Effects of maternal supplementation with rare earth elements during late gestation and lactation on performances, health and fecal microbiota of the sows and their offspring

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Abstract: The study was conducted to investigate the effects of maternal supplementation with rare earth elements (REE) on sows and their offspring. During late gestation, 120 multiparous sows were divided randomly into the control group (Basal diet) and REE-G group (Basal diet supplemented with 200 mg REE/kg). After delivery, REE-G group was further divided into two groups: REE-L (Change to basal diet during lactation) and REE-L+ group (REE diet all the time). Our results showed that maternal REE supplementation improved the antioxidant and immunity of sows and piglets. Additionally, REE supply during late gestation significantly decreased the coefficient of within-litter variation (CV) in birth weight and increased the weaning weights and the average daily gain (ADG) of piglets. During lactation, the insulin-like growth factor-1 (IGF-1) levels in piglets of REE-L+ group were higher, while no difference between REE-L- and control group. More beneficial bacteria (Christensenellaceae and Ruminococcaceae) were found in the REE-L+ group while some opportunistic pathogens (Proteobacteria and Campylobacter) were relatively suppressed. Fecal microbiota showed correlation with antioxidase, inflammatory factors and ADG. Collectively, our findings indicated that REE added in both gestation and lactation was more conducive to establish a healthier status for sows and their offspring.

Keywords: REE; performances; fecal microbiota; oxidative stress; sow; offspring
INTRODUCTION

Maternal nutrition and health condition during the transition from late gestation to lactation are not only essential for the sows themselves, but also for the neonatal suckling piglets [1]. Previous study indicates that during the perinatal period, mothers are easier to experience aggravated oxidative stress and inflammatory responses [2]. Indeed, gestational sows exhibit notable changes in gut microbiome and stress responses would increase [3] with any increase in the systemic exposure to microorganisms of maternal origin. The gut microbiota plays an important role in nutrient metabolism and immune system in the host, moreover intestinal microflora changes may directly influence the maternal pregnancy-associated metabolic alterations [4].

Rare earth elements (REE) include the lanthanides lanthanum (La), Cerium (Ce), and other 15 elements, which have been extensively applied in agriculture, medicine, and other fields [5], REE has also been used as useful feed additives in a variety of animals for decades [6-9]. It was suggested that dietary supply of low dosages of La and Ce could increase the activity of digestive enzyme in small intestine of laying hens and broilers [10, 11] and improve the feed conversion ratio, as well as functions as antioxidants and enhance cellular defense [12]. In addition, the antibacterial activities of Ce” to E. coli cells [13] and the suppressive effect of La on inflammatory response [14] were either found. La and Ce were also reported to improve the immunity of gibel carp [15]. Besides, it was also found that supplementing LaCl3 had an impact on rumen microbial flora (increased the relative abundance of F. succinogenes and decreased R. flavefaciens). In addition to the above species, REE supplementation also has certain effects on the growth and microflora of pigs [16-18]. A previous study reported in vivo preferential antimicrobial action of REE against Gram-negative bacteria despite little effective influence of REE on the fecal microbiota of 9-week-old piglets was monitored using PCR-DGGE analysis [18]. Importantly, the underdevelopment of intestinal microbiota has the potential to give rise to disorders. Thus, knowledge of the interactions of the microbiota with diets is essential for advancement in modulating and improving livestock health status.

However, to date, the effects of dietary maternal REE supply during perinatal period on sows and their offspring, and the experimental evidence of the relationship between the gut microbiota, antioxidant capacity, immune status and growth are still absent. To test the hypothesis that REE is beneficial to the performance and health of sows and even affect their offspring (longitudinal progression in time), we evaluate the effects of maternal REE supplementation during late gestation and lactation on reproductive performances of the sows, plasma biochemical changes and fecal microbiota of the sows and their piglets, as well as the growth performances of piglets.

