**Immune modulation effect of porcine placenta extracts in weaned the pig**


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**ABSTRACT:** In a previous study, we established a collection of appropriate porcine placental extracts using PBS at 80°C (PE-PBS80) as a food supplement to increase immune activities in a mice model. In this study, piglets were treated with 0.1%, 0.3%, and 0.5% PE-PBS80 for 3 wk after weaning. Experiments were performed at 2 separate farms using 2 different pig varieties. Composition of white blood cells, lymphocyte activation, and cytokine concentrations were analyzed to assess the immune modulation effect. In Exp. 1, the number of white blood cells increased significantly in the PE-PBS80 treatment and T- and B-cell activation increased as well ($P < 0.01$). Interestingly, piglets in all treatments in Exp. 2 were naturally infected by a rotavirus at the third day of the experiment but recovered after d 10. Increased lymphocyte activation was observed in the PE-PBS80 treatment ($P < 0.01$) regardless of viral infection. Additionally, unlike in Exp. 1, the percentage of granulocytes and concentrations of interferon-γ, IL-1β, and IgG increased in the PE-PBS80 treatment ($P < 0.01$) and were more active in the 0.3% PE-PBS80 treatment compared with the control and the other treatment. In conclusion, 0.3% PE-PBS80 treatment modulated immune activities in antigen-infected piglets. Therefore, the PE-PBS80 pig placental extract, particularly the 0.3% supplement to the normal diet, could be useful as an alternative feed supplement to modulate immune activity during the early piglet period.

**Key words:** cytokine, granulocyte, immune modulation, lymphocyte activation, pig, porcine placenta extract


**INTRODUCTION**

Agricultural antibiotics have been widely used as feed additives in the swine industry since the early 1950s. They continue to be effective for stimulating growth rate, improving feed efficiency, and reducing mortality and morbidity in pigs (Hays, 1977; Cromwell, 2001). Although the use of antibiotics in human medicine has been the primary cause of antibiotic-resistant disease, infection from antibiotic-resistant bacteria from domestic animals that were overtreated with antibiotics also could be a cause of antibiotic-resistant disease (Bonten et al., 2001; Smith et al., 2005). After Denmark and then the European Union banned the use of antibiotics for promoting growth, the prevalence of resistant bacteria has declined in farm animals and in retail meat and poultry (Aarestrup et al., 2001; Emborg et al., 2003). In Korea, alternative food supplements, which enhance the immune system and growth rate of domestic animals, have been studied because the use of antibiotics for commercial feed was abolished in September 2011.

The placenta is a temporary organ that connects to the developing fetus to allow nutrient uptake, antibody provisions, and gas exchange via the blood supply of the mother. In Asia, it has been used for wound treatment and
regeneration (Hong et al., 2002; Datta and Bhattacharyya, 2004). Moreover, placental extracts modulate the immune system in mammals. Placental extracts can induce IL-8 secretion in a human monocytic cell line via activation of transcription factors and kinases and stimulate activation of macrophages in mice (Chakraborty et al., 2006; Kang et al., 2007). When placenta is supplied to sows as a feed after farrowing, it induces increased IgG in the plasma and milk of the sows (Lee et al., 2006).

In this study, the effects of porcine placental extracts on immune modulation were investigated, and the possibility of using the placental extract as an alternative food supplement for piglets is discussed.

**MATERIALS AND METHODS**

All animal care protocols were approved by the Konkuk University Institutional Animal Care and Use Committee.

