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Effects of mineral nano particles on gene expression in piglet adipose tissue

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Abstract
In the present study, we aimed to evaluate the effect of supplementation of mineral nano particles on expression genes associated with piglet growth. The Duroc-Landrace-Yorkshire piglets weaned at 25 days were used for this study. Piglets of the experimental group was fed with basal diets supplemented with 12.8 mg/kg nMn, 160 mg/kg nFe, 16 mg/kg nCu, 0.8 mg/kg Co, 0.8 mg/kg nSe, and 160 mg/kg nZn. The commercially normal minerals were used for the control group. The qRT-PCR showed that the nano mineral supplementation induced the increase of CTGF and GHR transcript expression, while reduced the FST transcript expression in piglets for 20 days of treatment. After 40 days of treatment, the piglets using nano minerals showed the higher transcript expression of CTGF and GHR. However, the transcript expression of FST was reduced in group with nano minerals treatment. The result indicated that the ratio of mineral nano supplement could induce the changes of gene expression in piglet adipose tissue.

Keywords: Adipose tissue, mineral nano particles, transcript expression, weaned piglets

1. Introduction
Mineral nutrition plays an important role in animal health and production. Nano minerals have been considered as the factors could improve bioavailability by the increase in the surface area, higher surface activity, high catalytic efficiency and stronger adsorbing ability [1]. Nano minerals are used for enhancing the bioavailability of minerals in livestock which is helpful in improving growth, production and health status of animals [1]. The presence of exogenous ZnO could increase the *in vitro* intestinal epithelial regeneration capacity, leading to an increase in the level of intestinal insulin-like growth factor 1 (IGF-1 gene) in piglets [2]. The supplement of nanoparticles of ZnO also enhanced the growth performance, powered utility and provided benefits in weaned piglets [3]. The supplement of 150 ppb of nano selenium showed a higher average daily gain in guinea pigs, comparing to organic and inorganic selenium for 70 days [4]. The demonstrated that supplementation of 50 ppm nano Cu could improve their growth rate [5]. Moreover, nano Cu supplement could increase the activity of the lipase and phospholipase enzymes in the small intestine of pigs, comparing to sulfate Cu. Another study showed that the supplement of nano Cu for pig could induce a higher total globulin protein and superoxide dismutase than sulfate Cu, so it could be said that nano Cu have increased the immune system's activity. The higher serum level of total protein HDL and lipase activity were reported in pigs treated with the supplementation of 200 µg chromium from nano Cr [6]. The effects of nano particles on gene profile expression of pig adipose tissue have been still unclear. In this study, we investigated the changes of transcript expression of piglet adipose tissue which were treated with nano minerals.

2. Materials and Methods
Animal and experimental design
The piglets weaned at 25 days were used for this study. The piglets received the same basal diet (per kilogram diet) including: corn (55.7%), extruded soybean (12%), soybean meal (18.3%), fish meal (4%), whey powder (4%), glucose (2%), vitamin premix (1%), mineral premix (1%) for the control group. The piglets of the experimental group was fed with basal diets supplemented with 12.8 mg/kg nMn, 160 mg/kg nFe, 16 mg/kg nCu, 0.8 mg/kg Co, 0.8 mg/kg nSe, and 160 mg/kg nZn. The piglets of the control group at day 20 (group C-20) and day 40 (group C-40) and experimental group from day 20 (group IV-20) and 40 (group IV-40) were used for transcript expression evaluation.

Quantitative Real time RT-PCR

The total RNA of pig ear tissues was extracted using a Ribospin™ Total RNA Purification Kit (Gene All Biotechnology, Korea), according to the manufacturer's instructions. Piko Real 96 Real-Time PCR System (Thermo Scientific, United States) was applied for qRT-PCR. qRT-PCR reactions were performed with qPCR SyGreen 1-Step Lo-ROX kit (Biosystem, England). qRT-PCR was conducted in 20 µl for each reaction, including 1 µl of total RNA, 2 µl of primers (forward and reverse) (Table 1) [7], 10 µl Mix Ro-Lox, 1 µl RTAse, and 6 µl dH2O. The qRT-PCR reactions were performed by one cycle of 45 °C for 15 min, one cycle of 95 °C for 2 min, 40 cycles of 95 °C for 10 sec, 60 °C for 15 sec; and 71 cycles of 60 °C for 30 sec. 18S was used as internal control, the $2^{-\Delta\Delta Ct}$ method was applied for Ct value analysis [8].

