INTRODUCTION

Piglets often suffer from a multitude of psycho-social (maternal and littermate separation, mixing stress) and environmental (transport, abrupt changes in diet, increased pathogen exposure) stressors during the postweaning period (Peace et al., 2011). This situation generally increases susceptibility to intestinal dysfunction, which is characterized by villous atrophy, intestinal inflammation, pathogens infection, and intestinal barrier function injury (Pluske et al., 1997; Boudry et al., 2004; Smith et al., 2010). Specific dietary interventions can offer a viable and practical approach to alleviate intestinal dysfunction at weaning.

Spray-dried animal plasma (SDAP) has been repeatedly shown to have beneficial effects on health.

ABSTRACT: The objective of this study was to evaluate the effects of dietary addition of spray-dried chicken plasma (SDCP) as a replacement for spray-dried porcine plasma (SDPP) on serum biochemistry, intestinal barrier function, immune parameters, and the expression of intestinal development–related genes in weaning pigs. One hundred and forty-four 25-d-old weaning piglets with BW of 6.43 ± 0.39 kg were randomly allotted to 1 of 4 dietary treatments: 1) CON (basal diet; control), 2) SDPP (containing 5% SDPP), 3) SDPP + SDCP (containing 2.5% SDPP and 2.5% SDCP), and 4) SDCP (containing 5% SDCP). After a 28-d trial, 6 pigs from each treatment were randomly selected to collect serum and intestinal samples. On d 14 after the initiation of the trial, pigs in the SDPP, SDPP + SDCP, and SDCP groups had an increase (P < 0.05) in serum concentrations of total protein and IgG and a decrease (P < 0.05) in activities of alanine aminotransferase and diamine oxidase compared with the CON group. In the jejunum, supplementation with SDPP and SDCP reduced (P < 0.05) the concentration of tumor necrosis factor-α (TNF-α) and upregulated (P < 0.05) the mRNA levels of zonula occludens 1 (ZO-1), zonula occludens 2 (ZO-2), occludin (OCLN), Toll-like receptor 2 (TLR2), glucagon-like peptide 2 (GLP2), and IGF-1 compared with the CON group. In the ileum, feeding SDPP, SDPP + SDCP, and SDCP decreased (P < 0.05) the concentrations of TNF-α and secretory IgA (sIgA) and upregulated (P < 0.05) the mRNA levels of claudin 1 (CLDN-1) and TLR2 compared with feeding CON. However, there were no differences among the SDPP, SDPP + SDCP, and SDCP groups. Furthermore, supplementation with SDCP reduced (P < 0.05) the concentration of IL-10 and upregulated (P < 0.05) the mRNA levels of GLP-2, mucin 2 (MUC2), and trefoil factor family 3 (TFF3) in the ileum compared with feeding CON. Collectively, the current results indicate that dietary addition of SDCP has a beneficial influence on the health condition of weaning pigs by alleviating liver damage, promoting intestinal development, improving intestinal barrier function, and reducing overstimulation of immune response. The efficacy of SDCP is comparable to that of SDPP.

Key words: barrier function, immunity, intestine development, spray-dried chicken plasma, spray-dried porcine plasma, weaning pigs

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INTRODUCTION

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status and growth performance in weaning pigs (van Dijk et al., 2001; Moretó and Pérez-Bosque, 2009). These effects appeared to be more efficient under an environment with a greater pathogen load (Coffey and Cromwell, 1995). Meanwhile, several studies demonstrated the beneficial effects of SDAP in various disease challenge models (Bosi et al., 2004; Corl et al., 2007; Pérez-Bosque et al., 2008). In addition, feeding SDAP decreased both proinflammatory and anti-inflammatory cytokine levels simultaneously and prevented Staphylococcus aureus enterotoxin B–induced activation of lymphocyte population in gut-associated lymphoid tissue, including Peyer’s patches, lamina propria, and intraepithelial lymphocytes (Pérez-Bosque et al., 2008; Gao et al., 2011). Moreover, pigs fed SDAP have relieved intestinal barrier dysfunction and diarrhea (Peace et al., 2011). A recent study reported that SDAP also provided protection for pigs challenged with multiple mycotoxins from naturally contaminated corn (Weaver et al., 2014).

