Regulation of N-acetyl cysteine on gut redox status and major microbiota in weaned piglets

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ABSTRACT: This study was conducted to explore the regulation of N-acetyl cysteine (NAC) on gut redox status and proliferation of selected microbiota in weaned piglets. A total of 150 newborn piglets from 15 litters were randomly divided by litter to the control group (normally suckling), the weaning group (fed the basal diet), and the NAC group (basal + NAC diet) with 5 litters per group. Activities of total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and inhibition capacity of hydroxyl radical (IHR), and contents of malondialdehyde (MDA), H2O2, and NO in the ileum, colon, and cecum were analyzed to profile oxidative stress states. The real-time absolute quantitative PCR reaction was employed to quantify the amounts of total bacteria, Lactobacillus, Bifidobacterium, and Escherichia coli. The N-acetyl cysteine, as a universal antioxidant, was used to improve the redox status. Results showed that weaning stress resulted in the occurrence of gut oxidative stress and changes of gut microbiota (P < 0.05). Compared with the weaned piglets, the activities of ileal, colonic, and cecal T-AOC; ileal and colonic GSH-Px; cecal SOD; and colonic and cecal IHR were enhanced (P < 0.05), and the concentrations of ileal and cecal H2O2, ileal and colonic NO, and colonic MDA were reduced (P < 0.05) in the NAC-treated piglets. An increase (P < 0.05) in gut Lactobacillus and Bifidobacterium, accompanied with a decrease (P < 0.05) in Escherichia coli counts, was also observed in the NAC group. Bivariate correlation indicated that Lactobacillus and Bifidobacterium were positively correlated (P < 0.05) with the activities of T-AOC, GSH-Px, and SOD and inversely related (P < 0.05) to increased levels of H2O2, NO, OH, and MDA, and Escherichia coli showed a strong positive association (P < 0.05) with increased levels of free radicals and MDA and a negative association (P < 0.05) with the activities of antioxidant enzymes in intestines of weaned piglets. We concluded that NAC constructively regulated on the changes of the gut redox status and microbiota in piglets in response to weaning stress. The observed correlations implied that the NAC effects on the gut microbiota were confirmed, partly through an effect on oxidative stress in piglets, providing evidence that gut microbiota may be potentially improved by the modulation of the redox status by an antioxidant, which has relevance for gut health and function.

Keywords: gut, microbiota, N-acetyl cysteine, oxidative stress, piglets
and the regulation of gut microbiota in weaned piglets have also been paid more attention (Castillo et al., 2006; Konstantinov et al., 2006; Cheng et al., 2010). However, the underlying relationship between oxidative stress and microbiota in weaned piglets is still unclear. To our knowledge, this was the first study conducted to explore the underlying relationship between oxidative stress and proliferation of selected intestinal bacteria in weaned and unweaned piglets. The objective of the present study was to investigate the regulation of NAC on the changes of gut redox status and major microbiota in the intestines of weaned and unweaned piglets and explore the potential mechanism that NAC may modulate gut microbiota directly or indirectly or both by changing the redox status.

MATeRIALS AND METHODS

The animal experiment was approved by the Shanghai Jiao Tong University Institutional Animal Care and Use Committee.

Animals and Experimental Design

A total of 150 newborn piglets (Duroc × Landrace) from 15 litters were randomly divided by litter to control, weaning, and NAC groups with 5 litters per group. Piglets were kept with the sow in conventional farrowing pens and suckled. From 14 to 25 d of age, the control piglets and the weaning piglets had ad libitum access to the basal diet (Table 1), and the NAC-treated piglets were fed the basal diet supplemented with 500 mg/kg of NAC. The dosage of supplemental NAC was chosen based on the studies by Hou et al. (2013) and Zhu et al. (2013). Up to 21 d of age, the weaning and NAC-treated piglets were weaned and moved from the farrowing pens to nursery pens without mixing any litters, and the control piglets remained in the farrowing pens to suckle until the end of the experiment. All of the experimental piglets were given ad libitum access to water, and the room temperature in the farrowing and nursery building was kept at approximately 30°C.

