NON RUMINANT NUTRITION

Effects of mineral methionine hydroxy analog chelate in sow diets on epigenetic modification and growth of progeny

Ki Beom Jang,† Jong Hyuk Kim,† Jerry M. Purvis,‡ Juxing Chen,‖ Ping Ren,‖ Mercedes Vazquez-Anon,‖ and Sung Woo Kim†,1

†Department of Animal Science, North Carolina State University, Raleigh, NC 27695, ‡N.G. Purvis Farm Inc., Robbins, NC 27325, ‖Novus International, Inc., St. Charles, MO 63304

1Corresponding author: sungwoo_kim@ncsu.edu

Abstract

The study was conducted to determine the effects of mineral methionine hydroxy analog chelate (MMHAC) partially replacing inorganic trace minerals in sow diets on epigenetic and transcriptional changes in the muscle and jejunum of progeny. The MMHAC is zinc (Zn), manganese (Mn), and copper (Cu) chelated with methionine hydroxy analog (Zn-, Mn-, and Cu-methionine hydroxy analog chelate [MHAC]). On day 35 of gestation, 60 pregnant sows were allotted to two dietary treatments in a randomized completed block design using parity as a block: 1) ITM: inorganic trace minerals with zinc sulfate (ZnSO4), manganese oxide (MnO), and copper sulfate (CuSO4) and 2) CTM: 50% of ITM was replaced with MMHAC (MINTREX trace minerals, Novus International Inc., St Charles, MO). Gestation and lactation diets were formulated to meet or exceed NRC requirements. On days 1 and 18 of lactation, milk samples from 16 sows per treatment were collected to measure immunoglobulins (immunoglobulin G, immunoglobulin A, and immunoglobulin M) and micromineral concentrations. Two pigs per litter were selected to collect blood to measure the concentration of immunoglobulins in the serum, and then euthanized to collect jejunal mucosa, jejunum tissues, and longissimus muscle to measure global deoxyribonucleic acid methylation, histone acetylation, cytokines, and jejunal histomorphology at birth and day 18 of lactation. Data were analyzed using Proc MIXED of SAS. Supplementation of MMHAC tended to decrease (P = 0.059) body weight (BW) loss of sows during lactation and tended to increase (P = 0.098) piglet BW on day 18 of lactation. Supplementation of MMHAC increased (P<0.05) global histone acetylation and tended to decrease myogenic regulatory factor 4 messenger ribonucleic acid (mRNA; P = 0.068) and delta 4-desaturase sphingolipid1 (DEGS1) mRNA (P = 0.086) in longissimus muscle of piglets at birth. Supplementation of MMHAC decreased (P<0.05) nuclear factor kappa B mRNA in the jejunum and DEGS1 mRNA in longissimus muscle and tended to decrease mucin-2 (MUC2) mRNA (P = 0.057) and transforming growth factor-beta 1 (TGF-β1) mRNA (P = 0.057) in the jejunum of piglets on day 18 of lactation. There were, however, no changes in the amounts of tumor necrosis factor-alpha, interleukin-8, TGF-β, MUC2, and myogenic factor 6 in the tissues by MMHAC. In conclusion, maternal supplementation of MMHAC could contribute to histone acetylation and programming in the fetus, which potentially regulates intestinal health and skeletal muscle development of piglets at birth and weaning, possibly leading to enhanced growth of their piglets.

Key words: chelated minerals, growth, histone acetylation, intestinal health, piglets, sows
Introduction

With intensive genetic selection for the prolificacy of sows, the swine industry is challenged with improving the piglet survival rate in connection with the increased litter size and decreased litter uniformity (Kim and Hansen, 2013). It is well known that maternal nutrition can have an influence on not only the development of fetal organ and tissue but also milk yield (Kim et al., 1999; McPherson et al., 2004; Farmer, 2018). Deficiency of maternal nutrients would exert a negative impact on fetal and postnatal performance due to intrauterine growth retardation and inefficiency in milk secretion (Kim and Hansen, 2013). It is well known that maternal nutrition can have an influence on not only the development of fetal organ and tissue but also milk yield (Kim et al., 1999; McPherson et al., 2004; Farmer, 2018). Deficiency of maternal nutrients would exert a negative impact on fetal and postnatal performance due to intrauterine growth retardation and inefficiency in milk secretion (Kim and Hansen, 2013). Therefore, effective nutritional strategy is critical to improve sow and litter performance.

There is mounting evidence that maternal nutrition can elicit epigenetic modification of the fetal genome and expression of imprinted genes (Wu et al., 2004). Characteristics and variable patterns of epigenetic modification could cause changes in intestinal and muscle tissues under various environmental conditions (Jorgensen and Ro, 2019). The epigenetic modification represents a change not in the underlying deoxyribonucleic acid (DNA) sequence but in the way that how the cells read the genes. For epigenetic modification, DNA methylation and histone acetylation are caused by which methyl groups are transferred to DNA molecules and acetyl residue transferred to histone proteins, respectively, leading to a change of global gene expression (Stern and Berger, 2000; Moore et al., 2013). According to Anderson et al. (2012), epigenetic gene regulation could be modified by nutritional influence, leading to changes in gene expression. Therefore, the enzyme cofactors and methyl donor might be expected to lead DNA methylation and histone acetylation with the changes in global gene expression and biological reactions (Waterland, 2006; Delage and Dashwood, 2008). A previous study showed that the supplementation of methionine analog as a methyl donor in diets improved the protein accretion and growth rate as well as an immune response in broiler chickens (Zhang and Guo, 2008). Therefore, DNA methylation and histone modifications can be altered by the overall availability of amino acids and micronutrients, which play an important role in regulating the availability of enzyme groups causing the epigenetic modification.