It has been reported that spray-dried chicken plasma (SDCP) was able to improve intestinal digestive function and regulate intestinal selected microflora in weaning piglets (Zhang et al., 2015). However, little information exists regarding the effects of SDCP on immune parameters, and the expression levels of intestinal development–related genes in weaning pigs. Therefore, the objective of this study was to evaluate the effects of dietary addition of SDCP on intestinal barrier function, immune parameters, and the expression levels of intestinal development–related genes in weaning pigs.

MATERIALS AND METHODS

The experimental protocol involved in the present study was approved by the Animal Care and Use Committee of Sichuan Agricultural University. The spray-dried porcine plasma (SDPP) and SDCP were provided by a commercial company (Shanghai Genon Biological Products Co., Ltd., Shanghai, China). The plasmas utilized for production of SDPP and SDCP were collected at veterinary-inspected abattoirs from animals designated as fit for human consumption. The nutrient compositions of SDPP and SDCP used in this study have been presented by Zhang et al. (2015).

Experimental Animals and Design

One hundred and forty-four 25-d-old piglets (Duroc × Landrace × Yorkshire, weaned at 21 ± 1 d with BW of 6.43 ± 0.39 kg) were used in a 28-d experiment. At the beginning of the experiment, pigs were randomly allotted to 1 of 4 dietary treatments with 6 replicate pens (3 males and 3 females per pen) according to their initial BW and sex. The 4 dietary treatments included 1) CON (control, a basal diet), 2) SDPP (containing 5% SDPP), 3) SDPP + SDCP (containing 2.5% SDPP and 2.5% SDCP), and 4) SDCP (containing 5% SDCP).

Diets and Feeding Management

The diets were formulated to be isoenergetic and isonitrogenous and to meet or exceed the NCR (2012) nutrient requirements. Diets were fed in meal form throughout the experiment. Details of ingredient composition and calculated nutrient level of diets has been presented by Zhang et al. (2015).

The experiment was performed at the Research Base of the Institute of Animal Nutrition of the Sichuan Agricultural University. All piglets were housed in a temperature-controlled weaning room with completely slatted floors. Each pen (2.5 × 1.8 m) was equipped with a 1-sided feeder and a stainless-steel nipple drinker to allow the piglets ad libitum access to feed and water. After an adaptation period of 4 d, piglets were fed their respective diets 4 times per day at 0800, 1200, 1600, and 2000 h for a 28-d period. The initial room temperature was maintained at 28°C for the first week and was gradually decreased to 25°C by the end of the trial.

Sample Collection

Fourteen and 28 d after the initiation of the experiment, blood samples were collected into glass tubes without anticoagulant by jugular vein puncture from 6 randomly selected pigs in each treatment. After centrifugation (3,000 × g for 15 min at 4°C), serum samples were collected and stored at −20°C. Serum biochemical indicators (i.e., total protein, albumin, globulin, alkaline phosphatase, alanine aminotransferase [ALT], aspartate aminotransferase [AST], and glucose) on d 14 and 28 were measured using an automatic biochemical instrument (Biochemical Analytical Instrument, Beckman CX4, Beckman Coulter Inc., Brea, CA). Porcine-specific ELISA kits were used to quantify circulating IgA, IgG, and IgM (E101–102 for IgA, E101–104 for IgG, and E101–100 for IgM; Bethyl Laboratories, Montgomery, TX) in accordance with the
manufacturer’s instructions. The concentration of serum d-lactate was determined by a commercial kit (Sino-German Beijing Leadman Biotech Ltd., Beijing, China) and a CX4 chemistry analyzer (Beckman Coulter). The activity of the serum diamine oxidase was measured using an assay kit according to the manufacturer’s instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All measurements were done in triplicate at minimum.