MATERIALS AND METHODS

Animals and experimental treatments

One hundred and twenty multiparous sows (Landrace × Yorkshire, 3-5 parturition) were selected and housed individually in their own pen. From the day 90 of gestation (G90-114), sows were randomly assigned to two groups: the control group (corn and soybean meal-based diet, control diet) (n=60) and the REE-G group (the control diet supplemented with 200 mg REE mixture/kg, REE diet) (n=60). During the lactation (L1-21), REE-G group (n=60) was further divided into REE-L-group (REE diet during late gestation + control diet during lactation) (n=30) and REE-L+ group (REE diet throughout the late gestation and the lactation) (n=30). The control group were continued to be fed a control diet during the lactation. Complete diets were formulated to provide all nutrients at or above requirement and were shown in Table 1. As REE sources, a REE mixture containing 5.72% of La, 3.26% of Ce and other carrier components as chelating agent were used. All REE mixture samples were obtained from Shenzhen SQA Industrial Co., Ltd. in Guangdong, China. This experiment was approved by the Animal Care and Use Committee of Wuhan Polytechnic University (WHPU20140608-1, Wuhan, China).

Table 1. Composition of diet for sows during the late gestation and lactation.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Late gestation</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, %</td>
<td>-</td>
<td>46.54</td>
</tr>
<tr>
<td>Red sorghum, %</td>
<td>25.00</td>
<td>25.00</td>
</tr>
<tr>
<td>Unprocessed barley, %</td>
<td>46.72</td>
<td>-</td>
</tr>
<tr>
<td>Rice bran meal, %</td>
<td>10.00</td>
<td>-</td>
</tr>
<tr>
<td>Soybean meal, %</td>
<td>9.28</td>
<td>22.31</td>
</tr>
<tr>
<td>Palm meal, %</td>
<td>5.00</td>
<td>-</td>
</tr>
<tr>
<td>Soymeal oil, %</td>
<td>-</td>
<td>1.58</td>
</tr>
<tr>
<td>Moutain flour, %</td>
<td>1.48</td>
<td>1.60</td>
</tr>
<tr>
<td>Monocalcium phosphate, %</td>
<td>0.83</td>
<td>0.09</td>
</tr>
<tr>
<td>Sodium chloride, %</td>
<td>0.47</td>
<td>0.51</td>
</tr>
<tr>
<td>Sodium bicarbonate, %</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>0.20</td>
<td>0.34</td>
</tr>
<tr>
<td>DL-Methionine, %</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>L-Threonine, %</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>L-Tryptophan, %</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>Choline chloride, %</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Premix a, %</td>
<td>0.40</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Nutrient composition

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Late gestation</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, %</td>
<td>89.02</td>
<td>87.51</td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>13.20</td>
<td>16.50</td>
</tr>
<tr>
<td>Ether extract, %</td>
<td>2.18</td>
<td>4.09</td>
</tr>
<tr>
<td>Crude ash, %</td>
<td>6.13</td>
<td>5.49</td>
</tr>
<tr>
<td>Crude fiber, %</td>
<td>4.92</td>
<td>2.53</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.77</td>
<td>0.85</td>
</tr>
<tr>
<td>Total phosphate, %</td>
<td>0.69</td>
<td>0.54</td>
</tr>
<tr>
<td>Available phosphate, %</td>
<td>0.30</td>
<td>0.34</td>
</tr>
<tr>
<td>STTD phosphate, %</td>
<td>0.32</td>
<td>0.33</td>
</tr>
<tr>
<td>Net energy, kJ/kg</td>
<td>2178.20</td>
<td>2511.40</td>
</tr>
</tbody>
</table>

*The premix provided the following per kg of diets: Cu, 250 mg; Fe, 150 mg; Zn, 200 mg; Mn, 40 mg; Se, 0.4 mg; I, 0.3 mg; vitamin E, 20 mg; vitamin A, 11250 IU; vitamin D₃, 2500 IU; vitamin K₂, 2.5 mg; vitamin B₁₂, 0.08 mg; biotin, 0.01 mg; pantothenic acid, 12.5 mg; folic acid, 1.25 mg; niacin, 25 mg.

Litter performance measurement

At parturition, the individual birth weight was recorded to calculate the total birth weight per litter and the average birth weight for the live piglets as well as the coefficient for within-litter birth weight variation. Also, total litter size and born alive were recorded. On day 21 of lactation, weights of weaned piglets were measured to calculate the average daily gain (ADG) during the neonatal stage.