Zinc (Zn), Manganese (Mn), and Copper (Cu) are essential minerals for the embryonic and fetal development (Hostetler et al., 2003). Previous studies also showed that these trace minerals had key functional involvements in the intestinal immune system (Shannon and Hill, 2019) and muscle growth (Zhao et al., 2014) of pigs. Zinc enhanced intestinal barrier function with improved gene expression of claudin-1 and occludin in broiler chicken (Zhang et al., 2012). Supplementation of Mn could protect the chick embryo from stress conditions by enhancing antioxidant and antiapoptotic activation (Zhu et al., 2017). Supplementation of Cu has been related to influence embryo development, oxidase activity, and organ development in fetus (Fell et al., 1965; Grace et al., 1986; Goonaratne and Christensen, 1989).

Organic minerals are broadly used in the swine industry to enhance the bioavailability of minerals, reduce risks of heavy metal contamination, and reduce excretion to the environment (Hollis et al., 2005; Mateo et al., 2007; Mahan et al., 2014). Organic minerals include chelated or complexed forms with amino acids, organic acids, peptides, polysaccharides, and proteins (AAFCO, 2018). Liu et al. (2014) reported that mineral methionine hydroxy analog chelate (MHAC) had a greater digestibility and retention of Zn, Cu, and Mn in growing pigs compared with trace mineral sulfates. According to Zhao et al. (2014), growing pigs fed with 80 mg/kg Cu-methionine hydroxy analog chelate (Cu-MHAC) in the diets had greater loin depth compared with 160 mg/kg CuSO₄, indicating that less MHAC could be used to replace high inorganic mineral in the diets. Supplementation of Zn-MHAC in broiler breeder diets reduced intestinal inflammation and improved intestinal integrity and immunity in progeny chicks by the upregulation of an anti-inflammatory gene, A20 protein, via reducing DNA methylation and increasing histone acetylation (Li et al., 2015). Considering the biological functions of Zn, Mn, and Cu in fetal and postnatal development as well as the biological efficacy of organic minerals, MMHAC could effectively enhance the development of the skeletal muscle and intestinal immunity of progeny by epigenetic modifications when fed to sows during pregnancy.

It is hypothesized that the supplementation of MMHAC in sow diets may enhance epigenetic modifications in skeletal and intestinal tissues in fetuses by enhancing muscle development and modulating intestinal inflammatory status. To test the hypothesis, the objective of this study was to determine the effects of MMHAC partially replacing Zn, Mn, and Cu sulfates in sow diets on sow and litter performance, global DNA methylation, histone acetylation, and gene expression related to muscle development and intestinal inflammation of progeny.

Materials and Methods

The protocol for this study was reviewed and approved by the North Carolina State University Institutional Animal Care and
Animals, experimental design, and diets

A group of sows were bred and checked for the pregnancy at day 35 of gestation. Sixty pregnant sows (average parity: 3.8 ± 2.0) were assigned to two dietary treatments at day 35 in a randomized completed block design with parity of sows as a block (first and second parities vs. multiparities). The experimental diets included 0.02% mineral premix on each treatment. Treatments were 1) ITM: ZnSO₄, MnO, and CuSO₄ and (2) CTM: same Zn, Mn, and Cu concentrations as ITM treatment, but 50% of ITM was replaced with Zn-, Mn-, and Cu-MHAC (MINTREX Zn, Mn, and Cu, Novus International Inc., St Charles, MO; Table 1). The experimental diets contained corn and poultry fat as major energy feeds and soybean meal as a major protein supplement with supplemental amino acids, phytase, yeast, and choline. The dietary formulation is not shown to protect proprietary information of the N.G. Purvis Farm but the analyzed mineral composition in the diets is shown in Table 2. The inclusion levels of trace minerals met the NRC (2012) requirements.

According to standard operating procedures of the commercial farm, all sows received assigned gestation diets at 2 kg/d until farrowing regardless of their body weight (BW) or parity. Pregnant sows were weighed and moved to a farrowing building on day 109 of gestation. Upon farrowing, sows were given ad libitum access to experimental diets until weaning (average day 21 of lactation). Feed intake of sows was recorded daily. All piglets were weighed at birth, on day 9, and on day 18 of lactation. Sows were weighed on day 18 of lactation.

Sample collection and preparation

Upon farrowing, the 1 male piglet representing the fourth or fifth birth of 32 litters (16 piglets per treatment) was separated for blood and tissue sampling before approaching the colostrum to determine the effects of MMHAC supplementation during gestation. For this study, only the male piglets were selected to determine the effects of MMHAC supplementation on the measures behind unexpected sex effects (Tarleton et al., 2001; Jiao et al., 2009; Haren et al., 2011). Blood sampling was conducted with BD sterile vacutainers (BD, Franklin Lakes, NJ) to collect serum, and then the piglets were euthanized to collect jejunal and muscle tissues. Blood samples were centrifuged to separate serum and stored at −80 °C until analysis. Upon euthanasia, jejunal tissues were separated from mid-jejunum at 1 m (at farrowing) or 3 m (on day 18 of lactation) after the duodenoejunal junction. Lumen content was removed by gentle washing with sterilized saline solution. A part (3 cm) was placed in a 50-mL tube with 10% buffered formaldehyde for fixation and immunohistochemistry. Another two parts (3 cm each) were stored in liquid nitrogen and stored at −80 °C for DNA methylation and histone acetylation measurements. Another part (1 cm) was collected and stored in 1.2 mL RNALater (Thermo Fisher Scientific Inc., Rockford, IL, USA) using 2 mL tube for 24 h at 4 °C and then stored at −20 °C until analysis for gene expression. The remaining part (9 cm) was used to collect mucosal tissues and then stored at −80 °C for cytokines measurement. Two samples of muscle were collected from the left longissimus dorsi of carcase between 10th and 11th ribs. Longissimus dorsi muscle is the part having accuracy and precision to assess the body and carcass composition of the carcase (Gresham et al., 1994). One was frozen in liquid N and stored at −80 °C until analysis for DNA methylation and histone acetylation measurements. The other sample (~0.5 cm in all three dimensions) was collected and stored in 1.2 mL RNALater using 2 mL tube for 24 h at 4 °C and then stored at −20 °C until analysis for gene expression.