Sample Collection

At 25 d of age, one median-weight piglet was randomly selected from each litter (total of 5 piglets/treatment) and euthanized by intramuscular injection of sodium pentobarbital (50 mg/kg BW). The ileum, colon, and cecum digesta were collected under sterile conditions and immediately stored at −80°C for the evaluation of microbiota quantity using SYBR Green real-time absolute quantitative PCR technique. The ileum, colon, and cecum tissues were obtained, opened longitudinally, and rinsed thoroughly with physiological saline. Then the specimens were immediately frozen in liquid N2 and stored at −80°C for the detection of oxidative and antioxidant status.

Determination of Intestinal Redox Status

After homogenization of ileum, colon, and cecum tissues in saline solution (1:10, w:v) and centrifugation at 2,057 × g for 20 min at 4°C, the supernatants were diluted to the optimal concentration for intestinal redox status measurement. Activities of total antioxidant capacity (T-AOC), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px); inhibition capacity of hydroxyl radical (IHR), indirectly reflecting the hydroxyl radical (OH•) level; contents of malondialdehyde (MDA) as an indicator of lipid peroxidation; H2O2; and NO were analyzed with commercially available kits, according to the manufacturer’s instructions (Nanjing Jiancheng Bioen-
eering Institute, Nanjing, China). Activity of T-AOC was detected with reduction from ferric iron to ferrous iron and measured the changes in absorbance at 520 nm. Activity of SOD was detected by xanthine oxidase method with the changes in absorbance recorded at 550 nm. The GSH-Px activity was measured with 5, 5'-dithiobis(p-nitrobenzoic acid) and the changes in absorbance at 412 nm were recorded. Activities of IHR were detected by the fenton reaction method with the changes in absorbance read at 550 nm. The MDA concentration was analyzed with 2-thiobarbituric acid, and the changes in absorbance were recorded at 405 nm. A UV-visible spectrophotometer (Tongfang Inc., Shanghai, China) was used to measure the all absorbance levels.

Quantification of Intestinal Selected Microbiota

Genomic DNA of ileum, colon, and cecum digesta was extracted following a dung genome extraction kit (Tiangen, Beijing, China). The 16S rRNA gene-targeted PCR specific primers and the optimal annealing temperature of the PCR reaction were employed in this study (Table 2). After PCR amplification with a Taq DNA polymerase kit (Takara Bio Inc., Otsu, Japan) and electrophoresis on a 1.5% agarose gel, PCR products were purified according to the manufacturer’s protocol (Dongsheng, Guangzhou, China). The purified PCR products were linked to the pMD18-T vector system (Takara Bio Inc.) and then transferred to Escherichia coli DH5α (Tiangen) to clone. After checking the size of the cloned inserts with PCR amplification, the extracted plasmids of the positive clones were sequenced commercially, obtaining the positive plasmids (Invitrogen, Carlsbad, CA).

The real-time absolute quantitative PCR reaction was applied to quantify the abundance of total bacteria, Lactobacillus, Bifidobacterium, and Escherichia coli.

### Table 2. 16S rRNA gene-targeted specific primers

<table>
<thead>
<tr>
<th>Item</th>
<th>Primer, 5'–3'</th>
<th>Annealing temperature, °C</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bacteria</td>
<td>F: ACTCCTACGGGAGCCGACG</td>
<td>60</td>
<td>Morgan et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>R: ATTACCGGGCTGCTGGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>F: AGCAGTAGGGGACATGTTCCA</td>
<td>60.8</td>
<td>Kanno et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>R: CACCCGTACCATGGAGGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>F: GATTCCTGCTAGGATAGGACGC</td>
<td>62.3</td>
<td>Echarri et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>R: CTGATAGGACGCGACCCCAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>F: CATGGCCGCGTGTATGAGAAA</td>
<td>60</td>
<td>Jensen et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>R: CGGGTAACGTCAATGACAA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1F = forward; and R = reverse.

Standard curves were generated with 10-fold serial dilutions of the respective positive plasmids (10^8 to 10^4 copies/μL). The concentration of the positive plasmids was plotted against the cycle threshold (Ct) value. The Ct value in each sample was determined in triplicate, resulting in 15 values per treatment, and then the mean values were calculated. The reaction was performed in a total volume of 20 μL (Mastercycler ep Realplex; Eppendorf, Hamburg, Germany). A control without the template was included in all batches. Polymerase chain reaction conditions were as follows: 95°C for 30 s, 35 cycles of 95°C for 5 s, and annealing and extension with annealing temperature for 20 s, followed by a product melting curve to determine the specificity of amplification.

