Impact of weaning and an antioxidant blend on intestinal barrier function and antioxidant status in pigs

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ABSTRACT: The objective of this experiment was to investigate the influence of weaning stress and an antioxidant blend on gut health and free radical metabolism in postweaning pigs. A total of 96 pigs from 12 litters were randomly divided by litter to 3 groups with 4 litters each. The control group and the weaning group were fed the basal diet, and the antioxidant group was fed the basal diet supplemented with an antioxidant blend. The control group was suckling normally during the experimental period and the other 2 treatments were weaned at 21 d of age. Morphology in different parts of the intestines was used as a measure of intestinal barrier function. Activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and the concentrations of malondialdehyde (MDA), NO, H2O2, and O2 were measured in serum. Activities of the digestive enzymes, including sucrase, maltase, amylase, lipase, and pepsin, were measured at 24 d of age for all treatments. Gene expressions of free radicals, digestive enzymes, or antioxidant enzymes were selected for quantitative reverse transcription-PCR analyses. Results showed that weaning resulted in reductions (P < 0.05) in the villus height and width, and activity of digestive enzymes. Activity of SOD decreased (P < 0.05) and the concentrations of MDA, NO, and H2O2 increased (P < 0.05) after weaning. The expression results indicated that the genes related to the antioxidant enzymes and digestive enzymes were down regulated (P < 0.05) after weaning. Tumor protein 53, which regulates reactive oxygen-species generation, tended to increase (P < 0.10) in the weaning group. The concentration of PPARγ coactivator-1α (PGC-1α), which plays an important protective role against oxidative stress by regulating the expression of mitochondrial antioxidants, was reduced (P < 0.05) in weaning pigs and increased (P < 0.01) in antioxidant pigs compared with the control pigs. Results indicated that intestinal dysfunction occurred after weaning and there was an inhibition of the antioxidant system. The antioxidant blend has the potential to prevent free radical-induced damage and suppress oxidative stress by modulating the expressions of tumor protein 53 and PGC-1α genes.

Key words: antioxidant, gut health, oxidative stress, reactive oxygen species

INTRODUCTION

Weaning pigs is a stressful event associated with gastrointestinal disorders, causing diarrhea, and increased disease susceptibility (Boudry et al., 2004). However, the mechanisms that link weaning stress and intestinal disease are unknown. Overproduction of reactive-oxygen species and reactive-nitrogen species may result in oxidative stress (Valko et al., 2007) and changes in the antioxidant defense systems, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px; Han et al., 2009, 2011). Supplementation of natural antioxidants, such as vitamin E, vitamin C, tea polyphenols, and probiotics, has been practiced to relieve stress in livestock husbandry (Bonnette et al., 1990; Eicher et al., 2006; West et al., 2008; Deng et al., 2010). However, results of clinical and laboratory studies indicate that, rather than working independently, antioxidants interact in a complex network to recycle and regenerate each

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other, and protect against stress-induced diseases (Sies et al., 2005). Although stress is thought to play a role in postweaning intestinal disease, weaning-induced free-radical metabolism in weaning pigs has not been reported previously. The present study was conducted to investigate the effects of weaning stress and an antioxidant blend on free-radical metabolism and gut-barrier function in weaning-induced intestinal dysfunction.

MATERIALS AND METHODS

The experiment was approved by the Shanghai Jiaotong University Institutional Animal Care and Use Committee.

Animals and Experiment Design

A total of ninety-six 14-d old pigs (Duroc × Landrace) from 12 litters were randomly divided by litter to 3 treatment groups with 4 litters per group. The 3 treatment groups were designated as control, weaning, and antioxidant groups. The size of the litters, in which pigs were allotted to each group, were reared as indicated in Table 1. Pigs were kept with their dams in conventional farrowing pens and suckled until 21 d of age. From 14 to 35 d of age, the control pigs and weaning pigs had ad libitum access to the basal diet (Table 2), and the antioxidant pigs were fed the basal diet supplemented with 6.75 g/kg of an antioxidant blend, including 200 mg vitamin C, 100 mg vitamin E, 450 mg tea polyphenols, 1 g lipoic acid, and 5 g microbial antioxidants fermented by *Bacillus*, *Lactobacillus*, photosynthetic bacteria, and beer yeast. The microbial antioxidants were inactivated after fermentation.