Mineral composition in colostrum and milk

Colostrum and milk samples were stored at −20 °C and freeze-dried (24D 48, Virtis, Gardiner, NY, USA). The dried milk samples were submitted to the Environmental and Agricultural Testing Service (EATS) laboratory, Department of Crop and Soil Sciences, at North Carolina State University. The minerals (Zn, Mn, Cu, and Ca) concentration in colostrum and milk samples was determined using atomic absorption spectrometry (Perkin-Elmer 3100, Shelton, CT). Mineral compositions and immunohistochemistry

Morphological evaluation and immunohistochemistry

Jejunal tissue samples were fixed in 10% formalin buffer for 3 wk and sent to the Histology Laboratory of North Carolina State University (Raleigh, NC, USA) for hematoxylin and eosin

| Table 1. Micromineral concentration in dietary treatments¹ |
|---------------|-------------|-------------|
| Mineral source, mg/kg | ITM | CTM |
| ZnSO₄      | 125.0 | 62.5 |
| Zn-MHAC   | 0.0  | 62.5 |
| MnO        | 40.0 | 20.0 |
| Mn-MHAC   | 0.0  | 20.0 |
| CuSO₄     | 16.0 | 8.0  |
| Cu-MHAC   | 0.0  | 8.0  |

¹The same amount of methionine provided by organic minerals as a form of Zn-MHAC, Mn-MHAC, and Cu-MHAC (MINTREX, Novus International, St. Charles, MO) was also supplemented as a form of MHA in the ITM diet.

| Table 2. Analyzed mineral concentrations in diets (as-fed basis) |
|------------------|-------------|-------------|
| Item             | ITM¹        | CTM²        |
| **Gestation diet**|
| Zn, mg/kg        | 192.7       | 173.7       |
| Mn, mg/kg        | 89.3        | 81.3        |
| Cu, mg/kg        | 29.3        | 20.7        |
| Ca, %            | 0.83        | 0.84        |
| Total P, %       | 0.78        | 0.78        |
| **Lactation diet**|
| Zn, mg/kg        | 208.5       | 182.5       |
| Mn, mg/kg        | 91.0        | 92.0        |
| Cu, mg/kg        | 52.0        | 34.5        |
| Ca, %            | 1.09        | 1.07        |
| Total P, %       | 0.66        | 0.65        |

¹ITM, conventional inorganic sources of trace minerals (0.2% inclusion level in the diets).

²CTM, 50:50 MMHAC and inorganic minerals (0.2% inclusion level in the diets).
staining as well as immunohistochemistry for detecting Ki67- antibody as a biological marker for measuring the proliferation of enterocytes. A total of 15 villus and 15 crypts in each slide were selected to measure villus height (VH), villus width, crypt depth (CD), and percent of Ki67- enterocyte using a microscope (Olympus CX31 microscope, Tokyo, Japan). The ratio of VH to CD (VH:CD) was calculated. The histomorphology was measured as previously described by Duarte et al. (2019).

Immunoglobulins concentration was analyzed using the method described by Shen et al. (2011). Total concentrations of immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) in the colostrum and milk of sows, as well as in the jejunal mucosa and serum of nursery pigs, were measured by ELISA according to the manufacturer’s protocols (Bethyl Laboratories Inc., Montgomery, TX). Before analysis, all samples of colostrum, milk, and mucosal tissue were diluted to 1:800,000, 1:50,000, and 1:1,600 to measure IgG, IgA, and IgM, respectively.

IgG, IgA, and IgM
Immunoglobulins concentration was analyzed using the method described by Shen et al. (2011). Total concentrations of IgG, IgA, and IgM in the colostrum and milk of sows, as well as in the jejunal mucosa and serum of nursery pigs, were measured by ELISA according to the manufacturer’s protocols (Bethyl Laboratories Inc., Montgomery, TX). Jejunal mucosa samples were weighed and suspended into 1.0 mL phosphate-buffered saline. The suspended samples were homogenized on ice (Tissuemiser, Thermo Fisher Scientific Inc., Rockford, IL, USA). The homogenate was centrifuged at 14,000 × g for 20 min. The supernatant was used to determine concentrations of total protein in jejunal mucosa and muscle for analysis. Total protein concentration in the mucosa was determined by using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific Inc., Rockford, IL, USA); 50 ng of histone protein from jejunal mucosa or 180 ng of histone protein from jejunum was used to measure histone acetylation using EpiXtract Total Histone Extraction Kit (Epigentek, Farmingdale, NY). Total concentration of histone concentration was measured using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific Inc., Rockford, IL, USA); 50 ng of histone protein from longissimus muscle or 180 ng of histone protein from jejunum was used to measure histone acetylation using EpiQuik Global Acetyl Histone H3-K9 Quantification Kit (Epigentek, Farmingdale, NY). Acetylated histone was expressed as μg/mg protein as described in the manufacturer’s protocol.