### Statistical Analysis

All results were presented as mean ± SEM. Analysis of variance was performed and differences among means were examined with Tukey honestly significance difference test. Bivariate correlation was conducted and Pearson’s correlation coefficients were calculated by combining the data from the control and weaning groups to address associations between intestinal redox status and major microbiota of piglets. Differences were considered statistically significant at P < 0.05. All statistical analyses were done using a software (SPSS 19.0; SPSS Inc., Chicago, IL).

### RESULTS

#### Intestinal Antioxidant Enzymes

As presented in Table 3, compared with the control group, the activities of T-AOC and GSH-Px in the ileum were decreased (P < 0.05) after weaning. They were enhanced (P < 0.05) by feeding the NAC-supplemented diet compared to the weaning group. In the colon, weaning decreased (P < 0.05) the activities of
T-AOC and GSH-Px and increased ($P < 0.05$) the MDA concentration compared to the control piglets (Table 4). Conversely, the activities of T-AOC and GSH-Px were increased ($P < 0.05$), and the MDA concentration was decreased ($P < 0.05$) in the NAC-treated piglets compared to the weaned piglets. In the cecum, compared to the control piglets, weaning resulted in reductions ($P < 0.05$) in activities of T-AOC and SOD and elevation ($P < 0.05$) in the MDA concentration. Diets supplemented with NAC enhanced ($P < 0.05$) the activities of T-AOC and SOD (Table 5).

**Intestinal Free Radicals**

In the ileum, colon, and cecum, the concentration of $\text{H}_2\text{O}_2$ were increased ($P < 0.05$) and the activity of IHR was decreased ($P < 0.05$) in the weaning group compared to the control piglets (Tables 3, 4, and 5). After weaning, the concentration of NO in the ileum and colon were increased ($P < 0.05$) compared to the control piglets (Tables 3 and 4). In the ileum, compared to the weaning group, the concentrations of $\text{H}_2\text{O}_2$ and NO were decreased ($P < 0.05$) by diets containing NAC (Table 3). In the colon, the NO concentration was decreased and the IHR activity was increased in the NAC group compared to the weaning group (Table 4). In the cecum, the $\text{H}_2\text{O}_2$ concentration was decreased ($P < 0.05$) and the IHR activity was increased ($P < 0.05$) in the NAC group compared to the weaned piglets (Table 5).

**Selected Intestinal Microbiota**

The functions describing the relationship between Ct and X (Log $10$ 16S rRNA gene copies/g contents) for the different assays were: $\text{Ct} = -3.09X + 32.40$ ($R^2 = 0.998$) for total bacteria, $\text{Ct} = -2.90X + 30.34$ ($R^2 = 0.998$) for *Lactobacillus*, $\text{Ct} = -2.99X + 30.59$ ($R^2 = 0.996$) for *Bifidobacterium*, and $\text{Ct} = -3.04X + 34.02$ ($R^2 = 0.999$) for *Escherichia coli*.

As shown in Fig. 1, compared with the control piglets, early weaning reduced ($P < 0.05$) the proliferation of ileal, colonic, and cecal total bacteria; ileal and colonic *Lactobacillus*; and ileal *Bifidobacterium* and promoted ($P < 0.05$) the proliferation of *Escherichia coli* in the ileum, colon, and cecum. Conversely, in the NAC-treated piglets, an increase ($P < 0.05$) in the counts of ileal and colonic total bacteria and *Lactobacillus*, and ileal, colonic, and cecal *Bifidobacterium*, accompanied by a decrease ($P < 0.05$) in ileal and cecal *Escherichia coli* counts, was observed compared to the weaned piglets.

