | Parity 2 | | | | *p*–Values [1] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Dietary Treatment** | **CON** | **PRO20** | **PRO40** | **CON** | **PRO20** | **PRO40** | **SEM** | **Mn** | **Lin Mn** | **Mn (Parity)** |
| $Log_{10}$Conc, pg/mL (*N*) | 13 | 13 | 13 | 11 | 11 | 13 | | | | |
| GM-CSF | 2.38 | 2.57 | 2.37 | 2.33 | 2.57 | 2.47 | 0.15 | 0.24 | 0.61 | 0.94 |
| IFN-γ | 2.94 | 3.35 | 3.29 | 3.01 | 3.22 | 3.09 | 0.30 | 0.38 | 0.30 | 0.86 |
| IL-1α | 2.03 $^{ab}$ | 1.95 $^{ab}$ | 1.89$^{b}$ | 2.32 $^{a}$ | 2.08 $^{ab}$ | 1.82$^{b}$ | 0.15 | 0.05 | 0.01 | 0.45 |
| IL-1β | 3.10 $^{ab}$ | 2.96 $^{ab}$ | 2.94 $^{ab}$ | 3.38 $^{a}$ | 3.18 $^{ab}$ | 2.90$^{b}$ | 0.18 | 0.08 | 0.02 | 0.52 |
| IL-1rα | 3.00 | 3.12 | 3.03 | 3.37 | 3.15 | 2.99 | 0.14 | 0.26 | 0.12 | 0.24 |
| IL-2 | 3.03 $^{abc}$ | 2.96 $^{abc}$ | 2.86 $^{bc}$ | 3.33 $^{a}$ | 3.11 $^{b}$ | 2.72 $^{c}$ | 0.17 | 0.02 | 0.01 | 0.38 |
| IL-4 | 3.53 $^{ab}$ | 3.36 $^{ab}$ | 3.30 $^{b}$ | 3.84 $^{a}$ | 3.48 $^{ab}$ | 3.21 $^{b}$ | 0.19 | 0.03 | 0.01 | 0.61 |
| IL-6 | 2.43 $^{ab}$ | 2.41 $^{ab}$ | 2.31 $^{b}$ | 2.79 $^{a}$ | 2.55 $^{ab}$ | 2.34$^{b}$ | 0.17 | 0.11 | 0.04 | 0.45 |
| IL-8 | 1.47 | 1.04 | 1.52 | 1.25 | 1.30 | 1.17 | 0.18 | 0.50 | 0.93 | 0.24 |
| IL-10 | 3.29 $^{ab}$ | 3.31 $^{ab}$ | 3.07 $^{b}$ | 3.62 $^{a}$ | 3.35 $^{ab}$ | 2.95 $^{b}$ | 0.19 | 0.01 | 0.01 | 0.52 |
| IL-12 | 2.89 $^{ab}$ | 2.90 $^{ab}$ | 2.83 $^{b}$ | 3.07 $^{a}$ | 2.96 $^{ab}$ | 2.76 $^{b}$ | 0.09 | 0.04 | 0.02 | 0.38 |
| IL-18 | 3.51 $^{ab}$ | 3.52 $^{ab}$ | 3.35$^{b}$ | 3.82 $^{a}$ | 3.62 $^{a}$ | 3.25 $^{b}$ | 0.16 | 0.01 | 0.01 | 0.43 |
| TNF-α | 2.51 | 1.81 | 2.21 | 2.43 | 2.09 | 1.96 | 0.28 | 0.20 | 0.10 | 0.69 |
| Prolactin[2], ng/mL | 30.95 | 28.88 | 30.52 | 32.03 | 30.28 | 28.23 | 3.55 | 0.75 | 0.47 | 0.91 |
| (*N*) | 13 | 13 | 13 | 11 | 11 | 13 | | | | |
| Progesterone[3], ng/mL | 25.83 | 26.86 | 23.04 | 21.56 | 25.45 | 21.12 | 2.40 | 0.09 | 0.36 | 0.60 |
| (*N*) | 13 | 13 | 13 | 11 | 11 | 13 | | | | |

a-bLS Means within a row that do not share a letter superscript differ significantly ($p < 0.05$).1P-values reported are for the main effect of manganese (Mn), the preplanned linear orthogonal contrast (Lin Mn), and the effect of Mn within parity (Mn(Parity)). 2. Prolactin was measured from plasma obtained on d 3 ± 1 of lactation. 3. Progesterone was measured from plasma obtained on 10 ± 1 d post breeding.