Table 1: Primers were used in this study for qRT-PCR

Gene	Primer (5'–3')	Gen Bank accession number
Connective tissue growth factor (CTGF)	F: GTGTGACGAGCCCAAGGA R: GGGCCAAACGTGTCTTCCA	NM 213833
Growth hormone receptor (GHR)	F: GGACTGTGGATCAAAAGTGTTTCTC R: GTTGAGGCCAATGGGTGGAT	NM 214254
Follistatin (FST)	F: GGAAGTCCAGTACCAAGGCAAAT R: GCTGCCTGGACAGAAAACATC	NM 001003662
18S ribosomal RNA	F: AGGGCATCACAGACCTGTTATTG R: CCCCAACTTCTTAGAGGGACAAG	NR 002170

Statistical Analysis

All experimental data were preliminary processed by Excel 2007. The statistical analysis was performed using one-way ANOVA where $P<0.05$ was considered statistically significant.

3. Results

Effect of supplementation of mineral nano particles on gene expression in pig adipose tissue

In this study, the pig adipose tissue from the control group and the experimental group was applied to estimate transcript expression of several genes which related to the pig growing including connective tissue growth factor (CTGF), Follistatin (FST), and Growth hormone receptor (GHR). The qRT-PCR analysis showed that the transcript expression of CTGF was down-regulated in group C-20, IV-20, C-40, and IV-40 (Figure 1). However, the group IV-20 and IV-40 showed a higher CTGF transcript expression than group C-20 and C-40, respectively ($P \leq 0.05$).

The Figure 2 showed that the GHR transcript expression of pig from the group C-20 and C-40 was lower than the group NT ($P \leq 0.05$). The pig of the group IV-20 exposed an increase of GHR transcript expression, comparing to C-20 group ($P \leq 0.01$). Moreover, the pig from the group IV-40 showed the higher GHR transcript expression than group C-40 ($P \leq 0.05$).

As seen in the Figure 3, pigs from the group C-20, IV-20, C-40, and IV-40 exposed the reduced FST transcript expression, comparing to the group NT ($P \leq 0.001$). In the opposite to CTGF and GHR expression, the pig from the group IV-20 and IV-40 showed the lower FST transcript expression than the group C-20 ($P \leq 0.01$) and C-40 ($P \leq 0.001$).

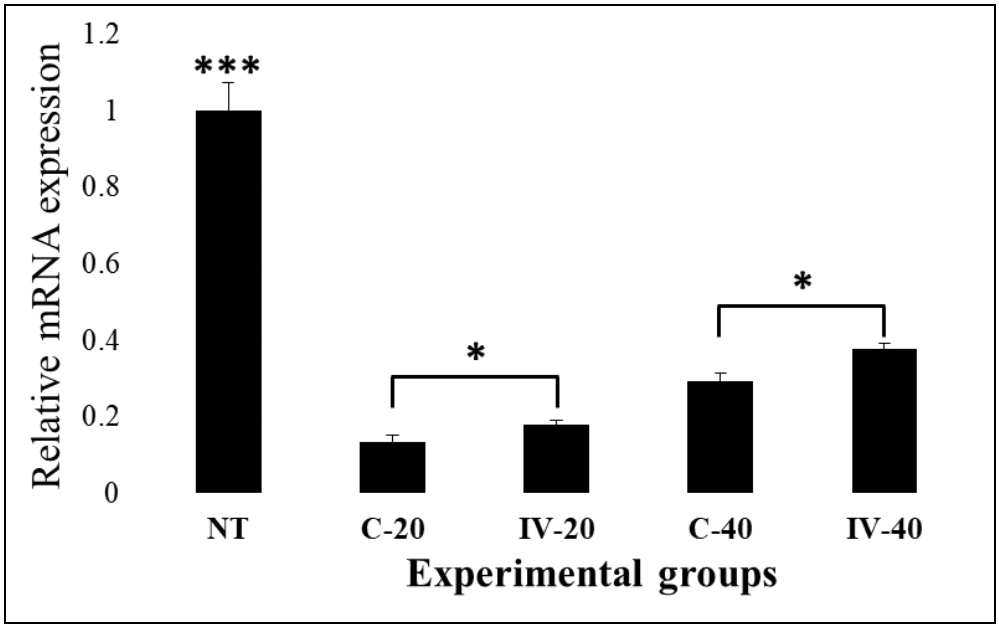

Fig 1: Quantitative Reverse transcriptase polymerase chain reaction analysis of CTGF. NT (non-treatment): piglets from day 0. C-20, IV-20: piglets at 20th day without and with treatment of nano mineral supplementation. C-40, IV-40: piglets at 40th day without and with treatment of nano mineral supplementation. ***$P<0.001$, NT vs. other groups; *$P<0.05$, C-20 vs. IV-20, and C-40 vs. IV-40.