**Preparation of Pig Placental Extracts**

The pig placental extract was prepared as described previously (Park et al., 2011). Pig placentas obtained from normal delivery were washed thoroughly with PBS and chopped into small pieces (<1 cm), and the sliced tissues were resuspended in PBS and freeze-dried to eliminate any residual liquid (Georgieva et al., 1995). To collect the placenta extraction, freeze-dried samples were resuspended with PBS at a concentration of 5:1 (wt/vol) and homogenized using an X620 universal homogenizer (Ultraturax, Staufen im Breisgau, Germany). The homogenized soluble materials were filtered using Quantitative Ashless 5A filter papers (Advantec, Toyo, Japan) for removal of the larger insoluble materials. The filtered aqueous materials were frozen and dried by lyophilization and stored at −80°C until use. For the aqueous PBS placenta extraction (PE), the freeze-dried placenta samples were ground using an HR2020 blender (Phillips, China) for 30 s, and then the ground materials (powders) underwent further extractions in which the placental powder was resuspended in PBS at a concentration of 5:1 (vol/wt) and incubated for the indicated period of time and temperature; 80°C for 30 min (PE-PBS80). After extraction, each of the extracts was filtered using Quantitative Ashless 5A filter papers to remove the larger insoluble materials, and the filtered soluble materials were freeze-dried by lyophilization and stored at −80°C until use. To prepare the 0%, 0.1%, 0.3%, and 0.5% PE-PBS80 diets, the placenta extracts were mixed with the diets.

**Experiment 1**

Experiment 1 was performed at Bok it nen swine farm in Hamyang, Gyeongnam, Korea. One hundred Berkshire piglets, 34 d old, were used to investigate the effects of the placental extracts. Piglets were raised in a 100 m² house, which was divided into 4 pens with a 1 m fence. Each pen had 25 piglets, and the house had a cross-shaped corridor 60 cm wide located in center of the rooms. Each dietary treatment was applied to a single pen. A drum-shaped feed trough and water supplier were located in the center of each pen. Feeding was not automatic but was done manually by the farmer. Placental extract mixed feed was supplied twice daily at 0700 and 1800 h for 3 wk. Control feeding did not include the PE-PBS80 or any antibiotics for 3 wk. Piglets with 0.1%, 0.3%, and 0.5% PE-PBS80 treatments were fed with an oral dose of 1, 3, and 5 g of PE-PBS80 mixed in 1 kg of commercial piglet feed without antibiotics, respectively (Korea Industrial Co. Ltd., Busan, Korea). The composition of the commercial piglet diet is represented in Table 1. Placenta extract was substituted with beet pulp at the same inclusion ratio. Water supply and the amount of diet were not controlled; however, use of antibiotics was strictly limited during the period of the experiment. The BW of piglets were not measured individually but were measured by the total BW of the 25 piglets in each pen with a weighing scale for pigs (CAS, Yangju, Korea) at d 0 (34 d from birth), d 14 (48 d from birth), and d 21 (55 d from birth) after commencing the experiment.

**Experiment 2**

Experiment 2 was performed under conditions similar to Exp. 1. The experiment was conducted at Baik woon swine farm located in Umsung, Chungbuk, Korea. One hundred 21-d-old, 3-way-crossed hybrid Yorkshire, Landrace, and Duroc piglets were raised in a 200-m² house, which was divided into 4 pens with a 1 m fence. Each pen had 25 piglets, and a 1-m-wide corridor surrounded the 4 pens. Feed trough and water supplier were located in the center of the each pen. Compositions of control and 0.1%, 0.3%, and 0.5% PE-mixed diets and feeding conditions were the same as Exp. 1 (Table 1). The BW of the piglets were not measured individually but were measured by the total BW of the piglets in each pen with a weighing scale for pigs (CAS) at d 0 (25 d from birth), 7 (32 d from birth), 14 (39 d from birth), and 21 (46 d from birth) after commencing the experiment.

**Plasma and Lymphocyte Preparation**

Blood samples were collected from individual piglets in each treatment pen at the same time that BW was measured. Approximately 2 to 3 mL of blood were col-
Whole blood were mixed with 2 mL of Histopaque-1077 which the supernatant was carefully collected and stored 20 min at 20°C. The lymphocytes were carefully isolated from the middle of the gradient. To separate the plasma, 1 mL of whole blood was incubated for 1 h at room temperature, and then the samples were centrifuged at 1,200 × g for 20 min at 20°C. The lymphocytes were carefully isolated from the middle of the gradient. To separate the plasma, 1 mL of whole blood was incubated for 1 h at room temperature, and then the samples were centrifuged at 1,200 × g for 15 min at room temperature, after which the supernatant was carefully collected and stored at −70°C until use.