Plasma and feces collection

On day 114 of gestation, eight sows per group were selected at random and the blood samples (10 mL) were collected via anterior vena cava into the heparin sodium anticoagulation tube. On day 21 of lactation, eight sows and eight piglets per group (one piglet per litter corresponding to the sow) were randomly selected and the blood samples of sows (10 mL) and piglets (5 mL) were collected in the same way. Plasma samples were then obtained by centrifuging the blood samples at 3000 × g for 10 min at 4°C and were immediately stored at -80°C until further analysis. At the same time, fresh feces of the lactating sows and the weaning piglets were collected and stored at -20°C until assay.

Analysis of antioxidase and inflammatory cytokines of plasma samples

The contents of total-antioxidant capacity (T-AOC), catalase (CAT), total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were tested using the commercial assay kits according to the manufacturers’ instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The concentrations of interleukin-10 (IL-10), interleukin-
1β (IL-1β), TNF-α were measured with the commercial porcine ELISA kit (Elabscience Biotechnology Co., Ltd) according to the manufacturer’s instructions.

**Fecal microbial analysis**

Microbial DNA was extracted from the feces samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer’s protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V3-V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 338F (5’-ACTCCTACGGGAGGCAGCAG-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’) by thermocycler PCR system (GeneAmp 9700, ABI, USA) [19]. The PCR reactions were conducted using the following program: 3 min of denaturation at 95°C, 27 cycles of 30 s at 95°C, 30 s for annealing at 55°C, and 45 s for elongation at 72°C, and a final extension at 72°C for 10 min.

Purified amplicons were pooled in equimolar and paired-end sequenced on the Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Raw fastq files were demultiplexed, quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (i) The reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window. (ii) Primers were matched with an allowance of 2 nucleotides mismatching, and reads containing ambiguous bases were removed. (iii) Sequences with overlap longer than 10 bp were assembled according to their overlap sequence. OTUs were clustered using UPARSE (version 7.1) with a cutoff of 97% similarity and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm against the Silva 128/16s bacterial database using threshold for confidence of 70%.

**Statistical analysis**

Data of the reproductive and growth performance, antioxidant and inflammation were analyzed using the general linear model (SPSS 22.0). The difference in alpha diversity was tested by using Kruskal-Wallis test (SAS 9.4) and P-values were adjusted with FDR (below 5%) [20]. P-values below 0.05 were considered statistically significant and all data were presented as mean ± SEM. Beta-diversities based on the Bray-Curtis and non-metric multidimensional scaling (NMDS) were calculated. Linear discriminant analysis (LDA) effect size (LEfSe) analysis was used to identify the differential genera. For piglets, only genera with an average relative abundance greater than 0.03% were considered. Correlations between bacterial communities and plasma parameters were assessed by Spearman’s correlation analysis using the “heatmap” and data were expressed as mean values.

**RESULTS**

**Effect of REE supplementation on reproductive performances of the sows and growth performances of their piglets**

Firstly, we investigated the reproductive performances of sows. As displayed in Table 2, there were no differences in total litter size, the number of piglet born alive, average birth weight and total piglet birth weight between the two groups. But the REE-G group showed a significantly reduction in CV of within-litter birth weight (P < 0.01), indicating an improvement of uniformity with the intervention of REE mixture during late gestation.

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary treatment</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>REE-G</td>
</tr>
</tbody>
</table>

**Table 2. Effects of REE supplementation during late gestation on reproductive performances of the sows at birth.**
We also evaluated the growth performances and hormone of piglets. The results in Table 3 showed that the average weaning weight in REE-L+ and REE-L- group on day 21 of lactation were significant higher ($P < 0.01$) than that of the control group. Since there was no difference in birth weight, the difference in weaning weight could attribute to the higher average daily gain (ADG) in REE-L+ and REE-L- group than that of the control group ($P < 0.01$). These results indicated a trans-generationally beneficial effect for REE supply during late gestation on the growth of their offspring during lactation, no depending on whether the REE was continuously supplied during the lactation or not. There was no difference in growth hormone (GH) secretion among piglets from the three groups (Fig. 1). The plasma insulin-like growth factor-1 (IGF-1) level in the REE-L+ group was significantly higher ($P < 0.01$) than that in the control group, while no difference between the REE-L- and the control group, indicating a specific beneficial role of further REE supplementation during the lactation.