**Correlation Between Intestinal Redox Status and Selected Microbiota**

As presented in Table 6, in the ileum, *Lactobacillus* was positively correlated ($P < 0.05$) with the activities of T-AOC, SOD, GSH-Px, and IHR and negatively related to the concentrations of $\text{H}_2\text{O}_2$ ($P < 0.05$) and NO ($P = 0.061$). *Bifidobacterium* was positively correlated with the activities of T-AOC ($P < 0.01$), SOD ($P < 0.05$), and GSH-Px ($P < 0.05$) and negatively related ($P < 0.01$) to the concentrations of $\text{H}_2\text{O}_2$ and NO. Conversely, *Escherichia coli* was positively correlated ($P < 0.05$) with the concentrations of $\text{H}_2\text{O}_2$ and NO and negatively correlated ($P < 0.01$) with the activities of T-AOC, SOD, GSH-Px, and IHR.

As shown in Table 7, in the colon, *Lactobacillus* had a positive correlation ($P < 0.01$) with the activities of T-AOC, GSH-Px, and IHR and a negative correlation with the contents of MDA ($P < 0.01$), $\text{H}_2\text{O}_2$ ($P < 0.01$), and NO ($P < 0.05$). *Bifidobacterium* showed a positive correlation with the activities of T-AOC ($P < 0.05$), SOD ($P < 0.01$), and GSH-Px ($P < 0.05$) and a negative correlation with the contents of MDA ($P = 0.075$), $\text{H}_2\text{O}_2$ ($P < 0.05$), and NO ($P = 0.053$). Conversely, *Escherichia*
coli showed a positive correlation ($P < 0.05$) with the contents of MDA, H$_2$O$_2$, and NO and a negative correlation with the activities of GSH-Px ($P < 0.01$) and IHR ($P < 0.05$).

As shown in Table 8, in the cecum, there was a positive correlation ($P < 0.05$) between total bacteria and the activities of T-AOC, SOD, and IHR and a negative correlation ($P < 0.05$) with the content of MDA. *Lactobacillus* was positively correlated to the activities of T-AOC ($P < 0.05$), GSH-Px ($P = 0.099$), and IHR ($P < 0.01$), whereas inversely correlated to the contents of MDA ($P = 0.063$), H$_2$O$_2$ ($P < 0.05$), and NO ($P < 0.05$). *Bifidobacterium* presented a positive correlation with the activities of T-AOC ($P < 0.01$), SOD ($P < 0.05$), and IHR ($P < 0.01$) and an inverse correlation ($P < 0.05$) with the contents of MDA and H$_2$O$_2$. However, *Escherichia coli* showed a positive correlation ($P < 0.01$) with H$_2$O$_2$ and a negative correlation ($P < 0.05$) with IHR.

**DISCUSSION**

We found that both the generation of oxidative stress and the changes in gut microbiota were induced by early weaning stress in piglets. Reactive oxygen species (ROS), such as superoxide anion (O$_2^-$), H$_2$O$_2$, and OH from mitochondria and other cellular sources may be toxic byproducts for both aerobic and anaerobic organisms. Moreover, NO at pathologically high concentrations can damage DNA, either directly by its free radical activity or by combining with O$_2^-$ to form peroxynitrite anion, causing damage to cells than NO alone (Hauser et al., 2004).

Our previous study has shown that weaning stress may induce oxidative stress in vivo, resulting in villus atrophy and reductions in the activities of digestive enzymes of weaned piglets (Zhu et al., 2012). Moreover, the gut microbial community underwent dramatic changes after weaning, accompanied by an obvious decline in counts of total bacteria, *Lactobacillus*, and *Bifidobacterium* and an increase in *Escherichia coli* counts in piglets. *Escherichia coli*, as a conditional pathogen in the gastrointestinal tract, are characterized by colo-

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**Table 6. Correlation between redox status and bacteria in the ileum of piglets fed experimental diets$^{1,2}$**

<table>
<thead>
<tr>
<th>Item</th>
<th>Correlation coefficient</th>
<th>Total bacteria</th>
<th><em>Lactobacillus</em></th>
<th><em>Bifidobacterium</em></th>
<th><em>Escherichia coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>T-AOC</td>
<td>0.108</td>
<td>0.759*</td>
<td>0.779**</td>
<td>–0.850**</td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>0.272</td>
<td>0.712*</td>
<td>0.674*</td>
<td>–0.843**</td>
<td></td>
</tr>
<tr>
<td>GSH-Px</td>
<td>0.233</td>
<td>0.638*</td>
<td>0.690*</td>
<td>–0.803**</td>
<td></td>
</tr>
<tr>
<td>IHR</td>
<td>0.239</td>
<td>0.724*</td>
<td>0.492</td>
<td>–0.790**</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>0.122</td>
<td>–0.144</td>
<td>–0.780**</td>
<td>0.466</td>
<td></td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>–0.425</td>
<td>–0.760*</td>
<td>–0.808**</td>
<td>0.672*</td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td>–0.190</td>
<td>–0.609</td>
<td>–0.390</td>
<td>0.662*</td>
<td></td>
</tr>
</tbody>
</table>