The prolactin concentrations in sows did not differ due to increasing dietary Mn ($p > 0.10$; Table 3). There was a tendency for the progesterone concentration to differ in response to the dietary Mn level ($p < 0.10$; Table 3), with concentrations being greater in the PRO20 sows compared to the CON and PRO40 sows.

### *3.4. Piglet Immune Marker Concentrations*

In piglets, the log plasma concentrations of all the tested immune markers did not differ in response to increasing the maternal dietary Mn concentrations ($p > 0.10$; Table 4).

### *3.5. Tissue MnSOD Activity and Mineral Composition in Piglets*

The dietary treatment had no effect on the MnSOD activity in the ileal or cardiac tissue of piglets ($p > 0.10$; Table 4). The hepatic MnSOD activity decreased in a linear manner across increasing levels of the maternal dietary Mn supplementation ($p = 0.03$; Table 4). There was a tendency for the Mn to affect the cardiac and hepatic zinc concentrations ($p = 0.09$, $p = 0.077$, respectively; Table 5). None of the other analyzed mineral concentrations differed across the dietary treatments ($p > 0.10$; Table 5).

**Table 4.** The effect of maternal supplemental dietary manganese on the log concentration of plasma immune markers in piglets 5 d post-weaning and MnSOD tissue activity at weaning.

| | Parity 1 | | | Parity 2 | | | | *p*–Values [1] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Dietary Treatment** | **CON** | **PRO20** | **PRO40** | **CON** | **PRO20** | **PRO40** | **SEM** | **Mn** | **Lin Mn** | **Mn (Parity)** |
| $Log_{10}Conc$[2], pg/mL (*N*) | 13 | 13 | 13 | 11 | 11 | 13 | | | | |
| GM-CSF | 1.21 | 0.97 | 1.35 | 1.36 | 1.67 | 0.88 | 0.24 | 0.57 | 0.42 | 0.06 |
| IFN-γ | 2.18 | 2.40 | 2.13 | 2.03 | 2.24 | 2.34 | 0.20 | 0.50 | 0.45 | 0.68 |
| IL-1α | 0.80 | 0.62 | 0.96 | 0.86 | 0.89 | 0.79 | 0.23 | 0.83 | 0.80 | 0.72 |
| IL-1β | 2.38 | 2.31 | 2.42 | 2.31 | 2.38 | 2.30 | 0.14 | 0.99 | 0.91 | 0.87 |
| IL-1rα | 3.17 | 3.02 | 3.15 | 3.24 | 3.18 | 3.17 | 0.12 | 0.58 | 0.64 | 0.76 |
| IL-2 | 1.80 | 1.76 | 1.89 | 1.64 | 1.87 | 1.81 | 0.24 | 0.78 | 0.51 | 0.90 |
| IL-4 | 2.25 | 1.81 | 2.04 | 2.17 | 2.06 | 2.12 | 0.20 | 0.28 | 0.41 | 0.76 |
| IL-6 | 1.57 | 1.33 | 1.52 | 1.57 | 1.44 | 1.49 | 0.12 | 0.13 | 0.50 | 0.87 |
| IL-8 | 1.46 | 1.30 | 1.38 | 1.78 | 1.42 | 1.66 | 0.17 | 0.12 | 0.44 | 0.44 |
| IL-10 | 2.30 | 2.17 | 2.13 | 2.27 | 2.42 | 2.13 | 0.14 | 0.23 | 0.14 | 0.57 |
| IL-12 | 3.19 | 3.15 | 3.13 | 3.13 | 3.18 | 3.17 | 0.06 | 0.97 | 0.88 | 0.71 |
| IL-18 | 2.93 | 2.83 | 2.92 | 2.89 | 3.07 | 2.85 | 0.12 | 0.69 | 0.70 | 0.17 |
| TNF-α | 1.74 | 1.84 | 1.82 | 1.85 | 1.63 | 1.49 | 0.15 | 0.51 | 0.25 | 0.16 |
| MnSOD [3,4], IU/mg (*N*) | | | | 11 | 11 | 13 | | | | |
| Ileum | . | . | . | 6.95 | 6.36 | 6.89 | 0.81 | 0.67 | 0.93 | |
| Heart | . | . | . | 8.79 | 5.73 | 6.77 | 1.25 | 0.21 | 0.23 | |
| Liver | . | . | . | 10.02 [a] | 7.46 [b] | 7.87 [ab] | 0.71 | 0.03 | 0.03 | |