**Blood Cell Analysis**

Piglets were randomly selected for blood analysis, and blood was collected from 15 piglets from each treatment. Blood analyses were performed in triplicate for each piglet. The concentrations of pig white blood cells (WBC) and the percentages of neutrophils, lymphocytes, eosinophils, basophils, and monocytes were compared among the 4 different treatments. Samples were measured immediately on a HemaVet 850 (CDC Technologies, Oxford, UK) analyzer according to the manufacturer’s procedures.

**Measurement of Lymphocyte Activation with Lipopolysaccharides and Concanavalin A Treatment**

Isolated pig lymphocytes (5 × 10⁵) from each treatment were added to 96-well plates and incubated for 1 h at 37°C. Lipopolysaccharide (LPS; 2.5 μg/mL; Sigma) was added to the cultured cells to identify B lymphocyte activity, and 2.5 μg/mL concanavalin A (Con A; Sigma) was added to assess T lymphocyte activities. Cells were then incubated for a further 48 h. Cell viability and proliferation were analyzed using an EZ-Cytox kit (Daeil Lab Service, Seoul, Korea) according to the manufacturer’s instructions. Then, the cell proliferation rate was determined on the basis of the absorbance at 550 nm using a Sunrise-‐Basic Tecan microplate reader (Tecan, Grödig, Austria).

**Measurement of IgG, Interferon-γ, and IL-1β in Blood**

Immunoglobulin and cytokine concentrations were assessed by ELISA. The IgG concentrations in the blood samples were measured using a porcine IgG ELISA quantitation kit (Bethly Laboratory Inc., Montgomery, TX). One microgram of anti-porcine IgG antibody was diluted using 100 μL of coating buffer and incubated for 1 h on a 96-well plate. The nonbinding excessive antibody was then aspirated, and the plates were washed 3 times using washing solution. Then 200 μL of blocking solution per sample were added and incubated for 30 min. Then 100 μL of reaction mixture were added to the samples, which were incubated for an additional 1 h. The samples were then washed 5 times with washing solution (provided by the manufacturer). Horseradish peroxidase (HRP)-conjugated secondary antibody (Bethly Laboratory) was added, and the samples were incubated for 1 h at room temperature. The HRP solution was then removed, and the samples were washed 5 times with washing solution. A solution of 3,3′,5,5′-tetramethylbenzidine (TMB; 100 μL) was added, and the samples were incubated for 20 min at room temperature. Finally, 100 μL of H₂SO₄ was added, and the concentration of each immunoglobulin was determined on the basis of the absorbance at 450 nm using a microplate reader. Concentrations of interferon (IFN)-γ and IL-1β were measured in undiluted plasma using a commercially available porcine cytokine detection ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. Briefly, 50 μL of the assay diluent RD1-63 was added to each anticytokine antibody-coated microplate, and then

<table>
<thead>
<tr>
<th>Table 1. Ingredient composition of diets, as-fed basis</th>
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<tbody>
<tr>
<td><strong>Ingredient</strong></td>
</tr>
<tr>
<td>----------------------------</td>
</tr>
<tr>
<td>Corn</td>
</tr>
<tr>
<td>Wheat flour</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Soy oil</td>
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<tr>
<td>Fat powder (source: lard)</td>
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<tr>
<td>Beet pulp1</td>
</tr>
<tr>
<td>Placenta extract</td>
</tr>
<tr>
<td>Soybean meal, dehulled</td>
</tr>
<tr>
<td>Fish meal</td>
</tr>
<tr>
<td>Meat bone meal</td>
</tr>
<tr>
<td>Whey</td>
</tr>
<tr>
<td>Whole-milk powder</td>
</tr>
<tr>
<td>Plasma protein</td>
</tr>
<tr>
<td>Chocolate by-product</td>
</tr>
<tr>
<td>Fortified milk powder</td>
</tr>
<tr>
<td>Soy protein concentrate</td>
</tr>
<tr>
<td>ZnO: 100%</td>
</tr>
<tr>
<td>Lysine: 80%</td>
</tr>
<tr>
<td>Methionine: 100%</td>
</tr>
<tr>
<td>Threonine: 100%</td>
</tr>
<tr>
<td>Choline: 50%</td>
</tr>
<tr>
<td>Vitamin-mineral premix2</td>
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<tr>
<td>Feed supplement3</td>
</tr>
</tbody>
</table>