When pigs were 21 d of age, weaning pigs and antioxidant pigs were weaned and moved from the farrowing pens to nursery pens without mixing any litters. The control pigs remained suckling in the farrowing pens until the end of the experiment. Room temperature in the farrowing and nursery building was maintained at about 30°C.

Sample Collection

At 21, 28, and 35 d of age, 3 pigs from each litter, resulting in a total of 12 pigs per treatment, were selected, and an amount of approximately 2 mL blood samples were collected by venipuncture method from precaval ein and placed in a tube for 2 h until centrifugation. The selected pigs had a BW that was close to the average for the litter. Serum was separated by centrifugation at 3,500 × g for 15 min at 4°C and stored at −20°C until analyses for free-radical and antioxidative physiological measures. At 24 d of age, 1 pig was randomly removed from each litter, resulting in a total of 4 pigs per treatment. These pigs were harvested and gut samples were collected. Gut segments (3 cm) were obtained at approximately 10, 50, and 90% of the small intestinal length (i.e., duodenum, jejunum, and ileum sections). Subsequently, the samples were kept in 4% neutral buffered formalin. Jejunum and stomach samples (approximately 2 g) were collected and stored at −80°C for subsequent gene expression and digestive enzyme activities analyses.

Determination of Free Radicals in Serum

A free-radical analyzer [TBR 4100, World Precision Instruments (WPI) Sarasota, FL], with software (LabTrax 2; MedTrax Software LLC, Glen Cove, NY), was used to record the NO, H₂O₂, and O₂ electrical potential traces in serum. Electrodes of ISO-NOP, ISO-HPO-2, and ISO-OXY-2 (TBR 4100, WPI) were used to monitor NO, H₂O₂, and O₂, respectively. Nitric oxide concentrations were determined using the NO electrode and calibration curves that were constructed with S-nitroso-N-acetyl-DL-penicillamine (0.2 to 500 nmol/L) in combination with the catalyst CuCl₂ to generate a known amount of NO in the solution (Zhang et al., 2000). The calibration curve was constructed by plotting the signal output vs. the concentration of the S-nitroso-N-acetyl-DL-penicillamine added at that time. For H₂O₂ measurements, the H₂O₂ electrode was calibrated with known concentrations of H₂O₂, using H₂O₂-equilibrated solutions from WPI. For O₂ measurements, the O₂ electrode was calibrated by 3-point calibration. Buffer solution with 0% O₂ was prepared by adding a slight excess of sodium dithionite to the buffer. Next, the sensor was exposed serially to 21% O₂ and 100% O₂ solutions. The calibration curve was constructed by plotting the signal output vs. percentage of O₂. Calibrations and all measurements were performed at room temperature. The reaction solution was constantly stirred with a magnetic bar controlled by a stirrer under the vial.

Table 1. Number of pigs in different groups

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Weaning</th>
<th>Antioxidant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Number of pigs</td>
<td>9</td>
<td>7</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 2. Dietary ingredient and analyzed energy and nutrient contents of the basic diets (as-fed basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, %</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>41.18</td>
</tr>
<tr>
<td>Soybean meal, fermented</td>
<td>5.00</td>
</tr>
<tr>
<td>Soybean meal, peeled</td>
<td>7.00</td>
</tr>
<tr>
<td>Extruded soybean</td>
<td>11.22</td>
</tr>
<tr>
<td>Fish meal</td>
<td>5.00</td>
</tr>
<tr>
<td>Plasma protein</td>
<td>4.00</td>
</tr>
<tr>
<td>Whey powder</td>
<td>15.00</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.50</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>0.90</td>
</tr>
<tr>
<td>Choline</td>
<td>0.10</td>
</tr>
<tr>
<td>Lactose</td>
<td>8.75</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.35</td>
</tr>
<tr>
<td>Vitamin premix1</td>
<td>0.50</td>
</tr>
<tr>
<td>Mineral premix2</td>
<td>0.50</td>
</tr>
<tr>
<td>Analyzed composition</td>
<td></td>
</tr>
<tr>
<td>DE, MJ/kg</td>
<td>14.48</td>
</tr>
<tr>
<td>CP (N × 6.25), %</td>
<td>20.50</td>
</tr>
<tr>
<td>Ca, %</td>
<td>0.85</td>
</tr>
<tr>
<td>P, %</td>
<td>0.67</td>
</tr>
<tr>
<td>Lys, %</td>
<td>0.55</td>
</tr>
<tr>
<td>Met, %</td>
<td>1.55</td>
</tr>
<tr>
<td>Thr, %</td>
<td>0.27</td>
</tr>
<tr>
<td>Tbr, %</td>
<td>1.01</td>
</tr>
</tbody>
</table>

1Provided per kilogram of mixed diet: vitamin A, 12,000 IU/kg; vitamin D3, 3,200 IU/kg; vitamin K3, 2.5 mg; vitamin E, 80 mg; vitamin B12, 2.5 mg; vitamin B6, 6.5 mg; vitamin B12, 5 mg; vitamin B12, 0.05mg; niacin, 45 mg; and D-pantothenic acid, 20 mg.