a-bLS Means within a row that do not share a letter superscript differ significantly ($p < 0.05$). 1.P-values reported are for the main effect of manganese (Mn), the preplanning linear orthogonal contrast (Lin Mn), and the effect of Mn within parity (Mn(Parity)). 2.This data is derived from plasma obtained from piglets on d 5 ± 1 post-weaning. One average sized piglet was bled from each litter, based on the average weaning weight for their litter. 3.MnSOD activity is expressed in the internationally recognized unit for enzymatic activity, IU/mg soluble protein. In addition, tissue samples were only collected from piglets during the second lactation of the study. 4. *p*–values reported (from left to right) are for the main effect of manganese (Mn), the preplanned linear orthogonal contrast (Lin Mn), and the effect of tissue on MnSOD activity. There was no Mn x Tissue interaction ($p = 0.653$).

**Table 5.** The effect of supplemental maternal dietary manganese on tissue mineral concentrations of piglets at weaning.

| **Dietary Treatment** | | **CON** | **PRO20** | **PRO40** | **SEM** | ***p*-Value** |
|---|---|---|---|---|---|---|
| Mineral Concentration [1,2] | *N* | 11 | 11 | 13 | | Mn |
| Ileum | | | | | | |
| Phosphorus, % | | 1.385 | 1.320 | 1.427 | 0.060 | 0.249 |
| Calcium, % | | 0.041 | 0.046 | 0.051 | 0.004 | 0.114 |
| Manganese, ppm | | 5.316 | 5.000 | 5.556 | 0.235 | 0.227 |
| Iron, ppm | | 116.000 | 114.000 | 118.000 | 11.000 | 0.937 |
| Copper, ppm | | 6.000 | 15.000 | 6.000 | 5.000 | 0.364 |
| Zinc, ppm | | 124.000 | 118.000 | 119.000 | 5.000 | 0.728 |
| Heart | | | | | | |
| Phosphorus, % | | 1.015 | 0.970 | 1.013 | 0.010 | 0.117 |
| Calcium, % | | 0.023 | 0.024 | 0.031 | 0.004 | 0.226 |
| Manganese, ppm | | 5.704 | 5.410 | 5.670 | 0.190 | 0.479 |
| Iron, ppm | | 238.000 | 222.000 | 219.000 | 16.000 | 0.589 |
| Copper, ppm | | 7.000 | 7.000 | 16.000 | 4.000 | 0.215 |
| Zinc, ppm | | 89.000 | 87.000 | 91.000 | 2.000 | 0.091 |
| Liver | | | | | | |
| Phosphorus, % | | 0.908 | 0.912 | 0.922 | 0.060 | 0.970 |
| Calcium, % | | 0.022 | 0.023 | 0.023 | 0.002 | 0.692 |
| Manganese, ppm | | 6.023 | 6.441 | 5.907 | 0.446 | 0.591 |
| Iron, ppm | | 845.000 | 1152.000 | 863.000 | 204.000 | 0.478 |
| Copper, ppm | | 172.000 | 159.000 | 148.000 | 27.000 | 0.662 |
| Zinc, ppm | | 262.000 | 265.000 | 199.000 | 24.000 | 0.077 |

1.Mineral concentration analysis performed via inductively coupled plasma (ICP) analysis. 2.Tissue samples were only collected from the second parity of the study.

### *3.6. Sow Milk Composition*

There was no interaction between the Mn and the parity for any of the measured milk components, therefore, averages were presented across the dietary treatments (Table 6).