Quantification of gene expression
Total ribonucleic acid (RNA) was isolated from the jejenum or longissimus muscle using Trizol reagent (Thermo Fisher Scientific Inc., Rockford, IL, USA); 1 μg of total RNA was used to synthesize complementary DNA using oligo dT and M-MLV Reverse Transcriptase (Thermo Fisher Scientific Inc., Rockford, IL, USA) according to the manufacturer’s instructions. Relative levels of messenger ribonucleic acid (mRNA) were measured by quantitative real-time polymerase chain reaction (PCR) using Applied Biosystems SYBR Green PCR Master Mix (Thermo Fisher Scientific Inc., Rockford, IL, USA) and a Q5S Real-Time PCR System. Results were expressed as the level relative to the corresponding housekeeping gene beta-actin. The Ct of housekeeping gene beta-actin was not statistically affected by dietary treatment. Therefore, beta-actin was chosen as a housekeeping gene for this study and quantified along with each gene, and the relative expression of each gene was normalized to beta-actin using delta–delta–Ct method as described previously (Livak and Schmittgen, 2001) and expressed as the level relative to beta-actin. All primers (Table 3) were verified for melting curve, efficiency (100% ± 10%), and linearity (r² ≥ 0.99) of amplification.

Statistical analysis
Data from this study were analyzed based on a randomized complete block design by the mixed model of SAS Software (Cary, NC). Sow was considered as an experimental unit for reproductive performance parameters, and piglet was the experimental unit for other parameters. Treatment was the fixed effects and the parity block was a random effect. The statistical difference among treatment means was considered significant with P < 0.05, whereas 0.05 ≤ P < 0.10 was considered as tendency.

Results
Sow and litter performance
Average parity of sow was not different between treatments (Table 4). The BW of sows at days 50 and 110 of gestation and BW change of sow during gestation were not different between treatments (Table 5). BW of sows, average daily feed intake (ADI), litter size, and litter weight during lactation were not different between treatments (Table 6). Sows fed a diet with MMHAC tended to have decreased (P = 0.059) BW loss during lactation compared with sows fed a diet with ITM. The BW of piglets at birth was not different between treatments. Piglets from sows fed a diet with MMHAC tended to have increased...
Table 3. Sequence of primers for jejunum and skeletal muscle

| Item   | Forward primers          | Reverse primers          | Accession number |
|--------|--------------------------|--------------------------|------------------|
| MEF2C  | GACATCCTGGAGAGCCTTGAGA  | TCAGGCTTGACCTGATGAGATGA  | NM_001044540.1   |
| MSTN   | CAGTAAACCCACGGCACTGTTA  | TCCTGCTGTTGCAAGGTTTA    | NM_214435.2      |
| MYOD1  | GAGGCAACATTTACAGGCGCA   | CAGGAGCTGGACAGACAGTT    | NM_001028241.1   |
| MYF5   | AGTTCCGGGAGCAGTTGAGAG  | GTGATTTTCTCTTGGACCCCG   | KC546667.1       |
| MRF4   | CTCAAGGACCTATCGAAAGG   | CACCCAGGAAAAACACAGG    | NM_001244627.1   |
| MEF2A  | CATGGGCAACACAGGCACCT   | TGCAAGCTAGTGGAGGAAAG    | NM_001244621.1   |
| DEGS1  | ATGGATGCGTGGTGATATTC   | GCATATAGGCGTCAAGAGG    | NM_001244621.1   |
| MTOR   | CAAACCCCGGAGGATGCAA    | ATATAAAAAAGTGCACTTCAAC  | XM_003127584.6   |
| NF-κB  (P50) | TGGTATACCTCATGAGAAAG | GCCACACTGGCCCTTTGT    | XM_021082584.1   |
| NF-κB  (P105) | TGGTAGTCTACACGAAAGCA | CAGCGAGGTGCAAAAACAGAGT | KC316024.1       |
| TGF-β  | AGTTCGGGGACGAGTTTGAG  | TCTGCTGCTCTTGGTCTTCTGT | AB057424.1       |
| IL-6   | GGAGTTGAGGAGGAGGAGG    | GCCCGAGCAATTACAGCGA    | KC316025.1       |
| IL-8   | CTGTTGCTGCTCTTGGCACCG  | GGCACACTGCGCTCTTGT    | XM_021082584.1   |
| MUC2   | CAAGGCGTCTCACCAGGACCA  | GGCAGATTTGAAACTCCCTTA  | XM_021093503.1   |
| DEGS1  | GCCGCTCTTCTACCAAGACCA  | CTTCTGGTGCCTGCTTGAT    | JF831365.1       |
| TNF-α  | CAATGCTCTTCTACGCGGACTG | TCTCATTTTCTGCCAACTTG   | XM_001242820.5   |
| --     | --                       | --                       | --               |

Table 4. Reproductive performance of sows in the previous parity

| Item         | ITM¹ | CTM² | SEM  | P-value |
|--------------|------|------|------|---------|
| N            | 24   | 23   |      |         |
| Gestation period | 115.5 | 115.7 | 0.2  | 0.447   |
| Sow parity   | 3.33 | 3.61 | 0.35 | 0.577   |
| Litter size, head |      |      |      |         |
| At birth, total | 14.2 | 14.7 | 0.9  | 0.689   |
| At birth, live | 13.4 | 13.4 | 0.8  | 0.996   |
| Stillborn    | 0.5  | 0.7  | 0.2  | 0.579   |
| Mummies      | 0.3  | 0.6  | 0.2  | 0.292   |

¹ITM, conventional inorganic sources of trace minerals (0.2% inclusion level in the diets).
²CTM, 50:50 MMHAC and inorganic minerals (0.2% inclusion level in the diets).