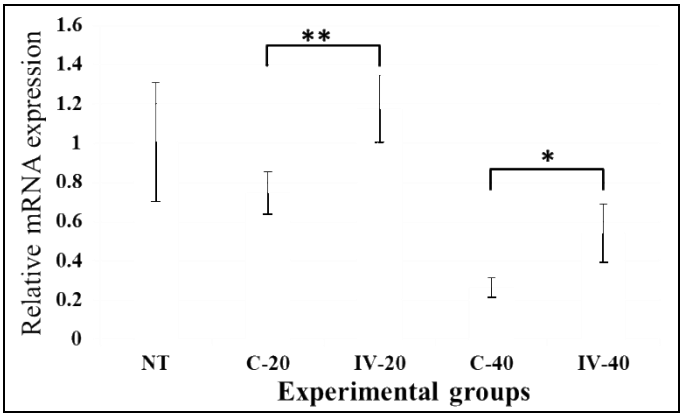

Fig 2: Quantitative Reverse transcriptase polymerase chain reaction analysis of GHR. NT (non-treatment): piglets from day 0. C-20, IV-20: piglets at 20th day without and with treatment of nano mineral supplementation. C-40, IV-40: piglets at 40th day without and with treatment of nano mineral supplementation. ***P*<0.01, C-20 vs. IV-20; **P*<0.05, C-40 vs. IV-40.

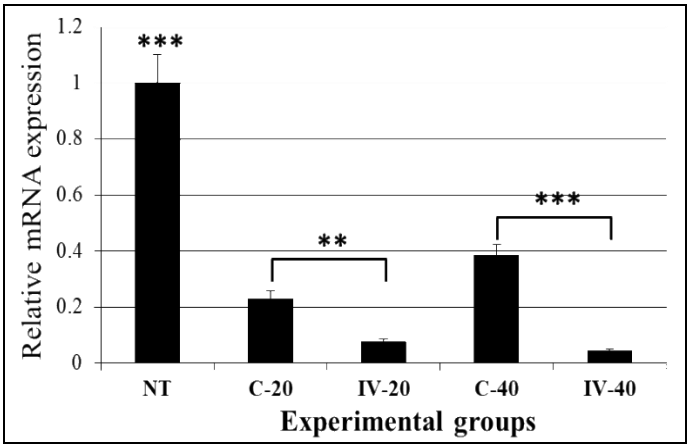

Fig 3: Quantitative Reverse transcriptase polymerase chain reaction analysis of FST. NT (non-treatment): piglets from day 0. C-20, IV-20: piglets at 20th day without and with treatment of nano mineral supplementation. C-40, IV-40: piglets at 40th day without and with treatment of nano mineral supplementation. ****P*<0.001, NT vs. other groups, and C-40 vs. IV-40, ***P*<0.01, C-20 vs. IV-20.

4. Discussion

The present study accessed the growth-related transcript expression between the control group and treatment group. The results revealed that nano mineral supplementation could induce the changes of transcript expression of CTGF, GHR, and FST.

CTGF is a cysteine-rich, secreted protein and potent angiogenesis factor since it functions in many other stages of angiogenesis [9]. The previous study showed that CTGF expression was down-regulated through the pig growth [7]. The qRT-PCR analysis demonstrated that the pigs from the group which treated with nano mineral supplementation exposed a higher CTGF transcript expression than the control group at 20th day and 40th day, suggesting that nano mineral supplementation could increase the CTGF transcript expression, leading to enhance angiogenesis in pigs.

The growth hormone shows its diverse actions in regulating growth, development and metabolism, mediated by growth hormone receptor (GHR) which is regulated developmentally and nutritionally in a tissue-specific manner [10, 11]. The GHR expression is also reduced during pig development [7]. In the present study, the pigs from group IV which treated with nano mineral supplementation also exposed a higher GHR transcript expression than the control group at 20th day and 40th day. This result revealed that nano mineral supplementation for pigs could enhance the GHR transcript expression.