There was no day effect between d 7 and d 14 milk samples for any measured component, therefore, averages were presented and an orthogonal contrast between colostrum (d 1) and milk (average of d 7 and d 14) was reported (Table 6). The protein percentage decreased ($p < 0.01$) between the colostrum and milk, while there was a tendency for the milk protein to decrease in response to the supplemented dietary Mn ($p = 0.08$). The colostral fat content differed in response to dietary treatment ($p < 0.05$), with The PRO20 sows having higher colostral fat than the PRO40 sows ($p < 0.05$), but similar values to the CON sows ($p > 0.10$). The CON sows had the highest percent milk fat when compared to the PRO20 and PRO40 sows ($p < 0.05$), whereas the PRO20 sows and the PRO40 sows had similar values ($p > 0.10$). The increased dietary supplementation of Mn did not affect the mineral composition of the colostrum or milk ($p > 0.10$; Table 6). The calcium content increased ($p < 0.01$) from the colostrum to the milk, while the copper and zinc concentrations decreased ($p < 0.01$).

**Table 6.** The effect of supplemental dietary manganese on the colostrum (d 1) and milk (d 7 and 14) composition of lactating sows.

| Dietary Treatment | CON | PRO20 | PRO40 | *p*–Values | |
|---|---|---|---|---|---|
| Milk composition (as-received) | | | | Mn | Col v. Milk |
| Protein[1], % | | | | | |
| Colostrum (*N*) | 15.4 (19) | 16.0 (18) | 13.5 (21) | 0.39 | 0.01 |
| Milk [2] (*N*) | 9.2 (16) | 8.7 (15) | 7.7 (20) | 0.08 | |
| Fat [1], % | | | | | |
| Colostrum (*N*) | 5.9 [ab] (19) | 6.2 [a] (18) | 4.4 [b] (17) | 0.05 | 0.02 |
| Milk [2] (*N*) | 7.8 [a] (15) | 5.5 [b] (12) | 6.1 [b] (16) | 0.01 | |
| Mineral Concentration [1,3] (as-received) | | | | | |
| Colostrum (*N*) | 19 | 17 | 17 | | |
| Milk [2] (*N*) | 15 | 11 | 15 | | |
| Phosphorus, % | | | | | |
| Colostrum | 0.11 | 0.12 | 0.12 | 0.21 | 0.85 |
| Milk [2] | 0.12 | 0.12 | 0.12 | 0.20 | |
| Calcium, % | | | | | |
| Colostrum | 0.09 | 0.11 | 0.12 | 0.22 | 0.01 |
| Milk [2] | 0.16 | 0.17 | 0.17 | 0.71 | |
| Manganese, ppm | | | | | |
| Colostrum | 0.25 | 0.25 | 0.26 | 0.32 | 0.13 |
| Milk [2] | 0.26 | 0.25 | 0.30 | 0.12 | |
| Iron, ppm | | | | | |
| Colostrum | 1.69 | 1.90 | 1.53 | 0.68 | 0.52 |
| Milk [2] | 1.68 | 1.84 | 2.17 | 0.63 | |
| Copper, ppm | | | | | |
| Colostrum | 2.59 | 2.60 | 2.21 | 0.52 | 0.01 |
| Milk [2] | 1.29 | 1.03 | 1.10 | 0.30 | |
| Zinc, ppm | | | | | |
| Colostrum | 9.05 | 10.34 | 8.66 | 0.48 | 0.01 |
| Milk [2] | 5.34 | 5.74 | 5.58 | 0.80 | |

a-bLS Means within a row that do not share a letter superscript differ significantly ($p < 0.05$). 1.There was no Mn x parity effect, so data were averaged over both lactation periods for protein and fat percentages and mineral composition. 2. There was no day effect ($p > 0.10$) between d 7 and d 14 samples for any component, therefore those samples were combined and an orthogonal contrast between colostrum and milk was reported. 3.Mineral analysis performed via inductively coupled plasma (ICP) analysis.

## 4. Discussion

Sow body weight is an important variable to monitor during gestation and lactation as it can affect subsequent reproductive performance and longevity [22]. Before farrowing, feed intake is restricted to prevent unnecessary weight gain and the onset of constipation in the days leading up to parturition [22]. In general, sows lose body weight after farrowing and throughout lactation until weaning [22,23]. The dietary treatment had no effect on sow body weight before farrowing, after farrowing, or after weaning. There was an effect of the Mn level within parity on the relative body weight change from d 110 to birth ($p < 0.02$), but there was no clear or logical pattern of change. This statistically significant observation

does not have a significant impact in the larger frame of the study, based on the absence of the observed differences in the other relative weight change variables.