2Provided per kilogram of mixed diet: folic acid, 1.5 mg; biotin, 0.15 mg; Fe, 150 mg as ferrous sulfate; Cu, 125 mg as copper sulfate; Zn, 200 mg as zinc oxide; Mn, 30 mg as manganese oxide; I, 0.3 mg as potassium iodide; and Se, 0.3 mg as selenium selenite.

Antioxidant Physiological Analyses

The antioxidant capacities of serum were determined using assay kits according to the manufacturer instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Superoxide dismutase activity was detected by monitoring the inhibition of nitro blue tetrazolium reduction. The GSH-Px activity was measured with 5,5′-dithiobis-p-nitrobenzoic acid and the change in absorbance at 412 nm was recorded. Malondialdehyde (MDA) concentration was analyzed with 2-thiobarbituric acid and the change in absorbance was read at 532 nm. All absorbance levels were measured using a UV-visible spectrophotometer (Tongfang Inc., Shanghai, China).

Digestive Enzyme Determinations

The intestinal and stomach tissues were homogenized in ice-cold deionized water at a ratio of 1:10 (wt/vol) and then centrifuged at 2,200 × g for 15 min at 4 °C, and the supernatant fractions were diluted to an appropriate concentration. Enzyme and supernatant-fraction protein determinations were accomplished as described by Kelly et al. (1991a). Amylase (EC 3.2.1.1) activity was determined using the method of Somogyi (1960). The activities of maltase (EC 3.2.1.20) and sucrase (EC 3.2.1.48) were determined according to Dahlqvist and Fiereck (1966). Pepsin (EC 3.4.23.1) activity was determined with the Ryle (1970) assay. One unit of enzyme activity was defined as the hydrolysis of 1 μM of substrate in 1 min.

Intestinal Histomorphometry

The samples of duodenum, jejunum, and ileum were kept in 4% neutral buffered formalin, processed using routine histological methods, and mounted in paraffin blocks. Six-micrometer-thick sections were cut and stained with Masson’s trichrome. All specimens were examined under a light microscope (Nikon Eclipse E-400 equipped with a digital camera head [DS-5M] and camera control unit [DS-L1], Nikon, Tokyo, Japan). Villus height, villus width, and crypt depth were measured using an image-analysis system.

Quantitative Reverse Transcription-PCR

Total RNA of jejunum tissues was extracted following the Trizol Reagent instructions (Invitrogen, Carlsbad, CA) and reverse transcribed using random primers according to the manufacturer’s protocol (TaKaRa, Otsu, Japan). The resulting cDNA was diluted and used as a PCR template to evaluate gene expression. The reaction was performed in a volume of 10 µL and carried out (ABI Prism 7700 Sequence Detection System; Applied Biosystems, Foster City, CA). A control without template was included in all batches. The PCR conditions were as follows: 95°C for 30 s, 35 cycles of 95°C for 5 s, annealing for 15 s, and 72°C for 10 s, followed by a melting curve. The β-actin gene was used to normalize variations in the amount of starting material. Dissociation curve analysis was done after each real-time experiment to ensure that there was only 1 specific product. Primers for genes of interest were designed based on the pig (Sus scrofa) sequence (Table 3) using primer design software (Primer Premier 5.0, PREMIER Biosoft International, Palo Alto, CA). Amplification efficiencies were calculated for each primer pair using 1:4 serial dilution of the cDNA template. The amplification efficiency of each gene was determined to be ³1.9 (Rosenheck et al., 2001). The relative expression was expressed as a ratio of the target gene to the control gene using the formula 2^(-ΔΔCt) according to Livak and Schmittgen (2001), where ΔΔCt = (Ct_Target - Ct_β-actin) treatment - (Ct_Target - Ct_β-actin)control. Relative expression
was normalized and expressed as a ratio to the expression in suckling pigs. Therefore, relative expression of target genes in suckling pigs was 1.0. Relative gene expressions in weaning pigs and antioxidant pigs represented the comparison vs. suckling pigs and reported as a fold change from the value of suckling pigs.