**RNA Extraction and Gene Expression Analysis**

Total RNA from the jejunum and ileum were isolated using TRIzol reagent (TaKaRa, Dalian, China) according to the manufacturer’s instructions. The yield and purity of mRNA were measured spectrophotometrically (Beckman Coulter DU800; Beckman Coulter), and an OD$_{260}$:OD$_{280}$ ratio (where OD is the optical density) ranging from 1.8 to 2.0 in all RNase-free water-treated RNA samples was considered a very low degree of contamination (Bustin, 2000). The integrity of RNA was checked by formaldehyde gel electrophoresis, and the 28S:18S ribosomal RNA band ratio was determined as ≥1.8. Reverse transcription reactions were performed using a PrimeScript RT reagent kit (TaKaRa) following the manufacturer’s instructions. Expression levels of zona occludens 1 (ZO-1), zona occludens 2 (ZO-2), claudin 1 (CLDN-1), occludin (OCLN), mucin 2 (MUC2), mucin 20 (MUC20), Toll-like receptor 2 (TLR2), trefoil factor family 3 (TFF3), glucagon-like peptide 2 (GLP2), epidermal growth factor (EGF), and IGF-1 in the jejunum and ileum were analyzed by real-time quantitative PCR with SYBR Green PCR reagents (TaKaRa), and analyses were performed using the Opticon DNA Engine (Bio-Rad, Hercules, CA). The real-time PCR reactions were performed using the following cycle program: a precycling stage at 95°C for 30 s and 40 cycles of denaturation at 95°C for 10 s and annealing at 60°C for 25 s with a final extension at 72°C for 5 min. A melting curve analysis was generated following each real-time quantitative PCR assay to check and verify the specificity and purity of all PCR products, which were further checked for size and specificity by agarose gel electrophoresis. Specific primers used in the experiment were synthesized commercially by Invitrogen (Shanghai, China) and are listed in the Table 1.

The reference gene transcript (β-actin) was used for normalization, and the relative mRNA expression levels

<table>
<thead>
<tr>
<th>Gene$^1$</th>
<th>Primer sequences (5’-3’)$^2$</th>
<th>Size, bp</th>
<th>$A_r$$^3$ °C</th>
<th>Reference</th>
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<tbody>
<tr>
<td>ZO-1</td>
<td>F: CAGAGGACCAAGGAGCGGTCC</td>
<td>105</td>
<td>60</td>
<td>this study</td>
</tr>
<tr>
<td></td>
<td>R: TGCTCAAGACATGGTGTCGC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZO-2</td>
<td>F: ATTCGCGACCATAGCAGACATAG</td>
<td>90</td>
<td>60</td>
<td>this study</td>
</tr>
<tr>
<td></td>
<td>R: GGCTCTTGTGTTCGTTTACG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLDN-1</td>
<td>F: ATTTCAAGGTCGGGACTCTTTAGTGC</td>
<td>214</td>
<td>60</td>
<td>this study</td>
</tr>
<tr>
<td></td>
<td>R: AGGGCTTTGTGGTTGGTAA</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>OCLN</td>
<td>F: TCAGGTCGACCCCTCACGAGTT</td>
<td>118</td>
<td>60</td>
<td>this study</td>
</tr>
<tr>
<td></td>
<td>R: AGGGAGGTGACCTTTCAGAAGG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUC2</td>
<td>F: CTGCTCGGGTGCTTGTTGGA</td>
<td>101</td>
<td>60</td>
<td>Pieper et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>R: GCCGGTGCTGTGGGTAC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUC20</td>
<td>F: GAAGGGGCGATGCTGGCTG</td>
<td>136</td>
<td>60</td>
<td>Pieper et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>R: GCCAGGGCTCCACTGCAATG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR2</td>
<td>F: AGTGGAAGAGGCTCCCAAGG</td>
<td>170</td>
<td>59.5</td>
<td>Chen et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>R: GAAGGACAGGAAGGCACAGAGA</td>
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<td></td>
<td></td>
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<tr>
<td>TFF3</td>
<td>F: AGTGGCGCGTCCTGCGCAAG</td>
<td>80</td>
<td>60</td>
<td>Liu et al. (2014)</td>
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<tr>
<td></td>
<td>R: GCAGCCCGGTGTGGTCAC</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>GLP2</td>
<td>F: ACTCACAAGGGGACGTACACTTCA</td>
<td>149</td>
<td>56</td>
<td>Han et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>R: AGGCCACCCCGACGTCTCCTCT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGF</td>
<td>F: ATCTCAAGGAGGGAGGCGATACC</td>
<td>165</td>
<td>60</td>
<td>Han et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>R: TCACGGGAGGTGAATAGAACC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-1</td>
<td>F: CTGGAGAGGCGAGAGGTGACT</td>
<td>137</td>
<td>58.5</td>
<td>Han et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>R: CCTGAACTCCCTCTACTGGGTTC</td>
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<tr>
<td>β-actin</td>
<td>F: TCCATCGTCCACCGCAATG</td>
<td>124</td>
<td>61</td>
<td>this study</td>
</tr>
<tr>
<td></td>
<td>R: TTCAGGAGGCCTGCCAGAGG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$ZO-1 = zona occludens 1, ZO-2 = zona occludens 2, CLDN-1 = claudin 1, OCLN = occludin, MUC2 = mucin 2, MUC20 = mucin 20, TLR2 = Toll-like receptor 2, TFF3 = trefoil factor 3, GLP2 = glucagon-like peptide 2, EGF = epidermal growth factor.