The lactation feed intake across all the treatments in this study approximated the industry mean of 6.5 kg/sow/day [24]. The increased lactation feed intake in the sows fed 20 ppm of Mn is in agreement with Tsai et al. [25] in regards to organic minerals improving sow growth characteristics. Nevertheless, Peters and Mahan [26] have reported no change in sow feed intake between mineral sources. Feed intake may be impacted by the type of mineral, but the previous literature on this subject is not consistent from one study to the next. Trace minerals are involved in many physiological pathways and affect many cellular processes, including hormone synthesis and distribution.

Progesterone is the pregnancy and conceptus maintenance hormone and is necessary for fetal growth [27,28]. Manganese is a cofactor for enzymes related to squalene synthesis, a precursor for steroid hormones like progesterone [29,30]; however, dietary treatment did not impact plasma progesterone concentrations in the current study. Current findings indicate that the dietary levels of Mn provided sufficient support of progesterone synthesis and did not differ based on Mn supplementation.

Prolactin stimulates the production of milk in mammals and has key roles in mammary development [31]. Increasing prolactin secretion in late gestation [32] or during lactation [33] led to greater sow milk yield. Prolactin is known to be a peripheral marker of Mn toxicity in rats and could also serve as a sensitive biomarker of cumulative exposure to Mn [34]. More specifically, Mn stimulates dopamine depletion, thereby increasing prolactin secretion and circulating concentrations [34]. In this study, prolactin concentrations were not affected by feeding increasing amounts of Mn from an organic source, even though organic minerals have better absorption and body retention compared to the inorganic form [35].

Efficient reproductive performance is a key component in swine husbandry. Increasing litter size while minimizing labor costs is a goal in piglet production, but there are many routes that may lead to this goal [3,4]. Plumlee et al. demonstrated that sows fed Mn-deficient diets gave birth to weak and poorly structured piglets [13]. Therefore, it was anticipated that sows fed increasing amounts of supplemental Mn would have improved Mn utilization for mineral deposition and bone development in the conceptus during gestation. It was predicted in the current study that more piglets would be born to the PRO20 and PRO40 sows and would be heavier in comparison to the CON piglets due to improved dam nutrient intake and deposition in fetal piglets. Increased maternal Mn supplementation may lead to improved Mn utilization and, as a result, lead to more piglets being born alive by the improvement of the embryonic survivability and the oxidative defense of the sow.

The sows in the current study had litter size characteristics (total number, total live born, total number weaned) slightly below the US industry average [24]. The piglet weights at birth and pre-weaning survivability were similar to the industry means. There was a numerical increase in the pre-weaning survivability when comparing the CON to PRO20 litters (79.4% vs. 85.4% over two parities, respectively). This is likely linked to the greater birthweight of the PRO20 piglets. Increased birthweight has been shown to improve pre-weaning survivability [36,37]. Improved piglet birthweights, weaning weights, and pre-weaning average daily rates of gain are important factors for efficient piglet production [3,4]. Piglets that are born heavier have a reduced risk of pre-weaning mortality and generally will gain more weight during the pre-weaning period, which was the case in the present study. Heavier piglets at weaning also typically result in improved average daily gain during the grow-finish phase of production [38]. Although the PRO20 sows did have significantly less total born and total live born piglets, there was not a feasible explanation for the heavier piglet birthweights for those particular sows.

Maternal immunity is essential for suckling piglets because there is no placental transfer of immunoglobulins to the developing offspring in swine. As is the case with most mammals, immunity must be passed from the dam to the neonate via colostrum in order

for the newborn piglet to fight off pathogenic organisms [39–41]. Sow colostrum and milk contain a variety of immunomodulatory agents: prolactin, nucleotides that enhance the activity of natural killer (NK) cells, macrophages, T helper cells, and cytokines [39]. These immune markers and agents are used by the animal and can usually be found circulating in the plasma [40]. It was anticipated that increasing Mn in sow diets may impact immune markers due to improved Mn utilization by the immune system and the reduction of oxidative stress. In a recent study, plasma cytokine profiles of sows during early gestation and the second half of pregnancy were characterized by the increased production of IL-1α and IL-4 and the reduction of the production of IFN-γ [42]. Following parturition, sows experience metabolic stress, and pro-inflammatory cytokine concentrations increase after farrowing [42].