(P = 0.098) BW of piglets on day 18 after birth. The concentration of Zn, Mn, Cu, and Ca in colostrum and milk were not affected by the supplementation of MMHAC in sow diets when measured on days 1 and 18 of lactation (Table 7).

Intestinal health, global DNA methylation, histone acetylation, and gene expression in the jejenum and longissimus muscle of piglets

No differences in jejunal histomorphometry and crypt cell proliferation of piglets were observed between treatments on days 1 and 18 of lactation (Table 8). Concentration of IgG, IgA, and IgM in colostrum, milk, serum, and jejunal mucosa of piglets did not differ between treatments on days 1 and 18 of lactation. (Table 9). Concentration of TNF-α, IL-8, TGF-β1, and MUC2 in the jejunal mucosa and myogenic regulatory factor 4 (MRF4) in the muscle of the piglets were not affected by the supplementation of MMHAC in sow diets on days 1 and 18 of lactation (Table 10). Piglets from sows fed a diet with MMHAC had a higher (P = 0.05) status of global acetylation in the muscle tissue at birth than piglets from sows fed without MMHAC (Table 11).

Table 5. Supplemental effects of MMHAC in sow diets on the performance of sows during gestation

| Item         | ITM¹ | CTM² | SEM  | P-value |
|--------------|------|------|------|---------|
| N            | 29   | 28   |      |         |
| Sow parity   | 3.79 | 3.96 | 0.38 | 0.703   |
| BW of sows, kg |      |      |      |         |
| Day 50 of gestation | 238.4 | 240.8 | 5.5  | 0.738   |
| Day 110 of gestation | 278.2 | 274.8 | 6.1  | 0.663   |
| BW change, kg |      |      |      |         |
| Day 50 to 110 of gestation | 39.9  | 34.0  | 3.2  | 0.184   |

¹ITM, conventional inorganic sources of trace minerals (0.2% inclusion level in the diets).
²CTM, 50:50 MMHAC and inorganic minerals (0.2% inclusion level in the diets).

(P = 0.068) at birth in the muscle of piglets from sows fed with MMHAC during gestation. Expression of delta 4-desaturase sphenolipid (1) (DEGS1) mRNA tended to be decreased (P < 0.10) at birth and was decreased (P < 0.05) on day 18 of lactation in the muscle of piglets from sow fed with MMHAC.

Discussion

This study demonstrated that dietary supplementation of MMHAC replacing inorganic minerals benefited sows by reducing BW losses during lactation and suckling piglets by increasing BW at weaning. The NF-κB is a signaling molecule that plays a key role in the regulation of inflammation pathway; it can be activated by external stimuli such as pathogens and inflammation and then translocate into the nucleus and stimulate the expression of proinflammatory cytokines, such as IL-1β, IL-6, IL-8, IL-12, IL-18, TNF-α, and interferon-γ (NF-κB). In this study, the expression of NF-κB mRNA in piglets was downregulated by supplementation of MMHAC in sow diets on day 18 of lactation.
inflammation in progeny piglet. Regulation of muscle-related gene expression indicated that MMHAC increased muscle fiber hypertrophy in suckling piglets. These outcomes partially explain how MMHAC reduced BW loss of sows and increased BW of suckling piglets. Results from this study indicated that genes related to muscle development at birth and intestinal health at weaning were modulated by feeding a diet with MMHAC.

The differentiation of muscle is regulated by a group of MRF, including myogenic differentiation 1 (MYOD1), myogenic factor 5 (MYF5), myogenin (MYOG), and MRF4 (Moncaut et al., 2013; Moretti et al., 2016). The MYOD1 and MYF5 are involved in the generation of myogenic precursors called myoblasts; MYOG and MRF4 are required for terminal differentiation of committed muscle progenitors into myofibers (Moncaut et al., 2013). The expression of MRF4 starts during fetal development, continues throughout postnatal stages, and is the predominant MRF in adult skeletal muscle (Hinterberger et al., 1991; Yan et al., 2013), suggesting that MRF4 may regulate skeletal muscle maturation and hypertrophy during late fetal development and postnatal muscle growth. Previous studies showed that the knockdown of MRF4 expression at both gene and protein levels induced muscle fiber hypertrophy, increased protein synthesis, and activated expression of a variety of muscle-specific genes in skeletal muscle, indicating that MRF4 negatively regulates skeletal muscle growth (Moretti et al., 2016). In this study, MMHAC increased global histone acetylation and crypt cell proliferation in the jejunum of suckling piglets (Table 8).