$^2$F = forward primer; R = reverse primer.

$^3$AT = annealing temperature.
of the target gene in comparison with the reference gene were calculated using the 2-ΔΔCT method (Livak and Schmittgen, 2001). Each standard and sample were run simultaneously in duplicate on the same PCR plate, and the average of each duplicate value expressed as numbers of copies was used for subsequent statistical analysis.

**Enzyme-Linked Immunosorbent Assays of Cytokine and Secretory Immunoglobulin A Concentration**

About 20-cm segments of jejunum and ileum were collected and cut into several 5-cm pieces, which were opened longitudinally and washed with cold saline solution to remove excess blood. Samples of intestinal mucosa were collected, rapidly frozen in liquid nitrogen, and stored at −80°C until analysis. After homogenization of mucosa samples in cold saline solution (1:9, wt/vol) and centrifugation at 5,000 × g for 5 min at 4°C, the supernatants were used for determination of cytokines and secretory immunoglobulin A (sIgA) levels using commercially available ELISA kits (Beijing 4A Biotech Co., Ltd., Beijing, China) according to the manufacturer’s procedures. The Htotal protein content of mucosa homogenates was determined using the Braford brilliant blue method. Concentrations of each cytokine were standardized to the protein in each sample.

**Statistical Analysis**

The pen was considered the experimental unit for analyses. All data were analyzed as a randomized complete block design using the GLM of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit. The results are presented as mean and SEM. Statistical differences among treatment were determined by Tukey’s multiple-range test. For significance determination, the α level was set at 0.05. A probability level of $P \leq 0.05$ was considered significant, whereas $P < 0.10$ was considered a tendency.

**RESULTS**

**Serum Biochemistry and Immune Parameters**

On d 14 after the initiation of experiment, pigs in the SDPP, SDPP + SDCP, and SDCP groups had an increase in serum concentrations of total protein and IgG and a decrease in the activity of ALT ($P < 0.05$). However, no differences were observed for serum concentrations of total protein and IgG and the activity of ALT among the SDPP, SDPP + SDCP, and SDCP groups (Tables 2 and 3). Furthermore, supplementation with SDPP or SDCP had no effect on the concentrations of serum IgA and IgM compared with feeding CON. On d 28, pigs in the SDPP + SDCP and SDCP groups tended ($P < 0.1$) to have an increased concentration of serum total protein compared with pigs in the CON group.

The effects of spray-dried animal plasma on the concentrations of intestinal cytokines and sIgA in weaning pigs are presented in Table 4. In the jejunum, pigs in the SDPP, SDPP + SDCP, and SDCP groups had decreased ($P < 0.05$) concentrations of TNF-α and tended ($P < 0.1$) to have a reduced concentration of IL-10 compared with pigs in the CON group. In the ileum, mucosal
concentrations of TNF-α and slgA were decreased ($P < 0.05$) in the SDPP, SDPP + SDCP, and SDCP groups compared with the CON group. Additionally, pigs fed SDCP had a decreased ($P < 0.05$) concentration of IL-10 and tended ($P < 0.1$) to have lower interferon γ (IFN-γ) levels compared with control pigs.