**Statistical Analyses**

All data were presented as mean ± SD. Comparison of variables was performed using ANOVA (SPSS Inc., Chicago, IL). Pig was the experimental unit. The statistical model included treatment (suckling vs. weaning) and diet (basal vs. basal + antioxidant blend). A probability of $P < 0.05$ was considered significant and differences were considered tendencies if $0.05 < P < 0.10$.

**RESULTS**

**Determination of Free Radicals in Serum**

As shown in Table 4, compared with the control group, the concentration of NO was increased ($P < 0.05$) and content of $O_2$ decreased in the weaning group on d 35 ($P < 0.05$), and the amount of $H_2O_2$ in the weaning group was increased on d 28 ($P < 0.05$). Compared with the weaning group, NO was less ($P < 0.05$) in the antioxidant group on d 35. The content of $O_2$ was increased in the antioxidant group on d 35 ($P < 0.05$). Supplementing the diet with an antioxidant blend decreased $H_2O_2$ ($P < 0.05$) in the antioxidant group on d 28 compared with the weaning group. However, an increase in NO ($P < 0.05$) in weaning pigs and antioxidant pigs, and an increase in $H_2O_2$ ($P < 0.05$) in wean-

**Table 3. Primers used for quantitative reverse transcription-PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Primer, 5'-3'</th>
<th>Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>NM_213824</td>
<td>F: CTGCCTTCTGGAAACACCC R: AAGGGCAGAAGGACGACA</td>
<td>199</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>AB106108</td>
<td>F: TGTGGATGAAAGACGATTTG R: GTGGTCCGTTGTTAGTGG</td>
<td>167</td>
</tr>
<tr>
<td>GSH-Px</td>
<td>NM_00115136</td>
<td>F: CAAGTCCTTTCAGACCTCA R: GAAGCCAAAGAACCACG</td>
<td>184</td>
</tr>
<tr>
<td>CAT</td>
<td>NM_214301</td>
<td>F: AAACGTCCCTTCCCGTCT R: CCTTGTTGAAACGGACGC</td>
<td>202</td>
</tr>
<tr>
<td>SOD1</td>
<td>NM_00190422</td>
<td>F: ACCTGGGCAATGTGACTG R: TCCAGCATTTTCCCGTCT</td>
<td>176</td>
</tr>
<tr>
<td>SOD2</td>
<td>NM_214127</td>
<td>F: GGCACAATCTGAGCCTTAACG R: CCTTGTTGAAACGGACGC</td>
<td>159</td>
</tr>
<tr>
<td>LPL</td>
<td>NM_21426</td>
<td>F: CTGCCATGCCAGGAAGT R: GGTGACCCCTGCTGTAAT</td>
<td>157</td>
</tr>
<tr>
<td>PGA</td>
<td>NM_213873</td>
<td>F: TGCCCTTGCTCTAGCTGCT R: ATGCTGTGCTATCAGCG</td>
<td>130</td>
</tr>
<tr>
<td>SI</td>
<td>XM_003132515</td>
<td>F: CCTACTGCTGGAGGCTTG R: CGGGACAGAAGGCTGAT</td>
<td>169</td>
</tr>
<tr>
<td>GAA</td>
<td>XM_003131141</td>
<td>F: AGGTCACTGGCTACTTTCG R: TCACACACGTGCTCTTCT</td>
<td>126</td>
</tr>
<tr>
<td>AMY2</td>
<td>NM_214195.1</td>
<td>F: CATATCAGGAGGGTGAT R: AAGAGAAGGCTCCTATCAG</td>
<td>198</td>
</tr>
<tr>
<td>β-actin</td>
<td>DQ452569</td>
<td>F: FGGACCTGACCGACTACCTCAT R: GGGCGACTGTGAGCTCTCT</td>
<td>181</td>
</tr>
</tbody>
</table>

1 $p53 = $tumor protein 53; $PGC-1α = $PPARγ coactivator-1α; $GSH-Px = $glutathione peroxidase; $CAT = $catalase; $SOD1 = $superoxide dismutase 1; $SOD2 = $superoxide dismutase 2; $LPL = $lipoprotein lipase; $PGA = $pepsinogen A; $SI = $sucrase-isomaltase; $GAA = $lysosomal alpha-glucosidase; $AMY2 = $amylase, alpha 2B.