| Table 6. Supplemental effects of MMHAC in sow diets on the performance of sows and litters during lactation |
|---|
| Item | ITM1 | CTM2 | SEM | P-value |
| N | 28 | 25 | | |
| Sow parity | 3.82 | 3.84 | 0.40 | 0.973 |
| BW of sows, kg | | | | |
| Day 50 of gestation | 238.4 | 240.8 | 5.5 | 0.738 |
| Day 110 of gestation | 272.8 | 274.8 | 6.1 | 0.663 |
| Day 1 of lactation | 261.0 | 257.8 | 5.3 | 0.635 |
| Day 20 of lactation | 244.1 | 250.9 | 5.1 | 0.314 |
| Jejunum, day 1 of lactation |
| VH, μm | 690 | 651 | 36 | 0.446 |
| Villus width, μm | 70 | 71 | 2 | 0.891 |
| CD, μm | 94 | 94 | 5 | 0.996 |
| VH:CD | 8.92 | 8.67 | 0.33 | 0.600 |
| Crypt cell proliferation, % | 42.3 | 46.1 | 2.9 | 0.369 |
| Jejunum, day 18 of lactation |
| VH, μm | 488 | 422 | 51 | 0.394 |
| Villus width, μm | 88 | 89 | 2 | 0.908 |
| CD, μm | 162 | 151 | 7 | 0.282 |
| VH:CD | 3.24 | 3.44 | 0.21 | 0.493 |
| Crypt cell proliferation, % | 37.3 | 36.2 | 4.6 | 0.874 |

| Table 7. Supplemental effects of MMHAC in sow diets on mineral composition in colostrum and milk (DM basis) |
|---|
| Item | ITM1 | CTM2 | SEM | P-value |
| N | 16 | 16 | | |
| Sow parity | 3.62 | 3.62 | 0.46 | 1.000 |
| Colostrum |
| Zn, mg/kg | 69.53 | 70.21 | 2.86 | 0.863 |
| Mn, mg/kg | 0.23 | 0.14 | 0.06 | 0.327 |
| Cu, mg/kg | 18.24 | 15.68 | 1.07 | 0.121 |
| Ca, % | 0.22 | 0.20 | 0.01 | 0.191 |
| Milk, day 18 of lactation |
| Zn, mg/kg | 31.05 | 29.28 | 1.52 | 0.411 |
| Mn, mg/kg | 0.55 | 0.52 | 0.07 | 0.801 |
| Cu, mg/kg | 6.36 | 6.58 | 0.27 | 0.567 |
| Ca, % | 1.04 | 0.93 | 0.04 | 0.104 |

| Table 8. Supplemental effects of MMHAC in sow diets on morphology and crypt cell proliferation in the jejunum of suckling piglets |
|---|
| Item | ITM1 | CTM2 | SEM | P-value |
| Jejunum, day 1 of lactation |
| VH, μm | 690 | 651 | 36 | 0.446 |
| Villus width, μm | 70 | 71 | 2 | 0.891 |
| CD, μm | 94 | 94 | 5 | 0.996 |
| VH:CD | 8.92 | 8.67 | 0.33 | 0.600 |
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1ITM, conventional inorganic sources of trace minerals (0.2% inclusion level in the diets).
2CTM, 50:50 MMHAC and inorganic minerals (0.2% inclusion level in the diets).

In this study, MMHAC increased global histone acetylation in the longissimus muscle of piglets at birth, indicating that MMHAC potentially increased the expression of genes that are related to muscle development in the fetus during gestation.
During gestation, the number of muscle fibers in pigs is predetermined through embryonic development (Wigmore and Stickland, 1983). After myogenesis, muscle growth is achieved by muscle fiber hypertrophy (Dwyer et al., 1994; Murray et al., 2018). Yan et al. (2013) reported the impact of maternal nutrition on the fetal development in pigs, especially related to fetal skeletal muscle and intestine development. In particular, maternal nutrition during the embryonic stage has relatively minor effects on skeletal muscle development, because only a very small number of myofibers would be formed during early gestation. Dwyer et al. (1994) indicated that the critical stage for fetal skeletal muscle development is mid-to-late gestation in pigs.

Histone H3K9 acetylation is a primary acetylated site of histone H3 and an active chromatin epigenetic tag (Turner, 2000). Histone acetylation modulated by histone acetyltransferases (HATs) can be one marker of epigenetic modifications, which can be associated with the regulation of the muscle-specific genes transcription, repression, and activation (Moresi et al., 2015). The HDAC inhibitors, which inhibit HDACs and thus block histone deacetylation, are potential therapeutic agents for promoting muscle regeneration (Jang et al., 2018). To assess the efficacy of MMHAC, a new inhibitor of HDACs, the effects of MMHAC in sow diets on global methylation and acetylation in the jejunum and longissimus muscle of suckling piglets were evaluated.

### Table 9. Supplemental effects of MMHAC in sow diets on immunoglobulin concentration in colostrum and milk of sows and serum and jejunal mucosa of suckling piglets

| Item                        | ITM1 | CTM2 | SEM    | P-value |
|-----------------------------|------|------|--------|---------|
| Colostrum, day 1 of lactation | IGG, mg/mL | 118.68 | 111.95 | 22.42   | 0.832   |
|                             | IGAM, mg/mL | 18.20 | 17.34  | 2.72    | 0.819   |
|                             | IGAM, mg/mL | 8.68  | 7.79   | 1.41    | 0.648   |
| Milk, day 18 of lactation   | IGG, mg/mL | 2.32  | 2.02   | 0.34    | 0.503   |
|                             | IGAM, mg/mL | 4.69  | 5.14   | 0.32    | 0.331   |
|                             | IGAM, mg/mL | 2.92  | 2.81   | 0.29    | 0.779   |
| Serum, day 1 of lactation   | IGG, mg/mL | 0.139 | 0.129  | 0.025   | 0.762   |
|                             | IGAM, mg/mL | 0.008 | 0.006  | 0.001   | 0.262   |
|                             | IGAM, mg/mL | 0.064 | 0.060  | 0.005   | 0.614   |
| Jejunal mucosa, day 1 of lactation | IGG, mg/mL | 2.966 | 2.717  | 0.192   | 0.339   |
|                             | IGAM, mg/mL | 0.175 | 0.132  | 0.020   | 0.164   |
|                             | IGAM, mg/mL | 0.938 | 1.037  | 0.129   | 0.580   |
| Jejunal mucosa, day 18 of lactation | IGG, mg/g | 2.501 | 1.942  | 0.307   | 0.226   |
|                             | IGAM, mg/g | 0.605 | 0.558  | 0.032   | 0.307   |
|                             | IGAM, mg/g | 0.220 | 0.190  | 0.032   | 0.518   |

1ITM, conventional inorganic sources of trace minerals (0.2% inclusion level in the diets).
2CTM, 50:50 MMHAC and inorganic minerals (0.2% inclusion level in the diets).