### Intestinal Barrier Function

On d 14, dietary supplementation of SDPP, SDPP + SDCP, and SDCP decreased ($P < 0.05$) serum concentration of d-lactate content and activity of diamine oxidase compared with feeding CON (Table 5). The serum concentration of d-lactate and activity of diamine oxidase in the SDCP group were not different from those in the SDPP group. On d 28, the concentration of serum d-lactate in the SDPP, SDPP + SDCP, and SDCP groups tended ($P < 0.1$) to decrease compared with that of the CON group.

The mRNA expression levels of intestinal barrier–related genes in the jejunum and ileum of weanling pigs are shown in Fig. 1A and 1B, respectively. In the jejunum, the expression levels of ZO-1, ZO-2, OCLN, and TLR2 were upregulated in the SDPP, SDPP + SDCP, and SDCP groups compared with the CON group ($P < 0.05$). Meanwhile, pigs fed SDPP and SDCP had a higher ($P < 0.05$) expression level of OCLN when compared with the SDPP + SDCP group. In the ileum, the mRNA levels of CLDN-1 and TLR2 in the SDPP, SDPP + SDCP, and SDCP groups were upregulated compared with those of the control group ($P < 0.05$). In addition, the pigs fed SDPP and SDPP + SDCP had a higher ($P < 0.05$) mRNA level of ZO-1 compared with the CON group. Also, pigs in the SDCP group exhibited higher ($P < 0.05$) expression levels of MUC2 and TFF3 compared with the CON group.

### Intestinal Development–Related Genes Expression

The mRNA expression levels of intestinal development–related genes (GLP2, EGF, and IGF-1) in the jejunum and ileum of weanling pigs are presented in Fig. 2A and 2B, respectively. In the jejunum, an increase in GLP2 and IGF-1 mRNA expression levels was observed in pigs fed SDPP, SDPP + SDCP, and SDCP compared with those of the CON group ($P < 0.05$). Meanwhile, pigs in the SDPP group showed a higher ($P < 0.05$) expression level of IGF-1 when compared with pigs in the SDPP + SDCP group. In the ileum, pigs fed SDCP exhibited higher ($P < 0.05$) mRNA expression level of GLP2 compared with the CON group. In addition, higher mRNA expression of EGF was also found in the SDPP + SDCP group than in the CON group ($P < 0.05$). However, there were no differences in the expression levels of GLP2 and EGF among the SDPP, SDPP + SDCP, and SDCP groups.

### DISCUSSION

Spray-dried plasma protein, a by-product of animal harvest, comprises a mixture of functional proteins and other biologically crucial elements (Campbell et al., 2010). Numerous studies have shown that the health benefits in the form of immune regulation and improvement of intestinal barrier function were attributed to the SDAP effects (Moretó and Pérez-Bosque, 2009; Peace et al., 2011). The results of our previous study have shown that SDCP increased growth performance, decreased diarrhea incidence, promoted small intestinal development, improved the digestive function, and reduced the population of *E. coli* (Zhang et al., 2015).

The liver plays an important role in immunity and metabolism by regulating the production of enzymes, hormones, blood proteins, and immune factors (Wu et al., 2013). When hepatic cells are injured or cell membrane permeability increases, ALT and AST mainly existing in hepatic cells are released into the blood circulation and consequently result in an increase in the serum activities of ALT and AST, which reflects the metabolic status and health condition of hepatic cells (Wolf, 1999).