2 F = forward; R = reverse.

**Table 4. Concentration of free radicals in serum from pigs fed experimental diets**

<table>
<thead>
<tr>
<th>Item</th>
<th>Control (n = 12)</th>
<th>Weaning (n = 12)</th>
<th>Antioxidant (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO, nM</td>
<td>d 21 6.88 ± 1.00a</td>
<td>d 28 9.66 ± 0.21</td>
<td>d 35 9.48 ± 1.56a</td>
</tr>
<tr>
<td></td>
<td>d 28 9.66 ± 0.21b</td>
<td>d 28 9.66 ± 0.21</td>
<td>d 35 27.10 ± 3.68a</td>
</tr>
<tr>
<td></td>
<td>d 35 25.72 ± 1.32</td>
<td>d 35 22.67 ± 0.77</td>
<td>d 35 30.28 ± 3.51</td>
</tr>
<tr>
<td>H2O2, μM</td>
<td>d 21 33.19 ± 0.27b</td>
<td>d 28 31.61 ± 0.26a</td>
<td>d 35 27.10 ± 3.68a</td>
</tr>
<tr>
<td></td>
<td>d 28 31.61 ± 0.26a</td>
<td>d 28 47.25 ± 6.77c</td>
<td>d 35 30.28 ± 3.51</td>
</tr>
<tr>
<td></td>
<td>d 35 25.72 ± 1.32</td>
<td>d 35 22.67 ± 0.77</td>
<td>d 35 30.28 ± 3.51</td>
</tr>
<tr>
<td>O2, %</td>
<td>d 21 23.34 ± 0.70</td>
<td>d 28 23.34 ± 0.70</td>
<td>d 35 23.96 ± 0.40b</td>
</tr>
<tr>
<td></td>
<td>d 28 23.34 ± 0.70</td>
<td>d 28 23.34 ± 0.70</td>
<td>d 35 9.48 ± 1.56a</td>
</tr>
<tr>
<td></td>
<td>d 35 25.72 ± 1.32</td>
<td>d 35 22.67 ± 0.77</td>
<td>d 35 30.28 ± 3.51</td>
</tr>
</tbody>
</table>

a–c Means within a row without a common superscript differ ($P < 0.05$).
ing pigs were also observed on d 21 compared with the control pigs.

**Determination of Serum SOD, GSH-Px, and MDA**

As shown in Table 5, compared with the control group, the activity of serum SOD decreased \((P < 0.05)\) on d 28 and the content of MDA increased \((P < 0.05)\) on d 35 in the weaning group. Supplementing the diet with an antioxidant blend reduced \((P < 0.05)\) MDA to quantities that were not different from those observed in the suckling pigs. However, compared with the control group, the activity of SOD in the antioxidant group was less \((P < 0.05)\) on d 28. There was no difference in the activity of GSH-Px among the 3 groups.

**Digestive Enzyme Assay**

Activities of sucrase, maltase, amylase, lipase, and pepsin were reduced after weaning \((P < 0.05); \text{Table 6}\). Feeding the antioxidant blend increased the activity of maltase \((P < 0.05)\) compared with the weaning group. Weaning in conjunction with feeding the antioxidant blend did not alter the weaning-induced changes in intestinal sucrase, amylase, lipase, and gastric pepsin activity.

**Macroscopic Presentation and Histological Evaluation**

A decrease \((P < 0.05)\) in villus height and width, and an increase \((P < 0.05)\) in crypt depth in duodenum, jejunum, and ileum were observed after weaning (Table 7). Feeding the antioxidant blend increased \((P < 0.05)\) villus height in jejunum and villus width in jejunum and ileum compared with the weaning group. As shown in Figure 1, no histological damage was observed in the control group. In weaning pigs, the villus was scattered and desquamated seriously in duodenum, jejunum, and ileum. A greater villus in duodenum, jejunum, and ileum was observed in antioxidant pigs relative to weaning pigs.