$^1$Correlation between major microbiota and redox items: *$P < 0.05$, and **$P < 0.01$.

$^2$T-AOC = total antioxidant capacity; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; IHR = inhibition capacity of hydroxyl radical; and MDA = malondialdehyde.
Gut antioxidant status of weaned piglets and Lactic acid bacteria balance, accompanied by Bifido bacteria.

In our case, decreased levels of free radicals and increased Escherichia coli presented a positive correlation with free radicals and a negative correlation with activities of antioxidant enzymes to the levels nearly close to the control group. The effective regulation of NAC may be directly linked to its chemical structure or to the secondary indirect effect on the defense system of gut bacteria in partially hepatectomized rats (Oz et al., 2007). In the present study, correlation analysis distinctly indicated that oxidative stress was directly related to changes of gut microbiota in the weaned piglets. Excessive free radicals possess the potential to cause cell viability loss, opening tight junctions between enterocytes, and finally reducing colonization and proliferation of indigenous gut bacteria (Hall et al., 2001; Valko et al., 2007; Muccioli et al., 2010). Frequent reports have indicated that oxidative stress because of weaning stress-ors may lead to the loss of intestinal mucosal integrity and villus atrophy, which allows the translocation of indigenous gut bacteria through the lymphatic system (Deitch et al., 1995; Zhu et al., 2012) and more potential detrimental bacteria to adhere to the intestinal epithelium, competing with the potential beneficial bacteria such as Lactobacillus and Bifidobacterium. Furthermore, some ROS may damage bacteria directly. For instance, \( \text{H}_2\text{O}_2 \) may penetrate biological membranes via anion channels to directly destroy bacteria (Imlay, 2008). However, Escherichia coli can be adapted to the conditions of oxidative stress through various ways. Nevertheless, most Lactobacillus is lack of the general defense system (SOD), which may be responsible for the high sensitivity of most species of Lactobacillus to oxidative stress (Roy et al., 1993). Therefore, because of different tolerance of gut bacteria to oxidative stress mentioned before, the survival rate of Escherichia coli was different from that of Lactobacillus and Bifidobacterium under oxidative stress conditions. In our case, results indicated that Escherichia coli were increased, and Lactobacillus and Bifidobacterium were decreased in the weaned piglets. Collectively, given the frequent evidences, we suggest that gut microbiota dysbiosis may be driven by the increased levels of oxidative stress in piglets in re-

### Table 7. Correlation between redox status and bacteria in the colon of piglets fed experimental diets\(^1,2\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Total bacteria</th>
<th>Lactobacillus</th>
<th>Bifidobacterium</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-AOC</td>
<td>0.538</td>
<td>0.878**</td>
<td>0.652*</td>
<td>-0.561</td>
</tr>
<tr>
<td>SOD</td>
<td>0.527</td>
<td>0.433</td>
<td>0.796**</td>
<td>-0.511</td>
</tr>
<tr>
<td>GSH-Px</td>
<td>0.386</td>
<td>0.850**</td>
<td>0.659*</td>
<td>-0.865**</td>
</tr>
<tr>
<td>HIR</td>
<td>0.441</td>
<td>0.868**</td>
<td>0.569</td>
<td>-0.641*</td>
</tr>
<tr>
<td>MDA</td>
<td>-0.435</td>
<td>-0.788**</td>
<td>-0.586</td>
<td>0.683*</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 )</td>
<td>-0.581</td>
<td>-0.873**</td>
<td>-0.657*</td>
<td>0.740*</td>
</tr>
<tr>
<td>NO</td>
<td>-0.367</td>
<td>-0.633*</td>
<td>-0.626</td>
<td>0.655*</td>
</tr>
</tbody>
</table>

1 Correlation between major microbiota and redox items: \( *P < 0.05, \) and \( **P < 0.01. \)

2 T-AOC = total antioxidant capacity; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; HIR = inhibition capacity of hydroxyl radical; and MDA = malondialdehyde.