When looking at the immune status of sows post-farrowing in the present experiment, clear patterns emerge. For markers that did respond significantly to the dietary treatment, the CON sows generally had increased concentrations compared to the PRO20 sows, while the PRO40 sows had lower concentrations compared to the CON and PRO20 sows. It may be that the CON sows had a more primed and capable immune response when faced with the metabolic stress that accompanies parturition. On the other hand, the PRO40 sows may have experienced a suppression or deficiency in their immune response based on the lower plasma concentrations of various immune markers. It is also possible that the PRO40 sows overcame the metabolic stress associated with farrowing more quickly or had less metabolic stress to begin with, and the plasma immune marker concentrations had already begun to decrease. It would be of interest to obtain multiple blood samples before and after farrowing and throughout lactation to draw meaningful conclusions on the impact of feeding supplementary organic Mn on the immune status of sows.

Manganese has been linked to nutritional immunity, which is the idea that the body sequesters trace nutrients to impair or prevent the growth of certain pathogens [43]. In addition, trace elements can play messenger roles in immune system cascades [43]. The reduced plasma concentrations (log transformed) of IL-1α in the PRO40 sows compared to the CON and PRO20 sows are of importance because IL-1α is produced by activated macrophages and plays an important role in the regulation of immune responses [39,44]. It is an intermediary cellular signal in the pathway activating the pro-inflammatory cytokine, tumor necrosis factor- α (TNF-α). However, log plasma concentrations of the TNF-α were not affected by the dietary treatment. It has been determined that TNF-α concentrations peak 24–36 h following parturition in sows of differing immune status [45]. The plasma concentrations (log transformed) of the pro-inflammatory cytokines, IL-1β, IL-2, and IL-6 and the anti-inflammatory cytokine, IL-4, were also lower in the PRO40 compared with the CON and PRO20 sows, indicating altered immune status. IL-1β is released by macrophages and monocytes during cell injury, infection, invasion, and inflammation [39,44]. IL-2 is a signaling molecule that regulates the activities of white blood cells that are responsible for immune status, while the anti-inflammatory cytokine IL-4 induces the differentiation of naïve helper T-cells and reduces pro-inflammatory responses [39,44]. IL-6 is a pro-inflammatory cytokine that has been shown to suppress feed intake and stimulate the acute phase immune response [44]. There was no effect of Mn on the plasma concentration of IL-6. The plasma concentrations of IL-10, IL-12, and IL-18 were also lower in the PRO40 sows compared with the CON and PRO20 sows, especially in parity 2. IL-1rα is secreted by various cell types (for example: epithelial and adipocytes) and is a natural inhibitor of the pro-inflammatory effects of IL-1α and IL-1β [44]. IL-1rα may have suppressed the inflammatory effects of the IL-1α and IL-1β in this study. IL-10 is an anti-inflammatory cytokine with multiple, pleiotropic effects in immune regulation and inflammation, while IL-18 is a pro-inflammatory cytokine that facilitates type 1 responses along with IL-12, to induce cell-mediated immunity following infection [39,44]. IL-12 stimulates the production of IFN-γ, TNF-α, and NK cells [44]. Supplemental Mn concentrations exceeding 20 ppm may have a role in the disruption of secondary messenger cascades and, as a result, a reduction in immune marker expression.

Overall, plasma concentrations of immune markers that did change in response to dietary treatment seemed to do so in similar patterns, showing that the PRO40 sows had reduced immune marker concentrations (log transformed) compared to the CON and PRO20 sows, especially in parity 2. A reduction in concentrations in response to the Mn supplementation is not necessarily a negative result. As previously mentioned, an explanation may be that the PRO20 and PRO40 sows overcame the metabolic stress associated with parturition more quickly than the CON sows. The significant responses in log concentrations of these markers to the dietary Mn occurred in parity 2, and it is possible that feeding these diets for a longer period of time could have longer term effects on the sow's immune system. It is important to understand that these are log concentrations at a specific moment in time in the farrowing room; therefore, more definitive conclusions could be made about the immune status of the animal if samples were taken at various points in time. Looking at a variety of acute phase proteins like serum amyloid A, haptoglobin, albumin, and others could aid to better understand the immune responses observed in this study. The concentrations and activity of these acute phase proteins can provide a more complete understanding of the immune system around parturition in sows of differing immune status [45].