1Porcine placenta extract was substituted with beet pulp at same inclusion ratio.
2Vitamin-mineral premix included vitamin A: 6,000,000 IU, vitamin D: 1,150,000 IU, vitamin E: 20,000 mg/kg, vitamin K: 700 IU, vitamin B1: 600 mg/kg, vitamin B2: 3,100 mg/kg, Mn: 7,000 mg/kg, Fe: 43,000 mg/kg, Cu: 30,000 mg/kg, I: 110 mg/kg, Zn: 25,500 mg/kg, Co: 85 mg/kg, Se: 55mg/kg.
3Feed supplement: flavor, sweetener, enzyme, plant extract, direct fed microbials, acidifier, and antioxidant.
7.8 to 2,000 pg/mL of each cytokine standard were added to measure binding to the coated antibody as a quantitative control, whereas plasma samples were added to the microplates to measure plasma cytokine concentrations. The standards and plasma samples were then incubated for 2 h at room temperature. After incubation, the mixtures were aspirated, and the microplate wells were washed with washing solution. Interferon-γ and IL-1β polyclonal antibody (100 μL) conjugated with biotin were added to the microplate wells. All mixtures were incubated for 2 h at room temperature, and the excessive nonbinding materials were aspirated and washed. Streptavidin substrate solution (100 μL) was added and incubated for 30 min at room temperature. Finally, 100 μL of stop solution were added, and the cytokine concentrations were determined on the basis of absorbance at 450 nm using a microplate reader.

Statistical Analysis

The SPSS statistical package (Chicago, IL) was used for the data analysis. One-way ANOVA was performed on all results. All data from figures are expressed as mean ± SEM. The values among treatment groups in the same week were analyzed using the Tukey test. The P-value was obtained from the comparison between the 0% (control) and PE-PBS80 treated groups in the same week. The null hypothesis was rejected when the probability was $P < 0.01$.

RESULTS

Body Weight and Rotavirus Infection

In Exp. 1, the daily BW gain of control and 0.1%, 0.3%, and 0.5% PE-PBS treatments was 387, 427, 430, and 406 g, respectively (Table 2). The daily BW gain of control and 0.1%, 0.3%, and 0.5% PE-PBS treatments was 488, 322, 616, and 515 g in Exp. 2, respectively (Table 2). However, piglets in all treatments had severe diarrhea at d 3 of Exp. 2 and recovered at d 10. In Exp. 2, a rotavirus was detected in feces collected from 10 different points of each pen using a rotavirus antigen detection kit (Jaeil Bio, Ansan, Korea; data not shown). To identify the source of the rotavirus infection, the control feed, placenta extract feed, placenta extract alone, and water stored in tanks were subjected to rotavirus antigen detection using a rotavirus antigen detection kit (Jaeil Bio, Ansan, Korea), but rotavirus antigen contaminations were not found. In addition, the same samples were sent to Chungbuk Livestock and Veterinary Service (Chungju, Korea) to confirm our results, and none of the rotavirus contaminations were identified (data not shown).