**Quantitative Reverse Transcription-PCR**

After weaning, expression of catalase, sucrase-isomaltase, and \(GSH-Px\) mRNA abundance was 3.3-fold \((P < 0.05)\), 5.5-fold \((P < 0.05)\), and 2.4-fold less \((P < 0.05)\), respectively, in weaning pigs compared with control pigs (Figure 2). The mRNA abundance of sucrase-isomaltase and \(GSH-Px\) were 3.8-fold \((P < 0.05)\) and 3.4-fold greater \((P < 0.01)\) in pigs fed the antioxidant blend compared with pigs in the weaning group. Abundance of PPAR\(\gamma\) coactivator-1a (\(PGC-1\alpha\)) mRNA was 3.23-fold less \((P < 0.05)\) after weaning compared with pigs in the

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**Table 5. Antioxidant indices in serum from pigs fed experimental diets**

<table>
<thead>
<tr>
<th>Item</th>
<th>Control (n = 12)</th>
<th>Weaning (n = 12)</th>
<th>Antioxidant (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD, U/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 21</td>
<td>151.58 ± 5.54</td>
<td>147.63 ± 2.48</td>
<td>150.56 ± 7.53</td>
</tr>
<tr>
<td>d 28</td>
<td>148.23 ± 2.68(^a)</td>
<td>77.35 ± 5.26(^b)</td>
<td>124.91 ± 3.67(^c)</td>
</tr>
<tr>
<td>d 35</td>
<td>125.06 ± 5.82</td>
<td>108.14 ± 16.17</td>
<td>115.23 ± 8.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA, nmol/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 21</td>
<td>58.23 ± 2.05</td>
<td>61.01 ± 8.36</td>
<td>64.86 ± 14.29</td>
</tr>
<tr>
<td>d 28</td>
<td>65.57 ± 3.80</td>
<td>53.89 ± 6.65</td>
<td>65.31 ± 3.12</td>
</tr>
<tr>
<td>d 35</td>
<td>58.05 ± 3.42</td>
<td>55.50 ± 2.47</td>
<td>58.32 ± 5.70</td>
</tr>
</tbody>
</table>

\(^a\)-\(^c\) Means within a row without a common superscript differ \((P < 0.05)\).

\(1\)SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; MDA = malondialdehyde.

**Table 6. Digestive enzyme activities of gastrointestinal tissue in pigs fed experimental diets**

<table>
<thead>
<tr>
<th>Item</th>
<th>Control (n = 4)</th>
<th>Weaning (n = 4)</th>
<th>Antioxidant (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrease</td>
<td>15.08 ± 0.88(^a)</td>
<td>12.56 ± 0.58(^c)</td>
<td>14.68 ± 0.56(^e)</td>
</tr>
<tr>
<td>Maltase</td>
<td>32.61 ± 0.49(^b)</td>
<td>24.46 ± 0.62(^c)</td>
<td>31.93 ± 1.48(^c)</td>
</tr>
<tr>
<td>Amylase</td>
<td>0.16 ± 0.01(^a)</td>
<td>0.14 ± 0.01(^c)</td>
<td>0.15 ± 0.02(^e)</td>
</tr>
<tr>
<td>Lipase</td>
<td>75.91 ± 1.56(^a)</td>
<td>55.18 ± 3.45(^c)</td>
<td>63.01 ± 2.13(^c)</td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pepsin</td>
<td>37.04 ± 2.43(^a)</td>
<td>16.34 ± 3.74(^c)</td>
<td>19.09 ± 4.50(^c)</td>
</tr>
</tbody>
</table>

\(^a\)-\(^c\) Means within a row without a common superscript differ \((P < 0.05)\).

**Table 7. Morphological characteristics in intestinal tissues in pigs fed experimental diets**

<table>
<thead>
<tr>
<th>Item</th>
<th>Control (n = 4)</th>
<th>Weaning (n = 4)</th>
<th>Antioxidant (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villus height, (\mu)m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>420.00 ± 49.67(^a)</td>
<td>270.00 ± 33.65(^c)</td>
<td>320.00 ± 18.26(^c)</td>
</tr>
<tr>
<td>Jejunum</td>
<td>390.00 ± 21.21(^b)</td>
<td>270.00 ± 33.67(^c)</td>
<td>368.00 ± 22.80(^a)</td>
</tr>
<tr>
<td>Ileum</td>
<td>390.00 ± 52.92(^a)</td>
<td>288.00 ± 48.17(^a)</td>
<td>304.00 ± 32.09(^a)</td>
</tr>
<tr>
<td>Villus width, (\mu)m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>108.00 ± 8.37(^a)</td>
<td>68.00 ± 13.04(^c)</td>
<td>82.00 ± 13.04(^c)</td>
</tr>
<tr>
<td>Jejunum</td>
<td>104.00 ± 19.49(^a)</td>
<td>70.00 ± 14.14(^a)</td>
<td>90.00 ± 8.16(^a)</td>
</tr>
<tr>
<td>Ileum</td>
<td>110.00 ± 8.16(^c)</td>
<td>74.00 ± 11.40(^c)</td>
<td>98.00 ± 17.89(^a)</td>
</tr>
<tr>
<td>Crypt depth, (\mu)m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>255.00 ± 43.58(^c)</td>
<td>368.00 ± 35.64(^c)</td>
<td>298.00 ± 54.49(^a)</td>
</tr>
<tr>
<td>Jejunum</td>
<td>255.00 ± 43.59(^a)</td>
<td>350.00 ± 21.21(^a)</td>
<td>318.00 ± 38.34(^a)</td>
</tr>
<tr>
<td>Ileum</td>
<td>280.00 ± 46.37(^c)</td>
<td>348.00 ± 53.57(^c)</td>
<td>300.00 ± 29.44(^c)</td>
</tr>
</tbody>
</table>