Table 3. Effects of spray-dried animal plasma on serum immunoglobulins in weanling pigs

<table>
<thead>
<tr>
<th>Item, μg/mL</th>
<th>CON</th>
<th>SDPP</th>
<th>SDPP + SDCP</th>
<th>SDCP</th>
<th>SEM</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>d 14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>280</td>
<td>291</td>
<td>290</td>
<td>287</td>
<td>6</td>
<td>0.58</td>
</tr>
<tr>
<td>IgG</td>
<td>4,350$^b$</td>
<td>5,617$^a$</td>
<td>5,603$^a$</td>
<td>5,755$^a$</td>
<td>252</td>
<td>$&lt;0.05$</td>
</tr>
<tr>
<td>IgM</td>
<td>680</td>
<td>691</td>
<td>710</td>
<td>715</td>
<td>13</td>
<td>0.20</td>
</tr>
<tr>
<td>d 28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>309</td>
<td>313</td>
<td>326</td>
<td>317</td>
<td>6</td>
<td>0.20</td>
</tr>
<tr>
<td>IgG</td>
<td>5,890</td>
<td>6,252</td>
<td>6,241</td>
<td>6,117</td>
<td>120</td>
<td>0.11</td>
</tr>
<tr>
<td>IgM</td>
<td>771</td>
<td>781</td>
<td>796</td>
<td>790</td>
<td>18</td>
<td>0.80</td>
</tr>
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</table>

$^a,b$ Means in a row with different superscripts differ ($P < 0.05$); $n = 6$.

$^1$CON = control diet, SDCP = spray-dried chicken plasma, SDPP = spray-dried porcine plasma.
Weaning was associated with upregulation of the intestinal immune response and suppress the production of proinflammatory cytokines to maintain immune homeostasis (Opal and DePalo, 2000). At present, we observe that SDCP reduced mucosal IL-10, which was consistent with observations of other in vitro studies in which a reduced IL-10 was found in alveolar macrophage cells isolated from pigs fed SDPP (Tran et al., 2014). It has been demonstrated that SDAP could reduce the expression of several cytokines in immune organs and the number of lymphocytes in the mucosa (Touchette et al., 2002; Nofrarías et al., 2007). Therefore, the mechanisms responsible for the reduced proinflammatory and anti-inflammatory cytokines may be the result of a protective role of SDAP in preventing the immune system from possible activation in weaning pigs. On the other hand, the reduced activity of the immune system avoided unnecessary energy consumption and led to greater growth performance, which was in line with our previous results (Zhang et al., 2015).

Secretory immunoglobulin A, secreted by plasma cells existing in intestinal lamina propria, forms a major
Table 5. Effects of spray-dried animal plasma on serum concentration of d-lactate and activity of diamine oxidase in weaning pigs

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>SDPP</th>
<th>SDPP + SDCP</th>
<th>SDCP</th>
<th>SEM</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>d-Lactate, μg/mL</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>d 14</td>
<td>9.45a</td>
<td>7.76b</td>
<td>7.43b</td>
<td>7.62b</td>
<td>0.29</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>d 28</td>
<td>6.28</td>
<td>5.58</td>
<td>5.65</td>
<td>5.42</td>
<td>0.16</td>
<td>0.09</td>
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<td>Diamine oxidase, U/L</td>
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<tr>
<td>d 14</td>
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<td>9.28b</td>
<td>9.69b</td>
<td>10.05b</td>
<td>0.41</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>d 28</td>
<td>8.35</td>
<td>8.02</td>
<td>7.95</td>
<td>8.11</td>
<td>0.18</td>
<td>0.31</td>
</tr>
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</table>

a,bMeans in a row with different superscripts differ (P < 0.05); n = 6.

1CON = control diet; SDCP = spray-dried chicken plasma; SDPP = spray-dried porcine plasma.

component of the local immune barrier of the intestine and plays an integral role in intestinal protection (Ushida et al., 2008). Therefore, the content of sIgA in the intestine was used as an indicator to evaluate intestinal mucosal immunity. Previous studies with early-weaned pigs have reported that dietary plasma protein reduced lamina propria cell density (Jiang et al., 2000; Peace et al., 2011), thereby suggesting a decrease in the content of intestinal sIgA. In agreement with previous reports, we found that the concentrations of sIgA in both the jejunum and ileum were lower in pigs supplemented with SDPP, SDPP + SDCP, and SDCP compared with unsupplemented pigs. Therefore, these findings suggest that diets supplemented with SDPP or SDCP may stimulate the immune response in weaning pigs by regulating the production of cytokines and antibodies.