The Follistatin, an extracellular protein, plays an important role skeletal muscle, especially in interscapular brown and subcutaneous white adipose tissue [12, 13]. Moreover, FST gene expression has been found in many extra gonadal tissues in the rat, revealing a more global role of FST [14]. Another study demonstrated that FST expression is induced by pre-adipocyte differentiation [15]. In this study, the FST transcript expression in pigs from the group which treated with nano mineral supplementation was lower than control group at 20th day and 40th day. This result suggested that nano mineral supplementation could induce the reducing of adipose tissue formation in pigs.

5. Conclusion

In this study, we have demonstrated that the supplementation of mineral nano particles changed the transcript expression of some gene relating to piglet adipose tissue such as Connective tissue growth factor (CTGF), Growth hormone receptor (GHR), and Follistatin (FST). In the further research, the other genes Leptin receptor, Vascular endothelial growth factor, Connective tissue growth factor, Insulin-like growth factor, Angiopoietin will be estimated to complete the evaluation of the transcript profile in adipose tissue of piglets with treatments of mineral nano particles.

6. Acknowledgments

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7. References

1. Raje K, Ojha S, Mishra A, Munde VK, Rawat C, Chaudhary SK. Impact of supplementation of mineral nano particles on growth performance and health status of animals. A review. Journal of Entomology and Zoology Studies. 2018; 6(3):1690-1694.
2. Li X, Yin J, Li D, Chen X, Zang J, Zhou X. Dietary supplementation with zinc oxide increases IGF-I and IGF-I receptor gene expression in the small intestine of weanling piglets. The Journal of Nutrition. 2006; 136:1786-1791.
3. Yang ZP, Sun LP. Effects of nanometre ZnO on growth performance of early weaned piglets. Journal of Shanxi Agricultural Sciences. 2006; 3:024.
4. Bunglavan SJ. Effect of supplementation of selenium nano particles on growth and health status of guinea pigs. Thesis, PhD. Deemed University, Indian Veterinary Research Institute, Izatnagar, India. 2013, 140.
5. Gonzales-Eguia A, Fu CM, Lu FY, Lien TF. Effects of nanocopper on copper availability and nutrients digestibility, growth performance and serum traits of piglets. Livestock Science. 2009; 126(1):122-129.
6. Wang MQ, Xu ZR, Zha LY, Lindemann MD. Effects of chromium nanocomposite supplementation on blood metabolites, endocrine parameters and immune traits in finishing pigs. Animal Feed Science and Technology. 2007; 139:69-80.
7. Hausman GJ, Barb CR, Dean RG. Patterns of gene expression in pig adipose tissue: Insulin-like growth factor system proteins, neuropeptide Y (NPY), NPY receptors, neurotrophic factors and other secreted factors.

Domestic Animal Endocrinology. 2008; 35:24-34.
8. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using Real-Time Quantitative PCR and the 2-ΔΔCt Method. Methods. 2001; 25:402-408.
9. Shimo T, Nakanishi T, Nishida T, Asano M, Kanyama M, Kuboki T *et al*. Connective tissue growth factor induces the proliferation, migration, and tube formation of vascular endothelial cells *in vitro*, and angiogenesis *in vivo*. The Journal of Biochemistry. 1999; 126(1):137-145.
10. Katsumata M, Cattaneo D, White P, Burton KA, Dauncey MJ. Growth Hormone Receptor Gene Expression in Porcine Skeletal and Cardiac Muscles Is Selectively Regulated by Postnatal Undernutrition. The Journal of Nutrition. 2000; 130(10):2482-2488.
11. Kelly PA, Goujon L, Sotiropoulos A, Dinerstein H, Esposito N, Edery M *et al*. The GH receptor and signal transduction. Hormone Research. 1994; 42:133-139.
12. Lee SJ, Lee YS, Zimmers TA, Soleimani A, Matzuk MM, Tsuchida K *et al*. Regulation of muscle mass by follistatin and activins. Molecular Endocrinology. 2010; 24:1998-2008.
13. Schneyer AL, Sidis Y, Gulati A, Sun JL, Keutmann H, Krasney PA. Differential antagonism of activins, myostatin and growth and differentiation factor 11 by wild-type and mutant follistatin. Endocrinology. 2008; 149:4589-4595.
14. Michel U, Albiston A, Findlay JK. Rat follistatin: gonadal and extragonadal expression and evidence for alternative splicing. Biochemical and Biophysical Research Communications. 1990; 173:401-407.
15. Hirai S, Matsumoto H, Moriya NH, Kawachi H, Yano H. Follistatin rescues the inhibitory effect of activins A on the differentiation of bovine preadipocyte. Domestic Animal Endocrinology. 2007; 33:269-280.