Table 3. Effects of REE supplementation during the late gestation and lactation on growth performances of the piglets.

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary treatment</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>REE-L+ a</td>
</tr>
<tr>
<td>Weight at 21st day, kg</td>
<td>5.73 ± 0.10</td>
<td>6.21** ± 0.14</td>
</tr>
<tr>
<td>Daily weight gain, g/d</td>
<td>218.88 ± 4.50</td>
<td>241.75** ± 5.84</td>
</tr>
</tbody>
</table>

* $p < 0.01$ versus the control group.

a REE-L+, REE mixture supplied during both late gestation and lactation.

b REE-L-, REE mixture supplied only in late gestation.
Figure 1. Effect of maternal REE supplementation on hormone secretion of their piglets. n = 8 per group. Values are mean ± SEM. *P < 0.05, **P < 0.01.

Effect of REE supplementation on antioxidant capacity of the sows and their offspring

The antioxidant capacities in plasma with REE supplementation were presented in Fig. 2. For the farrowing sows, there was an increase in activity of T-SOD (P < 0.05), CAT (P < 0.01), and content of GSH-Px (P < 0.01) in response to the REE supplementation during late gestation. T-AOC showed non-significant changes between the two groups. MDA showed a downtrend (P = 0.082) in the REE-G group compared with the control group. For the lactating sows, the plasma content of T-AOC, GSH-Px, CAT in REE-L+ group (P < 0.01) and REE-L- (P < 0.05) group were significantly higher than that of the control group, but no difference between the REE-L+ and REE-L- groups, indicating a continuously beneficial effect of REE during late gestation on subsequent antioxidant capacity of lactating sows. For weaning piglets, no significant difference was observed in plasma T-AOC, GSH-Px, CAT, MDA content on day 21 of lactation among the three groups. Only plasma content of T-SOD in piglets of the REE-L+ group was higher (P < 0.05) than that of the control group, but there was no difference between the REE-L- group and control group. The results indicated a limited enhancing effect of maternal REE supply on antioxidant capacity of the piglets. Besides, further addition of REE was recommended during lactation.
Effect of maternal REE supplementation on plasma antioxidant defence system of the sows and their piglets. n = 8 per group. Values are mean ± SEM. *P < 0.05, **P < 0.01.

Figure 2. Effect of maternal REE supplementation on plasma antioxidant defence system of the sows and their piglets. n = 8 per group. Values are mean ± SEM. *P < 0.05, **P < 0.01.

Effect of REE supplementation on inflammation-related indicators in the sows and their offspring

As demonstrated in Fig. 3, REE supplementation during late gestation had no impact on plasma levels of IL-10, IL-1β, TNF-α of the farrowing sows. For lactating sows, there was no significant change in level of IL-10 and IL-1β among the three groups. However, the level of TNF-α in sows of the REE-L+ group decreased significantly (P < 0.01), in comparison to the control group. For weaning piglets, plasma IL-10 and IL-1β levels did not show differences among the three groups. However, the plasma level of pro-inflammatory factor TNF-α was greatly reduced in piglets from the REE-L+
and REE-L- group ($P < 0.01$; Fig. 3) compared with the control group, while there was no difference between the REE-L- and REE-L+ group. This indicated the intergenerational advantageous effect of REE supplementation to the sow’s diet during late gestation on immune response of their piglets, and among the cytokine we measured, TNF-α was the only one responsive to REE.

**Figure 3.** Effect of maternal REE supplementation on plasma inflammatory cytokines of the sows and their piglets. $n = 8$ per group. Values are mean ± SEM. *$P < 0.05$, **$P < 0.01$.