Quantitative Change in Blood Immune Cells

To investigate whether or not PE-PBS80 can influence immune cells, the number of WBC and granulocytes was calculated. Compared with 0% PE-PBS80-treated piglets for each week, a significant increase in WBC and monocytes was observed for the third and second weeks, respectively, in the 0.3% PE-PBS80-treated piglets in Exp. 1 (Figs. 1A and 1C), and a decrease in monocyte concentration was observed at wk 0 in the 0.1% and 0.3% PE-PBS80-treated piglets in Exp. 1 (Fig. 1C). Neutrophils, eosinophils, and basophils were not different among the treatments in Exp. 1 (Figs. 1B, 1D, and 1E). No significant difference was observed in WBC concentration in Exp. 2 (Fig. 2A). The percentage of neutrophils was increased significantly in the 0.5% PE-PBS80-treated piglets in the third week (Fig. 2B). Unlike Exp. 1, a significant increase in eosinophils, basophils, and monocytes was observed in the 0.3% PE-PBS80-treated piglets (Figs. 2C, 2D, and 2E). In addition, a significant decrease in monocytes was observed in the second week in the 0.3% PE-PBS80-treated piglets (Fig. 2C). In Exp. 1, the PE-PBS80 treatment showed an alteration only in monocyte concentration, whereas there was an altered variation of monocytes and granulocytes in Exp. 2.

Cytokines and IgG in PE-PBS80-treated Piglets

Alterations in the concentrations of IFN-γ and IL-1β, which are indicators of immune response and activation, were calculated. Only an increase of IL-1β in the third week of 0.3% PE-PBS80 treatment was observed among

<table>
<thead>
<tr>
<th>Item</th>
<th>Average BW, kg</th>
<th>Control (n = 25)</th>
<th>PE-PBS80 (n = 25)</th>
<th>PE-PBS80 (n = 25)</th>
<th>PE-PBS80 (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wk 0, 34 d</td>
<td>8.92</td>
<td>9.04</td>
<td>8.8</td>
<td>9.08</td>
<td></td>
</tr>
<tr>
<td>Wk 2, 48 d</td>
<td>13.56</td>
<td>13.8</td>
<td>13.68</td>
<td>13.88</td>
<td></td>
</tr>
<tr>
<td>Wk 3, 55 d</td>
<td>17.04</td>
<td>18</td>
<td>17.88</td>
<td>17.6</td>
<td></td>
</tr>
<tr>
<td>DWG, g</td>
<td>387</td>
<td>427</td>
<td>430</td>
<td>406</td>
<td></td>
</tr>
<tr>
<td>Wk 0, 25 d</td>
<td>4.88</td>
<td>4.81</td>
<td>5.25</td>
<td>5.03</td>
<td></td>
</tr>
<tr>
<td>Wk 1, 32 d</td>
<td>6.38</td>
<td>5.89</td>
<td>7.03</td>
<td>7.12</td>
<td></td>
</tr>
<tr>
<td>Wk 2, 39 d</td>
<td>9.38</td>
<td>7.58</td>
<td>12.02</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>Wk 3, 46 d</td>
<td>15.12</td>
<td>11.56</td>
<td>18.18</td>
<td>15.58</td>
<td></td>
</tr>
<tr>
<td>DWG, g</td>
<td>488</td>
<td>322</td>
<td>616</td>
<td>515</td>
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</table>

PE-PBS80 = porcine placental extracts using PBS at 80°C.
Immune modulation using porcine placenta extracts

In Exp. 1, compared with control (Figs. 3A and 3B). However, IFN-γ and IL-1β increased significantly in the PE-PBS80 treatment in Exp. 2. Increases in IFN-γ were observed from the first to third weeks in the 0.3% PE-PBS80-treated piglets and the third week in the 0.5% PE-PBS80-treated piglets (Fig. 3C). Interleukin-1β increased in the second week in the 0.1% PE-PBS80-treated piglets, the third week in the 0.3% PE-PBS80-treated piglets, and the first week in the 0.5% PE-PBS80-treated piglets (Fig. 3D). In addition, blood IL-1β concentration was decreased in the second and third weeks in the control and 0.1% and 0.5% PE-PBS80 treatments in Exp. 1, but decreased IL-1β concentration in the second week in the 0.3% treatment was recovered in the third week (***P < 0.01, n = 15). PE-PBS80, porcine placental extracts using PBS at 80°C.