The intestinal barrier is mainly formed by a layer of epithelial cells joined together by tight junctions. The intestinal epithelium is the major digestive and absorptive site of nutrients. Moreover, it also acts as a critical line of defense against pathogenic agents and luminal antigens (Boudry et al., 2004). Impairment of the intestinal barrier, characterized by increased intestinal permeability, will promote the translocation of intestinal bacteria and the entering of toxic or allergenic substances from the gut into the body (Wijtten et al., 2011). Therefore, an intact intestinal barrier is crucial for both nutrient absorption and intestinal health. Weaning stress was reported to cause remarkable disturbances in intestinal barrier function (Blikslager et al., 2007). In this study, we used serum concentration of d-lactate and the activity of diamine oxidase, 2 well-established markers, to evaluate intestinal permeability (Nieto et al., 2000; Song et al., 2010). When intestinal epithelial cells are injured, the adhesion of leukocytes and damage to intestinal endotheliocytes will increase the concentration of d-lactate and the activity of d-lactate (Nieto et al., 2000; Song et al., 2010). In the current experiment, dietary inclusion of SDPP or SDCP reduced serum concentration of d-lactate and the activity of diamine oxidase on d 14 postweaning, which indicates that SDCP had beneficial effects on intestinal integrity. These results are in line with the report by Pierce et al. (2005), who showed SDAP could reduce the ileal permeability to both low- and high–molecular weight markers.

Tight junction proteins, such as ZO-1, ZO-2, CLDN1, and OCLN, form a structure at the boundary of 2 adjacent cells, working as a selectively permeable barrier within the epithelial cell space (Li et al., 2012). However, early weaning induced sustained impairment in the intestinal barrier by decreasing tight-junction proteins expression (Hu et al., 2013). Our current study showed that SDCP increased the mRNA expression of tight-junction proteins in weaning pigs. These results were consistent with those of Pérez-Bosque et al. (2008), who reported that SDAP could prevent the increased permeability by facilitating the expression of ZO-1 and β-catenin in Staphylococcus aureus enterotoxin B–treated rats. In addition, the decreased mucosa permeability was associated with decreased diarrhea incidence (Moretó and Pérez-Bosque, 2009), which was in accordance with our previous result (Zhang et al., 2015). Furthermore, intestinal microflora also affects the expression of intestinal tight-junction proteins. Escherichia coli has been reported to destabilize and dissociate the ZO-1, OCLN, and CLDN 1 tight-junction complex (Muza-Moons et al., 2004). Therefore, the increased expression of tight-junction proteins in the SDPP and SDCP groups is in accordance with the reduced population of E. coli (Zhang et al., 2015). However, the specific molecular mechanisms by which intestinal bacteria mediated tight-junction alterations are still unclear.

Mucin 2 is one of the major secreted mucins expressed by intestinal goblet cells and acts as a protective barrier for the intestine (Dharmani et al., 2009). Mucus secretion was reported to be affected by dietary treatment (Brown et al., 1988), such as high-protein diets (Pieper et al., 2012), nondigestible carbohydrate (Hedemann et al., 2009), and zinc oxide (Liu et al., 2014). Our results show that pigs fed SDCP have an increased expression of MUC2 in the ileum, which is in line with the results of King et al. (2008). Further, Balan et al. (2011) showed that feeding an ovine serum immunoglobulin increased mucin (MUC2, MUC3, and MUC4) gene expression and goblet cell count. Therefore, we speculate that a SDPP- or SDCP-induced increase in expression of MUC2 may depend on the regulation of immunoglobulin on goblet cell count. Additionally, the proinflammatory cytokines TNFα, IL1β, and IL6 were also reported to regulate the expression of MUC2 through the SAPK/JNK (stress-activated protein kinase/c-Jun N-terminal kinase) or the JAK/STAT (Janus kinase/signal transducer and activator of transcription) pathway (Dharmani...
The trefoil factors (TFF) are essential for epithelial restitution, thus maintaining intestinal mucosal integrity (Taupin and Podolsky, 2003). Therefore, upregulation of TFF3 in the present study may benefit mucosal restore and intestinal barrier function. The Toll-like receptor (TLR2) activated by a specific microbe was reported to protect intestinal epithelial tight-junction-associated integrity, whereas TLR2-deficient mice suffered impaired epithelial barrier function via downregulating ZO-1 and claudin 3 during infection by a bacterial pathogen (Gibson et al., 2008). Therefore, the increased expression of TLR2 was associated with the increased tight-junction protein gene expression in pigs fed SDPP and SDCP.