\(^a\)-\(^c\) Means within a row without a common superscript differ \((P < 0.05)\).
Figure 1. Histological evaluation of intestinal tissues in the 3 groups: A) changes of histology in duodenum; B) changes of histology in jejunum; and C) changes of histology in ileum. See online version for figure in color.
control group. In the antioxidant group, the abundance of PGC-1α mRNA increased 4.97-fold ($P < 0.01$) and the pepsinogen A mRNA abundance increased 1.9-fold ($P < 0.05$) compared with the suckling pigs. Abundance of superoxide dismutase 1 in the antioxidant group and tumor protein 53 (p53) mRNA in the weaning group tended to be increased ($P < 0.10$) after weaning.

**DISCUSSION**

Weaning stress in pigs is a critical factor in relation to the compromised intestinal barrier function, increased disease susceptibility, and endocrine disorder causing serious economic losses after weaning. In agreement with earlier reports (Kenworthy, 1976; Kelly et al., 1991a,b), results of the present experiment indicated that weaning can cause villus shortening and crypt hyperplasia, as well as decreases in intestinal digestive enzyme activities. To the best of our knowledge, this is the first study analyzing the quantity of free radicals using electrochemical methods after weaning in pigs.

Reactive-oxygen species from mitochondria and other cellular sources may be toxic byproducts of metabolism with the potential to cause damage to lipids, proteins, and DNA (Valko et al., 2007). Molecular oxygen is essential for the survival of all aerobic organisms. In the process of aerobic energy metabolism, $O_2$ serves as the final electron acceptor for cytochrome-C oxidase, the terminal enzymatic component of the mitochondrial enzymatic complex that catalyzes the 4-electron reduction of $O_2$ to $H_2O$. Partially reduced and highly reactive metabolites of $O_2$ may be formed during the electron transfer reactions to produce superoxide anion ($O_2^-$) and $H_2O_2$. These partially reduced or activated derivatives of oxygen ($O_2^-$ and $H_2O_2$) are highly reactive and toxic, and can lead to the oxidative destruction of cells (Rao and Berk, 1992). The intestinal tissue from hemorrhagic shock in rats presents an up-regulation of inducible NO synthase expression (Hierholzer et al., 2004). Overproduced NO can react with $O_2^-$ to form peroxynitrite anion (ONOO$^-$), which in turn can be protonated to form peroxynitrous acid that is considered a strong oxidizing agent, causing damage to cells. To protect against the potentially damaging effects of free radicals, cells possess several antioxidant enzymes, such as SOD, which reduces $O_2^-$ to $H_2O_2$, catalase, and GSH-Px, which reduces $H_2O_2$ to $H_2O$. The decrease in SOD and $O_2$ and the increase in NO and $H_2O_2$ after weaning in our study indicated that an oxidative stress was present in weaning pigs, which caused increased free-radical generation. The increase in NO and $H_2O_2$ on d 21 after weaning might have been due to the transport and blood collection stress.