### Table 10. Supplemental effects of MMHAC in sow diets on inflammatory cytokines, MUC, and MYF in the jejunum and longissimus muscle of suckling piglets

| Item                        | ITM1 | CTM2 | SEM    | P-value |
|-----------------------------|------|------|--------|---------|
| TNF-α in jejunal mucosa, pg/mg | Day 1 of lactation | 0.97 | 0.80 | 0.08 | 0.168 |
|                             | Day 18 of lactation | 1.21 | 1.24 | 0.12 | 0.896 |
| IL-8 in jejunal mucosa, pg/mg | Day 1 of lactation | 3.75 | 2.89 | 0.82 | 0.451 |
|                             | Day 18 of lactation | 34.90 | 27.51 | 3.99 | 0.211 |
| TGF-β1 in jejunal mucosa, pg/mg | Day 1 of lactation | 35.08 | 26.26 | 6.65 | 0.360 |
|                             | Day 18 of lactation | 7.65 | 7.49 | 0.79 | 0.884 |
| MUC2 in jejunal mucosa, U/mg | Day 1 of lactation | 0.86 | 0.85 | 0.23 | 0.996 |
|                             | Day 18 of lactation | 0.40 | 0.43 | 0.04 | 0.601 |
| MYF6 in muscle, ng/mg | Day 1 of lactation | 0.45 | 0.41 | 0.06 | 0.665 |
|                             | Day 18 of lactation | 0.21 | 0.23 | 0.02 | 0.464 |

1ITM, conventional inorganic sources of trace minerals (0.2% inclusion level in the diets).
2CTM, 50:50 MMHAC and inorganic minerals (0.2% inclusion level in the diets).

### Table 11. Supplemental effects of MMHAC in sow diets on global methylation and acetylation in the jejunum and longissimus muscle of suckling piglets

| Item                        | ITM1 | CTM2 | SEM    | P-value |
|-----------------------------|------|------|--------|---------|
| Day 1 of lactation          | Jejunum | Methylation | 7.8 | 6.7 | 0.8 | 0.303 |
|                             | Acetylation | 360.8 | 343.6 | 13.8 | 0.383 |
|                             | Muscle | Methylation | 11.6 | 11.6 | 1.8 | 0.999 |
|                             | Acetylation | 748.9 | 1,137.4 | 112.7 | 0.021 |
| Day 18 of lactation         | Jejunum | Methylation | 13.4 | 14.9 | 1.2 | 0.378 |
|                             | Acetylation | 338.6 | 305.8 | 32.7 | 0.484 |
|                             | Muscle | Methylation | 9.7 | 8.6 | 2.1 | 0.705 |
|                             | Acetylation | 75.8 | 75.1 | 1.0 | 0.646 |

1ITM, conventional inorganic sources of trace minerals (0.2% inclusion level in the diets).
2CTM, 50:50 MMHAC and inorganic minerals (0.2% inclusion level in the diets).

### Table 12. Supplemental effects of MMHAC in sow diets on the expression of key mRNA related to jejunal inflammation in suckling piglets

| Item                        | ITM1 | CTM2 | SEM    | P-value |
|-----------------------------|------|------|--------|---------|
| Day 1 of lactation, × 10^5  | IL-8 | 1,344 | 1,018 | 178 | 0.193 |
|                             | MUC2 | 5,380 | 5,511 | 984 | 0.925 |
|                             | NF-κB (p50) | 701 | 693 | 93 | 0.944 |
|                             | NF-κB (p105) | 1,991 | 1,646 | 211 | 0.274 |
|                             | TGF-β1 | 2,000 | 1,600 | 370 | 0.500 |
|                             | TNF-α | 11 | 7 | 2 | 0.174 |
| Day 18 of lactation, × 10^5  | IL-8 | 1,134 | 787 | 220 | 0.262 |
|                             | MUC2 | 5,773 | 3,871 | 722 | 0.077 |
|                             | NF-κB (p50) | 752 | 507 | 84 | 0.048 |
|                             | NF-κB (p105) | 1,589 | 995 | 172 | 0.012 |
|                             | TGF-β1 | 2,000 | 1,400 | 230 | 0.057 |
|                             | TNF-α | 21 | 27 | 3 | 0.139 |

1ITM, conventional inorganic sources of trace minerals (0.2% inclusion level in the diets).
2CTM, 50:50 MMHAC and inorganic minerals (0.2% inclusion level in the diets).
inhibit histone deacetylation, could ultimately increase histone acetylation, thereby regulating gene expression. These HDAC inhibitors increased MyoD acetylation in myoblasts (Lezzi et al., 2002), myocyte enhancer factor 2 (MEF2) acetylation in human embryonic kidney 293 (HEK293) cells (Grégoire et al., 2007), and histone H3 acetylation, which leads to the upregulation of cardiac gene expression in cardiomyocytes (Otsuji et al., 2012). The HDAC inhibitors have also been reported to increase myofiber size and gene expression in cardiomyocytes (Otsuji et al., 2012). The HDAC histone H3 acetylation, which leads to the upregulation of cardiac embryonic kidney 293 (HEK293) cells (Grégoire et al., 2007), and MEF2C, 50:50 MMHAC and inorganic minerals (0.2% inclusion level in the diets).