### Table 8. Correlation between redox status and bacteria in the cecum of piglets fed experimental diets\(^1,2\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Total bacteria</th>
<th>Lactobacillus</th>
<th>Bifidobacterium</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-AOC</td>
<td>0.635*</td>
<td>0.692*</td>
<td>0.793**</td>
<td>-0.545</td>
</tr>
<tr>
<td>SOD</td>
<td>0.716*</td>
<td>0.480</td>
<td>0.687*</td>
<td>-0.382</td>
</tr>
<tr>
<td>GSH-Px</td>
<td>0.146</td>
<td>0.551</td>
<td>0.318</td>
<td>-0.440</td>
</tr>
<tr>
<td>HIR</td>
<td>0.658*</td>
<td>0.836**</td>
<td>0.794**</td>
<td>-0.860**</td>
</tr>
<tr>
<td>MDA</td>
<td>-0.732*</td>
<td>-0.607</td>
<td>-0.728*</td>
<td>0.390</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 )</td>
<td>-0.475</td>
<td>-0.746*</td>
<td>-0.664*</td>
<td>0.701*</td>
</tr>
<tr>
<td>NO</td>
<td>-0.276</td>
<td>-0.644*</td>
<td>-0.533</td>
<td>0.467</td>
</tr>
</tbody>
</table>

1 Correlation between major microbiota and redox items: \( *P < 0.05, \) and \( **P < 0.01. \)

2 T-AOC = total antioxidant capacity; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; HIR = inhibition capacity of hydroxyl radical; and MDA = malondialdehyde.
sponse to weaning stress. However, Oz et al. (2007) found, under some pathological conditions, *Escherichia coli* and *Klebsiella may*, in turn, act on local and distant tissues via releasing ROS. Future research may be greatly needed to confirm whether the changes of gut redox status influence the balance of the gut microbiota in weaned piglets.

We observed that alteration of gut microbiota was strongly related to the increased levels of oxidative stress in the weaned piglets. Qiao et al. (2013) have also found that altering the status of oxidative stress with lipoic acid can modulate the gut microbiota based on the in vitro assays. Besides, the survival rate of *Escherichia coli* was lower than that of *Lactobacillus* and *Bifidobacterium* under enhanced antioxidant environment (Qiao et al., 2013). We suggest that the rebalance of the monitored gut bacteria was partly attributed to the diminished oxidative stress in the NAC-treated piglets. Under NAC-enhanced antioxidant environment, oxidative damage to *Lactobacillus* and *Bifidobacterium* was lowered. Repaired intestinal barrier function may possibly contribute to the colonization and proliferation of *Lactobacillus* and *Bifidobacterium*. The proliferation of potential beneficial bacteria may inhibit the *Escherichia coli* multiplication. Consequently, the changes of gut microbiota were restored in the NAC-treated piglets. Similar diet-induced lower oxidative stress levels, which can drive the changes of the composition of the gut microbiota, have also been observed. For example, FOS and sorbitol with the capacities of diminishing free radicals, respectively, contributed to maintaining the abundance of *Lactobacillus reuteri* and *Lactobacillus* sp. AD102, possibly accounting for healthy colonic mucosa (Busserolles et al., 2002; Sarmiento-Rubiano et al., 2007; Mesa et al., 2008). Because of the high amount in oligosaccharides, breast milk favors the development of a simple flora, dominated by *Bifidobacterium*, promoting the generation of various health benefits in the colon mucosa (Bosscher et al., 2009).

In conclusion, NAC may possess a constructive regulation on the changes of the gut redox status and microbiota in response to weaning stress, enhancing gut health and function. The NAC effects on the gut microbiota were partly attributable to an effect on oxidative stress in the weaned piglets. Further studies should be conducted to more completely determine the mechanism regulating gut microbiota after weaning.

**LITERATURE CITED**


Gut antioxidant status of weaned piglets