Various natural antioxidant extracts have been used to protect pigs from weaning stress in intensive pig production. Fragu et al. (2004) suggested that 100 mg/kg vitamin E needs to be added to the diet for pigs during the initial 2 wk postweaning. Administration of live yeast to weaned pigs improves growth performance, villus height, epithelial cell proliferation, and the numbers of macrophages at various sites of the small intestine (Bontempo et al., 2006). Eicher et al. (2006) also reported that the combination of vitamin C and β-glucan enhanced postweaning growth and reduced tumor necrosis factor-α expression of intestinal and liver tissues, indicating an important immunomodulatory role of a combination treatment. This approach, however, showed only limited effectiveness because the pathogenesis of oxidative tissue injury involves multiple disturbances of cellular physiological processes, including mitochondrial dysfunction (Lu and Gong, 2009). These antioxidants always work in a complex network. They occupy distinct cellular compartments and, among them, there is active recycling (Chan et al., 1999). For example, vitamin C is a hydrophilic antioxidant that acts together with the membrane-localized vitamin E in protecting membrane lipids from peroxidation as it regenerates oxidized vitamin E (Kojo, 2004). It is likely that a combination of a variety of different antioxidants is needed to keep animal cells protected from oxidative stress.
phological and histological evaluation of intestinal tissues indicated that the antioxidant blend enhanced the antioxid-
ant defense system, reduced the effects of free radicals, and maintained intestinal barrier function.

Reactive oxygen-species generation induced by endo-
genous and exogenous stimulation leads to increased oxidative stress in a variety of tissues. There are some endogenous antioxidant systems within cells that neutralize reactive-oxygen species. These antioxidant en-
zyme systems are critical to maintaining proper cellular function. When the endogenous antioxidant network fails to provide a sufficient compensatory response to restore cellular redox balance, the system becomes overwhelmed (i.e., redox imbalance), leading to activation of stress-sensitive intracellular signaling pathways. One major consequence is production of gene products that cause cellular damage and are ultimately responsible for late complications of disease (Mates, 2000). Alteration of antioxidant enzymes in response to exogenous and endogenous factors leading to oxidative stress depends on many factors, including expression of the genes that encode for these enzymes (Rodriguez et al., 2004).

A major cellular antioxidant is reduced glutathione, which is regenerated most efficiently by glutathione re-
ductase and reduced nicotinamide adenine dinucleotide phosphate. Superoxide dismutase provides the efficient dismutation of O$_2^-$, leading to the formation of H$_2$O$_2$, which is removed by GSH-Px and catalase (Rodriguez et al., 2004). In the absence of an appropriate compensatory re-

tory response from the endogenous antioxidant network, GSH-Px, catalase, and SOD concentrations are reduced, and oxidative stress ensues. In this experiment, we ex-

amined the mRNA expression of genes related to the antioxi-
dant enzymes and digestive enzymes. All the mRNA expressions were consistent with the enzyme activities. In our opinion, weaning stress down regulated the expres-
sion of genes for GSH-Px, catalase, and sucrase-isomalt-
ase, whereas the mRNA increase of the abundance of these genes in the antioxidant group can be considered a regulation that was enabled by the mixture of antioxidants that were used.

Tumor protein 53 is a redox-active transcription factor that organizes and directs cellular responses in the face of a variety of stresses that lead to genomic instability (Liu and Chen, 2006). Activation of p53 can induce a range of responses, including cell cycle arrest, DNA repair, apoptosis, and senescence. These responses contribute to tumor suppression either by preventing or repairing genomic damage or through the elimination of potentially onco-
genic cells from the proliferating population (Vousden, 2006). Under normal or low cellular stress, decreased concent-
trations of p53 induce the expression of antioxidant genes. In severe cellular stress, high concentrations of p53 promote the expression of genes that contribute to free-
radical formation and p53-mediated apoptosis (Sablina et al., 2005). Thus, under normal or low stress conditions, p53 seems to have an antioxidant role that protects cells from oxidative DNA damage. Although this effect might depend on the concentration of p53, other cellular fac-
tors likely participate in a cell’s final fate. The relative proapoptotic and antiapoptotic functions of p53 seem to depend at least partly on the cellular p53 concentration, as well as on other factors, such as p53 subcellular localization and phosphorylation status (Tomko et al., 2006). In the present study, the greater content of p53 in weaning pigs indicated that p53 may play the proapoptosis role in the small intestine after weaning.

The PGC-1α gene is a transcriptional coactivator implicated in transducing physiological stimuli into meta-

BONNETTE ET AL. 2007.掠 oxidant enzymes and digestive enzymes. All the mRNA expressions were consistent with the enzyme activities. In our opinion, weaning stress down regulated the expression of genes for GSH-Px, catalase, and sucrase-isomaltase, whereas the mRNA increase of the abundance of these genes in the antioxidant group can be considered a regulation that was enabled by the mixture of antioxidants that were used.

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LITERATURE CITED