**Effect of REE supplementation on fecal microbiota of the sows and their offspring**

Fecal samples of the lactating sows and their piglets were analyzed by 16S rRNA gene sequencing. For lactating sows, a total of 897910 sequences were generated from 24 fecal samples after noise sequences clearance. Based on 97% sequence similarity, 912 operational taxonomic units (OTUs) were identified and then assigned to 17 phyla, 34 classes, 60 orders, 92 families, 216 genera and 361 species. For piglets, a total of 1094555 sequences were generated from 24 fecal samples, 925 OTUs were identified and then assigned to 23 phyla, 42 classes, 71 orders, 120 families, 311 genera and 548 species.

The differences in alpha-diversity of fecal microbiota in lactating sows and their piglets among the three groups were shown in Table 4. For both of the sows and the piglets, REE supplementation didn’t alter the fecal microbial diversity (Simpson, Shannon index) and richness (Sobs, ACE index). For beta-diversity analysis of sows, a NMDS plot based on bray-curtis distance (Fig. S1A) revealed the REE-L+ group sows had a distinct microbiota composition from that of the control sows. Meanwhile, REE-L+ group separated from the REE-L- group sows. However, REE-L- group could not be distinguished from the control group. As well, the microbiota of piglets from the REE-L+ group was separated from those in the control group (Fig. S1B).

At the phylum level, the relative abundance of *Firmicutes* in lactating sows was higher in REE-L- group (81.0%) than the control group (78.2%), and *Bacteroidetes* was higher in REE-L+ group (19.1%) than control group (13.0%) (Fig. S2A). In piglets, *Firmicutes* and *Bacteroidetes* were the dominant phyla in all three groups. And the abundance of *Firmicutes* phylum were higher, while *Proteobacteria* phylum were lower in REE-L- (5.3%) and REE-L+ group (6.7%), compared to the control group (14.8%) (Fig. S2B).
Significant differences in relative abundance of the fecal microbiota from phyla to genera were further identified using the LEfSe analysis. In lactating sows, proportions from the Spirochaetae phylum to Spirochaetaceae family were increased in REE-L+ group compared with the control group. *Treponema_2, Christensenellaceae_R-7_group, Prevotellaceae_UCG-001, Ruminiclostridium_1, Turicibacter, norank_f__Bacteroidales_BS11_gut_group* genus was enriched in REE-L+ (Fig. 5A), while *Clostridia* class from *Firmicutes* phylum and *Lachnospiraceae_XPB1014_group* genus showed an increased abundance in the control group. On the other hand, REE-L- group had little difference with the control (Fig. 5B). Besides, the abundance of *Succinivibrio, Desulfovibrio, Phascolarctobacterium, Prevotella_1* genus and *Rikenellaceae* family in REE-L+ group were higher than that of REE-L- group.

In piglets, Epsiloproteobacteria family and the genus Campylobacter, Micrococcus, *Prevotella_9, Tyzzerella* was observed to be enriched in the control group. *Ruminococcaceae_UCG-005, Ruminococcaceae_UCG-002, Ruminococcaceae_UCG-014, Lachnospiraceae_FCS020_group, Anaerotruncus* genus were significantly higher in REE-L+ group than that in the control group (Fig. 5D). More abundant genera Campylobacter, Helicobacter, Hungatella, Eisenbergiella and family Rikenellaceae and phylum Proteobacteria were enriched in the control group when compared with REE-L- group (Fig. 5E).