**Lymphocyte Activation**

Lipopolysaccharides and Con A were used to evaluate B- and T-cell activity, respectively. Activation of B and T lymphocytes were compared on dose effect; dose effect was analyzed among the treatments for the same week using 1-way ANOVA. Regarding dose effect, B-cell activation in the 0.3% treatment increased significantly in the second and third weeks, and T-cell activation in the same treatment increased in the second week in Exp. 1 (Figs. 5A and 5B). In Exp. 2, increased activation of B cells was observed at wk 0 and 2 for control and 0.1% treatment; however, decreased activation of B cells was observed in the first and third weeks of 0.3% and 0.1% treatments, respectively (Fig. 5C). Decrease in T-cell activation in the 0.3% treatment was observed in the first week, whereas the other treatment did not show any changes (Fig. 5D). Interestingly, the 0.3% PE-PBS80 treatment showed a significant decrease in both B- and T-cell activation (Figs. 5C and 5D, respectively).
DISCUSSION

In a previous study, PE-PBS80-treated mice without a challenge showed increased immune activities that increased blood cytokine concentrations, IgG concentrations, and activated lymphocytes (Park et al., 2011). Unlike previous results in mice experiments where all the tested immune variables were dramatically changed, the 0.3% treatment of uninfected normal piglets showed an alteration in the percentage of WBC and monocytes and lymphocyte activation compared with the control among the tested parameters in Exp. 1. It is speculated that some of the substances in PE-PBS80 were recognized as foreign antigens in mice but not in swine because PE-PBS80 was from the placenta of swine, suggesting that PE-PBS80 is tolerated in piglets. Furthermore, the percentage of monocyte and lymphocyte activation increased in Exp. 1, suggesting that a putative role of PE-PBS80 could be to activate monocytes and lymphocytes. Antigen-presenting cells (APC), such as macrophages and dendritic cells, are differentiated from monocytes (Cheong et al., 2010). They stimulate and control lymphocyte (B and T cell) activity in the lymph nodes (Ahrens et al., 2009; Cheong et al., 2010; Mousson and Girard, 2011); therefore, it is possible that PE-PBS80 might be involved in stimulating APC as an immune modulator, and these stimulated APC may play a role triggering lymphocyte activation in piglets. In Exp. 2, all piglets were infected by a rotavirus, providing valuable results on immune response in comparison with our previous study. Significant increases in monocytes, granulocytes, IgG, and IFN-γ were observed in the PE-PBS80 treatment in the second and third weeks compared with those in the other treatments. Indeed, the 0.3% treatment showed more active changes. These results suggest that PE-PBS80 may contain a component that activates the monocyte-mediated immune response and initiates or accelerates lymphocyte activation for IgG production.

Interestingly, during the first week in Exp. 2, the concentration of blood IFN-γ increased. Interferon-γ, a representative cytokine of immune cell activation, increases when cells are infected by antigens, and IFN-γ secreted from T and natural killer (NK) cells promotes B-cell differentiation, inhibits B-cell proliferation, and enhances the activity of macrophages and granulocytes (Schroder et al., 2004; Murphy et al., 2008). In this regard, the temporally decreased lymphocyte activation during the first week in Exp. 2 may be correlated with the increase in blood IFN-γ concentrations after the viral infection. Additionally, IFN-γ plays a role in B-cell differentiation and immunoglobulin production (Schoenborn et al., 2007; Murphy et al., 2008). Increased concentrations of IgG during the first week in the 0.3% PE-PBS80 treatment of Exp. 2 after the viral infection also may be related to blood IFN-γ concentrations, although B lymphocyte activity decreased significantly. Therefore, these results suggest that an active
component of PE-PBS80 may stimulate the secretion of IFN-γ and IgG in lymphocytes.