There were no significant differences observed in the log concentrations of immune markers in piglets at 5 d post-weaning. It is known that weaning induces the expression of pro-inflammatory cytokines in piglets, with peak expression primarily occurring 1 d post-weaning [46,47]. Based on the current data, it can be concluded that there were no long-term effects of maternal Mn supplementation on the immune status of their piglets at 5 d post-weaning.

The dietary treatment did not affect tissue mineral concentrations in the present study. In a related study, some tissues, such as the liver, showed similar trends in mineral concentration compared to the present study, independent of dietary treatment [48,49]. There is limited research on the impact of supplementing maternal diets with organic minerals on the mineral status of offspring. It was reported that maternal supplementation of chelated organic mineral sources (Cu, Fe, Zn and Mn) did not impact whole body tissue analysis in piglets when compared to inorganic sources [50]. Furthermore, as described by Papadopolous et al. [50], there was no difference in tissue concentrations of Mn in response to the maternal dietary addition of Mn, the element of interest in this study.

Weaning for piglets is a time of nutritional, immunological, social, and oxidative stress [47]. Oxidative stress results from the formation of reactive oxygen species (ROS), a byproduct of oxygen metabolism [12]. If left unchecked by antioxidant regulators, the accumulation of ROS can cause damage to membrane lipids, proteins, and DNA [51,52]. The tissue types of interest for the analysis of MnSOD activity in the present study were chosen based on the increased cellular energy requirement of hepatic, cardiac, and ileal tissues and localization of MnSOD to the mitochondria [12]. It was expected that increasing maternal Mn supplementation would increase MnSOD activity in tissues post-weaning. In weanling pigs fed the increasing dietary levels of Mn (0.24 to 32 ppm Mn), the hepatic MnSOD concentrations averaged 4.89 IU/mg and the cardiac concentrations averaged 10.0 IU/mg irrespective of Mn dose [53]. Weanling pigs fed 12 mg/kg had the increased red blood cell MnSOD activity 7 d post-weaning compared to those with no supplemented Mn [54]. The MnSOD values in the current study are higher than those previously reported. These differences are likely attributed to the supplemental Mn post-weaning rather than the maternal diet supplementation. In the current study, the liver MnSOD activity showed a reduction as the maternal Mn supplementation increased. This could be a result of suppressed oxidative stress in the PRO20 and PRO40 piglets at weaning. Cardiac tissue MnSOD activity, though not significantly different, showed a similar numerical reduction in activity from the CON to the PRO20 and PRO40 piglets.

Sow Mn supplementation influenced percent fat of both colostrum and milk and tended to change milk protein content. Mineral composition did not change due to supplemental Mn. The PRO20 sows had increased colostrum fat when compared to the PRO40

sows ($p < 0.05$), while colostrum fat in the PRO20 sows did not differ from the CON sows ($p > 0.05$). The CON sows had the highest milk fat percentage when compared to the PRO20 and PRO40 sows. Decreased percent fat of colostrum in response to increased dietary Mn may be due to increased milk demand from heavier the PRO20 and PRO40 piglets. Piglets from the PRO20 and PRO40 sows had significantly increased ADG when compared to CON piglets, suggesting consumption of increased amounts of milk. In conjunction, the PRO20 sows had a significantly increased overall ADFI. It is known that litter size can have an impact on sow milk yield [55]. The milk fat in thePRO20 and PRO40 may have been reduced or diluted, due to presumably higher milk yield. However, based on this data, there was no conclusive evidence that this was the case. The milk and colostral percent fat values from the current study are in line with those previously reported [56–58].

## 5. Conclusions

Supplementing gestating and lactating sow diets with Mn increased piglet birthweights and improved pre-weaning growth. The driver of increased pre-weaning piglet weight gain seems to be increased milk production, driven by increased sow lactation feed intake. Immune markers in lactating sows and their offspring at weaning responded inconsistently to Mn supplementation, suggesting that dietary Mn beyond what is provided by feedstuffs does not significantly impact either sow or piglet immune status at 3 d into lactation. Manganese supplementation to sow diets is critical to ensure increased piglet birthweights and pre-weaning growth rates. These improvements can have compounding effects as pigs continue to grow and progress through the nursery and grow-finish phases of production.

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