**Table 4.** Alpha-diversity of fecal microbiota from the lactating sows and the weaning piglets on day 21 of lactation.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>REE-L-</th>
<th>REE-L+</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sobs</td>
<td>494.63 ± 10.88</td>
<td>498.25 ± 13.36</td>
<td>519.13 ± 9.02</td>
<td>0.22</td>
</tr>
<tr>
<td>Ace</td>
<td>592.28 ± 13.10</td>
<td>611.76 ± 8.56</td>
<td>613.87 ± 7.13</td>
<td>0.34</td>
</tr>
<tr>
<td>Shannon</td>
<td>4.06 ± 0.11</td>
<td>3.89 ± 0.17</td>
<td>4.28 ± 0.08</td>
<td>0.15</td>
</tr>
<tr>
<td>Simpson</td>
<td>0.06 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>0.14</td>
</tr>
<tr>
<td>Piglet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sobs</td>
<td>382.50 ± 32.54</td>
<td>294.88 ± 20.84</td>
<td>359.13 ± 15.12</td>
<td>0.05</td>
</tr>
<tr>
<td>Ace</td>
<td>466.70 ± 37.06</td>
<td>371.10 ± 29.15</td>
<td>437.04 ± 17.96</td>
<td>0.08</td>
</tr>
<tr>
<td>Shannon</td>
<td>3.91 ± 0.10</td>
<td>3.51 ± 0.18</td>
<td>3.88 ± 0.09</td>
<td>0.23</td>
</tr>
<tr>
<td>Simpson</td>
<td>0.05 ± 0.01</td>
<td>0.09 ± 0.02</td>
<td>0.05 ± 0.01</td>
<td>0.11</td>
</tr>
</tbody>
</table>

* REE-L+, REE mixture supplied during both late gestation and lactation.

b REE-L-, REE mixture supplied only in late gestation.
Correlation of gut microbiota with plasma parameters of sows and their piglets

A Spearman's correlation matrix was generated to explore the potential correlation of differential genera with oxidative stress, immune response and growth performance in lactating sows and their offspring. As shown in Fig. 6A, for the lactating sows, the correlation analysis illustrated that the genus Lachnospiraceae_XPB1014_group was negatively correlated with the T-AOC (R = -0.497, P < 0.05). The genus Turicibacter was positively associated with CAT (R = 0.632, P < 0.01) and IL-10 (R = 0.423, P < 0.05). The genus Treponema_2 was positively correlated with T-AOC (R = 0.41, P < 0.05).

For piglets (Fig. 6B), the correlation analysis revealed that the genus Campylobacter was negatively correlated with T-AOC (R = -0.491, P < 0.05), T-SOD (R = -0.415, P < 0.05) and positively correlated with MDA (R = 0.448, P < 0.05), TNF-α (R = 0.497, P < 0.05) and IL-1β (R = 0.533, P < 0.01). The genus Hungatella was negatively associated with T-AOC (R = -0.422, P < 0.01), and the genus Ruminococcaceae_UCG-005 was negatively correlated with IL-10 (R = -0.405, P < 0.05), TNF-α (R = -0.611, P < 0.01) and IL-1β (R = -0.511, P < 0.05). The genus Ruminococcaceae_NK4A214_group showed positive relation to T-AOC (R = 0.533, P < 0.01), CAT (R = 0.538, P < 0.01) and GH (R = 0.466, P < 0.05).

The genus Anaerotruncus and Turicibacter was positively and negatively correlated with GH, respectively (R = 0.493 vs -0.425, P < 0.05). Ruminococcaceae_UCG-002 was negatively related with MDA (R = -0.423, P < 0.05) but positively with T-SOD (R = 0.428, P < 0.05) and ADG (R = 0.555, P < 0.01).
Figure 6. Spearman correlation analysis between differential genera and oxidative stress, inflammatory marker and growth performance of (A) the sows and (B) their piglets. n = 8 per group.

DISCUSSION

It was reported that REE has already been widely applied in livestock [21], however, in the case of pigs, most of the research were carried out on weaned, growing and fattening pigs [7, 18], with no reports about effects of REE on sows and their offspring. In the present study, we investigated the effects of maternal REE supply during late gestation and lactation on performances and health of the sows and their nursing piglets, the study also looked at the impact on fecal microbiota of the sows and piglets. Besides, we observed an improved uniformity of birth weight at delivery and ADG of piglets during the suckling stage, an elevated plasma antioxidase activity and a decreased TNF-α content in sows and piglets. Apart from the improvement in the health and performances, we also observed an alteration in composition of the fecal microbiota, the intestinal probiotic colonization in lactating sows and weaning piglets were increased. To the best of our knowledge, this study is the first to report that REE supplementation during the perinatal stage has beneficial roles on both the sows and their offspring.