Nutrition is an important factor of immune systems, and protein-energy malnutrition is associated with a significant impairment of cell-mediated immunity, phagocyte function, complement system, secretory immunoglobulin A concentrations, and cytokine production (Chandra, 1997). As a nutrition source, placental extracts contain many kinds of nutritional substances (e.g., cytokines, hormones, bioactive peptides, enzymes, growth factors, vitamins, and minerals), and treatments of placenta extracts may alter growth rate. It has been reported that an increase in IL-1 is associated with decreased feed intake and growth performance (Klasing et al., 1987). The IL-1β concentration was decreased in the second and third weeks in all treatments, except the 0.3% treatment of placenta extract increased the blood IL-1β in the third week in Exp. 1; however, IL-1β concentration was overall not altered in each treatment in Exp. 2, except in the first week in the 0.5% treatment. These data are accordant with another disease challenge study where pigs fed Choleraesuis had a reduction in growth performance compared with the control, but serum IL-1β did not change compared with the control (Fraser et al., 2006). It is hard to speculate about the correlation between the changes in IL-1β concentration and the growth performance in this study because of the lack of statistical analysis data for growth performance. However, it is possible that treatment of placenta extract in Exp. 2 may not involve an alteration of IL-1β concentration, which is related to growth performance, but may modulate secretion of IFN-γ, which is a key cytokine for innate and adaptive immunity against foreign pathogens.

The B- and T-cell response to LPS and Con A unexpectedly decreased in the second week in both experiments, except the 0.3% treatment in Exp. 1 increased B- and T-cell activation in the third week. This pattern could be caused by the stress of weaning, which could induce various immune responses in piglets (Niekamp et al., 2007), although the effect of other physiological and immunological factors cannot be ruled out. Differences in the blood concentration and B-cell activation at wk 0 may be related to weaning stress. Additionally, the 0.3% PE-PBS80 treatment showed a significant decrease in both B- and T-cell activation at wk 0.

Figure 3. Concentrations of interferon-γ and IL-1β in both experiments. (A) and (B) The results in Exp. 1. (C) and (D) The results in Exp. 2. IFN-γ = interferon-γ. P-value was detected in between 0% (control) and each PE-PBS80 treatment in the same week (***P < 0.01, n = 15). PE-PBS80, porcine placental extracts using PBS at 80°C.

Figure 4. Changes in IgG concentration in Exp. 2. P-value was detected in between 0% (control) and each PE-PBS80 treatment in the same week (***P < 0.01, n = 15). PE-PBS80, porcine placental extracts using PBS at 80°C.
results suggest that a temporarily decreased proliferation of lymphocytes also may be correlated with activation of innate immunity, including macrophage and granulocyte activation that occurred during the viral infection of Exp. 2.

According to Darwinian medicine, adaptive immunity is developed by conflict with foreign infection (Nesse and Williams, 1996). In vertebrates, only the skin, intestines, eyes, and respiratory organs interact with foreign substances. Allergic diseases, such as atopic dermatitis and asthma, are strongly correlated with intestinal colonization of helminths and microorganisms to develop the immune system during childhood; the proportion of their colonization induces a systemic imbalance of T-helper cells 1 and 2, which are regulated by regulatory T cells (Garn and Renz, 2007; Penders et al., 2007; Rook, 2009). Intestines are where nutrition is absorbed and immune cells are activated (Iwasaki and Kelsall, 1999; Ouellette, 1999; Murphy et al., 2008). Mature dendritic cells, which are initiated by interacting with foreign substances from foods and microorganisms, interact with lymphocytes, and the immune response is activated (Round and Mazmanian, 2009). On the basis of activation of the intestinal immune cells, substances in foods that are digested can activate immunity as well as provide nutrition. It could not be ruled out that PE-PBS80 absorbed through the gut may have induced the activation and response of the immune cells, particularly the granulocytes and lymphocytes in rotavirus-infected piglets. Therefore, these results suggest that PE-PBS80 modulated the systemic immune system by activating intestinal immune cells. In conclusion, the PE-PBS80 pig placental extract, particularly the 0.3% supplement to the normal diet, could be useful as an alternative feed supplement to modulate immune activity during the early piglet period.

**LITERATURE CITED**


![Figure 5. T- and B-cell activation in both experiments. (A) and (B) The results in Exp. 1. (C) and (D) The results in Exp. 2. Lymphocytes were treated with lipopolysaccharide (LPS; A and C) and concanavalin A (Con A; B and D). P-value was detected in between 0% (control) and each PE-PBS80 treatment in the same week (***(P < 0.01, n = 15). PE-PBS80, porcine placental extracts using PBS at 80°C; O.D., optical density.](image-url)