Gastrointestinal development of piglets was regulated by external and intrinsic factors (Pluske et al., 1997). The former mainly included intrauterine growth retardation, weaning stress, and diet, whereas the latter were mediators participating in enterocyte proliferation and differentiation. Insulin-like growth factor 1 is an important regulator of intestinal cell growth, differentiation, and barrier function (Jones and Clemmons, 1995; Burrin et al., 1996; Herman et al., 2004). In our study, pigs fed SDPP, SDPP + SDCP, and SDCP had increased expression of IGF-1 in the jejunum, which may partly contribute to intestinal growth and structural integrity. Meanwhile, numerous studies have reported that GLP-2 increased mucosal thickness and mass (Drucker et al.,

Figure 1. Effects of spray-dried animal plasma on mRNA level of intestinal barrier–related genes in (A) the jejunum and (B) ileum of weaning pigs. Each column represents the mean expression level with 6 independent replications. Letters above the bars (a–c) indicate statistical significance ($P < 0.05$) of genes expression among the 4 treatments. CON = control diet; SDCP = spray-dried chicken plasma; SDPP = spray-dried porcine plasma. ZO-1 = zonula occludens 1, ZO-2 = zonula occludens 2, CLDN-1 = claudin 1, OCLN = occludin, MUC2 = mucin 2, MUC20 = mucin 20, TLR2 = Toll-like receptor 2, TFF3 = trefoil factor family 3.
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Kato et al., 1999) and promoted intestinal digestive and absorptive functions (Petersen et al., 2002; Sangild et al., 2006). These lines of evidence indicated that GLP-2 was a potent gut trophic factor and a measure of intestinal growth and function. Our results showed that pigs fed SDCP had a higher mRNA level of GLP-2 in both the jejunum and ileum. Epidermal growth factor has been reported to play an important role in stimulating intestinal epithelium proliferation, differentiation, and maturation (Dignass and Sturm, 2001; Lee et al., 2006; Kang et al., 2010). The EGF was also found to reduce the enteropathogenic colonization in the intestinal epithelium (Buret et al., 1998). In the present study, pigs fed SDPP + SDCP showed a higher mRNA level of EGF in the ileum, which was in line with our previous report that feeding SDCP and SDPP reduced the population of Escherichia coli (Zhang et al., 2015). In addition, our previous results showed that pigs fed SDCP or SDPP had improved villus height of the duodenum and jejunum (Zhang et al., 2015). Therefore, results from the current data plus those of others indicate that the increased mRNA levels of IGF-1, GLP-2, and EGF induced by SDPP or SDCP may contribute to a better intestinal morphology and an intact intestinal mucosal barrier.

Figure 2. Effects of spray-dried animal plasma on mRNA level of intestinal development-related genes in (A) the jejunum and (B) ileum of weaning pigs. Each column represents the mean expression level with 6 independent replications. Letters above the bars (a–c) indicate statistical significance (P < 0.05) of genes expression among the 4 treatments. CON = control diet; SDCP = spray-dried chicken plasma; SDPP = spray-dried porcine plasma. GLP2 = glucagon-like peptide 2, EGF = epidermal growth factor.
In summary, the present study demonstrated that dietary inclusion of SDCP had a beneficial effect on health status of weaning piglets via protecting liver health, promoting intestinal development, improving the intestinal barrier function, and reducing the overstimulation of immune response. The efficacy of SDCP is comparable to that of SDPP.

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