A previous study has indicated that dietary Ce supplementation in female rabbit [22] increased total litter weight at birth, and post-partum weight of the offspring. In vitro research on mice also proposed an increased proportion of the developed embryo cells under the intervention of La [23]. The present study demonstrated that REE supplementation on sows during late gestation reduced the CV of within-litter birth weight, indicating an improvement of reproductive performance. In addition, maternal REE supply significantly boosted the growth of their nursing piglets as well. In mammals, postnatal growth is controlled by the activity of the somatotropic axis, where GH instructs the liver and peripheral tissues to produce IGF-1 to promote growth [24, 25]. IGF-1 not only mediates the growth-promoting effects of GH, but also promotes anabolism [26]. An earlier study showed that the level of GH in serum and ADG were increased when incorporating La to barrow’s diet [8]. Our results confirmed the growth-promoting effect of REE on their offspring and improvement of IGF-1 in plasma but no difference in GH was observed. The similar beneficial role on ADG but different results on level of GH between these two studies might be due to the complexity of GH/IGF-I system with growth regulation at multiple levels [27], and the different animal breeds. In our study, improvements observed in the ADG of the piglets could be attributed to the REE stimulated secretion of IGF-1, which was considered as an important growth factor. Furthermore, our results showed that
the level of IGF-1 in the REE-L+ group was significantly higher while no difference between the REE-L- and control group, indicating that REE supply during both gestation and lactation worked better than only in gestation.

In production, oxidative stress can give rise to suboptimal livestock health conditions and economic loss. Especially, oxidative status of the sows was not only closely related to their own health, but also that of their nursing piglets \[28, 29\]. The antioxidative effects of REE have been previously reported and confirmed \[30-34\] on various kinds of animals. In the current study, REE supply elevated the antioxidant enzyme activity of the gestating and lactating sows indicated by higher plasma CAT, GSH-Px etc., yet, only T-SOD content of piglets from REE-L+ group differed considerably from the control, indicating a limited improvement on antioxidant capacity of the piglets through maternal REE supply during late gestation and lactation.

Cytokines play a vital role in the immune and inflammatory responses and therefore their balance is crucial for the health and protection against infection \[35\]. Studies in pigs have suggested that the immune system is active in pregnant sows \[36\] and neonatal piglets \[37\]. To further investigate the effects of REE on immune status, we measured the level of pro-inflammatory cytokines including TNF-α and IL-1β as well as anti-inflammatory cytokine IL-10 in plasma of sows and piglets. We found that there was no difference in all these cytokines between the REE-G and control group, with only the level of TNF-α in sows from the REE-L+ group significantly decreased compared to the control while no difference between the REE-L- and the control group. TNF-α plays a central role in pathogenesis of a broad range of inflammatory diseases \[38\]. An earlier research on mice reported that LaCl3 was a potent inhibitor of pro-inflammatory factors and could greatly decrease the secretion of TNF-α and IL-1β \[14\]. The present study suggested that maternal REE supply during late gestation and lactation decreased the level of plasma TNF-α in both sows and the piglets yet no difference in IL-1β, which confirmed the effects of REE on suppressing excessive immune response in pigs. Based on our results, further addition of REE during lactation helped to maintain a better antioxidant and immune status for both the lactating sows and their offspring.

Role of intestinal microbiota in host physiological health had been recognized \[39, 40\], and pioneers in gut microbiology have emphasized the significance of diet: the microbe interactions may contribute to health status \[41\]. In this study, Clostridia were abundant in sows from the control group, and Clostridial flagella were known to exert a peculiar role in adhesion and pathogenicity \[42\]. In addition, the higher abundance of Lachnospiraceae_XPB1014_group found in control group was shown negatively related with T-AOC. Besides, this bacteria was reported in a previous study to be associated with the gut dysfunction in humans and mice \[43\]. As for the bacteria enriched in sows from the REE-L+ group, Christensenellaceae_R_7_group was regarded as potential beneficial bacteria due to its positive role in intestinal environment and immunomodulation \[44\]. Turicibacter was positively correlated with IL-10 and CAT in our study. It can degrade polysaccharides to short chain fatty acids (SCFAs) such as butyrate. Changes in these “butyrogenic” bacteria \[45\] may in turn influence metabolic, inflammatory bowel disorders. Indeed, butyrate, which can be used as a source of energy by the host, confers many benefits including anti-inflammatory effects \[46\]. Likewise, Phascolarctobacterium enriched in REE-L+ is a Gram-negative genus able to produce SCFAs.