### Table 13. Supplemental effects of MMHAC in sow diets on the expression of key mRNA related to muscle development of suckling piglets

| Item     | ITM | CTM | SEM | P-value |
|----------|-----|-----|-----|---------|
| **Day 1 of lactation, × 10⁴** |     |     |     |         |
| MEF2C    | 312 | 299 | 48  | 0.838   |
| MSTN     | 58  | 54  | 10  | 0.770   |
| MYOD1    | 218 | 165 | 43  | 0.375   |
| MYF5     | 101 | 134 | 18  | 0.191   |
| MRF4     | 3,529 | 2,436 | 408 | 0.088 |
| MEF2A    | 1,166 | 910  | 128 | 0.157   |
| DEGS1    | 19,870 | 26,424 | 2,599 | 0.086 |
| MTO      | 88  | 80  | 21  | 0.798   |
| **Day 18 of lactation, × 10⁴** |     |     |     |         |
| MEF2C    | 825 | 618 | 126 | 0.249   |
| MSTN     | 558 | 504 | 71  | 0.591   |
| MYOD1    | 1,385 | 995  | 169 | 0.114   |
| MYF5     | 241 | 225 | 24  | 0.636   |
| MRF4     | 9,207 | 10,555 | 1,091 | 0.390 |
| MEF2A    | 3,047 | 2,506 | 280 | 0.175   |
| DEGS1    | 54,844 | 33,777 | 4,182 | 0.001   |
| MTO      | 195 | 298 | 47  | 0.130   |

1ITM, conventional inorganic sources of trace minerals (0.2% inclusion level in the diets).
2CTM, 50:50 MMHAC and inorganic minerals (0.2% inclusion level in the diets).

Supplemental effects of MMHAC in sow diets on the expression of key mRNA related to muscle development of suckling piglets

- **Day 1 of lactation, × 10⁴**
  - MEF2C: 312, 299, 48, 0.838
  - MSTN: 58, 54, 10, 0.770
  - MYOD1: 218, 165, 43, 0.375
  - MYF5: 101, 134, 18, 0.191
  - MRF4: 3,529, 2,436, 408, 0.088
  - MEF2A: 1,166, 910, 128, 0.157
  - DEGS1: 19,870, 26,424, 2,599, 0.086
  - MTO: 88, 80, 21, 0.798

- **Day 18 of lactation, × 10⁴**
  - MEF2C: 825, 618, 126, 0.249
  - MSTN: 558, 504, 71, 0.591
  - MYOD1: 1,385, 995, 169, 0.114
  - MYF5: 241, 225, 24, 0.636
  - MRF4: 9,207, 10,555, 1,091, 0.390
  - MEF2A: 3,047, 2,506, 280, 0.175
  - DEGS1: 54,844, 33,777, 4,182, 0.001
  - MTO: 195, 298, 47, 0.130

This study also shows that weaning piglets from sows fed a diet with MMHAC had lower expression of NF-κB, MUC2, and TGF-β1 in the jejunum compared with piglets from sows fed a diet with ITM. The NF-κB is a transcription factor responsible for immune and inflammatory response. The MUC2 expression was not correlated with impaired intestinal barrier functions when considering...
decreased NF-κB gene expression and increased growth of piglets at day 18 of lactation. Therefore, reduced expression of MUC2 in piglets from sows fed a diet with MMHAC could also indicate that nutrients to make mucin could be saved potentially directing more nutrients for the growth of their piglets. Previous studies showed that chelated mineral sources can regulate intestinal inflammatory response (Liao et al., 2018; Shannon and Hill, 2019). The intestinal inflammatory response can be caused by various stimuli, such as viral antigen, bacteria invasion, cytokines, stress, and free radicals. Prasad et al. (2004) described that Zn had anti-inflammatory effect by decreasing gene expression of TNF-α and IL-1β through inhibiting NF-κB activation. Zinc amino acid complex supplementation reduced TNF-α levels in the pigs (Mayorga et al., 2018). Supplementation of Zn-MHAC in the breeder diets induced the reduction of intestinal inflammation in their progeny chicks compared with ZnSO₄. Supplementation of Zn-MHAC in the breeder diets induced the reduction of intestinal inflammation in their progeny chicks compared with ZnSO₄. 

In conclusion, maternal supplementation of MMHAC to sows during gestation improved intestinal health and skeletal muscle growth in progeny piglets by modulating the expression of mRNA for key regulatory proteins. In the intestine, supplementation of MMHAC reduced inflammation by downregulating NF-κB and TGFβ1 expression. In the longissimus muscle, supplementation of MMHAC potentially promoted skeletal muscle growth by upregulating histone H3K9 acetylation, by tendency of downregulating MRPs gene expression, and by differentially regulating DEGS1 gene expression. However, these changes in mRNA expression were not shown at protein levels. Collectively, maternal supplementation of MMHAC could potentially modulate histone acetylation and programming in the fetus during gestation, regulate intestinal health and skeletal muscle development of piglets, and, therefore, lead to enhanced growth of their piglets. However, the results from this study could not directly answer whether the regulation of genes expression measured is mediated by epigenetic modification such as histone acetylation which warrants future investigation.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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