Environmental factors and maternal bacteria quickly colonize offspring gut after parturition \[47\] through birth canal, breast feeding or skin contact \[48\] and shape the onset of an intestinal immune system and its future development. Remarkably, REE supplementation reduced the presence of Proteobacteria in offspring weaned piglets, which can be considered advantageous because the prevalence of Proteobacteria always indicates a labile microbial community and is associated with intestinal inflammation \[49\]. This phylum includes a variety of bacteria known to cause intestinal pathology in humans and animals \[50\]. Similarly, Campylobacter spp., some of which associated with diarrhea in piglets \[51\], were shown to be more abundant in the control group, and positively related to TNF-α but negatively related to T-SOD and T-AOC. Fortunately, opportunistic pathogens such as Proteobacteria, Campylobacter were suppressed in REE supplied group. Ruminococcaceae_UCG-005, Ruminococcaceae_NK4A214_group, and Ruminococcaceae_UCG-002 showing the positive relation with antioxidase and the negative relation with pro-inflammatory factor, were increased in piglets of the
REE-L+ group. Besides, Ruminococcaceae_UCG-002 genus was significantly associated to ADG of the suckling piglets, which is consistent with a recent study reporting that the genus was positively correlated with the body weight of newborn piglets [52]. In a recent report assessing gut bacterial composition in suckling piglets, the most discriminant bacterial family was the high abundance Ruminococcaceae in feces of healthy pigs when compared with the diarrhea pigs [53], which was in line with our study. Our results showed that Ruminococcus (a SCFAs-producing bacterium) was found to be abundant in the REE-L+ group. As mentioned before, SCFAs can provide most of the energy needed by colonic epithelial and gut immune cell, and also play roles in anti-inflammation, antioxidant and mucosal protection [54]. These further support the idea that beneficial alterations in the gut microbiome lead to changes in oxidative stress and inflammatory markers that affect performance and health. In fact, the antibacterial activities of rare earth ions have been previously reported [55], possible explanation could be that lanthanides impeded the physiological activities of undesirable bacterial species by strongly inhibiting the bacterial respiration and destroyed the micro-structure of cell membrane [56]. The results in our study suggest that maternal REE supplementation during late gestation and lactation might regulate oxidative stress and immune status by modulating gut bacteria and inhibiting the proliferation of potential pathogen in sows, accordingly modulate the microbiota establishment in their offspring thus promote hormone secretion and improve health condition of suckling piglets. However, whether sows or piglets, little difference was found between the REE-L- and the control group, indicating the necessity of further addition of REE during the lactation stage.

Conclusions

Taken together, our data evidence that REE supplementation improve the antioxidant and immune status of sows, beneficially changed microbial composition, and then led to the good physiological conditions of sows, which accordingly improved the reproductive performance of sows and growth performance of their offspring. The increased ADG of piglets is presumably due to the fact that the maternal REE addition boosted the secretion of IGF-1 and inhibited the potential pathogens. In addition, it was more effective to add REE mixture during both late gestation and lactation rather than only in late gestation. The findings of our study offer new insights into how a REE diet during late gestation and lactation affects profoundly on porcine health and performance, and also will facilitate the application of REE in animal production.

Acknowledgments: This work was financially supported by the National Key Research and Development Program of China (2018YDF0501002, 2016YFD0500506), the National Natural Science Foundation of China (31630074, 31872373), the Beijing Municipal Natural Science Foundation (S170001), the China Agriculture Research System (CARS-35), the 111 Project (B16044) and Jinxinnong Animal Science Developmental Foundation.

Author Contributions: Ying Ren and Junjun Wang designed research; Yi Xiong and Jiaman Pang analyzed data; Yi Xiong, Liangkang Lv, Zhi Feng and Yujun Wu performed research; Yi Xiong wrote the paper; Na Li and Shimeng Huang developed software necessary to perform and record experiments.

Competing interests: The authors declare no conflicts of interest.

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