Effects of antibacterial peptide on cellular immunity in weaned piglets1,2


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ABSTRACT: The aim of this study was to evaluate the effects of antibacterial peptide (ABP) sufficiency on cellular immune functions by determining the spleen cell cycle and apoptosis, peripheral blood T cell subsets, and T cell proliferation function in weaned piglets. A total of 90 piglets (Duroc × Landrace × Yorkshire) of both sexes were randomly allotted to 5 dietary treatments. Each treatment consisted of 3 replicates with 6 piglets per replicate. The dietary treatments consisted of the negative control (NC; basal diet), positive control (PC; basal diet supplemented with 400 mg/kg Astragalus polysaccharide), and ABP (basal diet mixed with 250, 500, and 1,000 mg/kg ABP). The experimental lasted for 28 d. Two piglets from each replicate were selected randomly for blood samples extraction from the jugular vein to obtain peripheral blood T cell subsets, and T cell proliferation function analysis was performed on d 32, 39, 46, and 53. Two piglets from each replicate were selected and euthanized to observe the spleen cell cycle and apoptosis on d 39 and 53. In ABP-sufficient piglets, the G0/G1 phase of the spleen cell cycle was much lower (P < 0.05) and the S and G2 + M phases and proliferation index (PI) were greater (P < 0.05) than in NC piglets. The percentage of apoptotic cells in the spleen significantly decreased under ABP sufficiency (P < 0.05). The proliferation function of peripheral blood T cells increased (P < 0.05) in ABP-sufficient piglets. Percentages of CD3+ and CD3+CD4+ ratios (d 39, 46, and 53) and CD4+CD8+ ratios (d 32, 39, 46, and 53) increased remarkably (P < 0.05) under ABP sufficiency compared with NC. These results suggest that ABP sufficiency could increase the T cell population and proliferation function of T cells and could induce decreased percentages of apoptotic cells. Overall, the cellular immune function was evidently improved in weaned piglets. We suggest optimal dosages of 500 mg/kg ABP for 4-wk addition and 1,000 mg/kg ABP for 2-wk addition.

Key words: antimicrobial peptide, apoptosis, lymphocyte cycle, lymphocyte proliferation, T cell subset, weaned piglets

INTRODUCTION

In the modern pig industry, most piglets are weaned at 3 to 4 wk of age; however, the immune function of weaned piglets can be considered mature only at about 7 wk of age (Yang and Schultz, 1986; Kim et al., 2004). During this critical period, some viral diseases, such as classical swine fever and porcine respiratory and reproductive syndrome, could cause great morbidity and mortality in weaned piglets, resulting in significant economic loss. Therefore, improving the immunity of weaned piglets is an important problem.

Antimicrobial peptides represent a series of short-chain peptides composed of dozens of amino acid residues. They are bioactive substances that are extracted, separated, and purified from a variety of plants, animals, and human tissues and cells in vivo (Wang and Wang, 2004). They have a broad range of functions, such as antibacterial (Koczulla and Bals, 2003), antiviral (Huang et al., 2013), antifungal (Rossignol et al., 2004).
2011), antitumor (Yan et al., 2012), antiparasitic (Torrent et al., 2012), and immune function enhancing (Yu et al., 2010), among others. In some studies, dietary supplementation of different antibacterial peptides (ABP) to mice, chicken, rabbit, and piglet diets improved E rosette ratios (Geng et al., 2011) as well as levels of IgG, IgM, IgA, and alexin C3 (Lv et al., 2011; Guo et al., 2012; Liu et al., 2012; S. D. Wu et al., 2012).

To the best of our knowledge, few reports on the effects of ABP on cellular immunity in weaned piglets are available. In this study, we investigate the effects of different concentrations of ABP on cellular immunity in weaned piglets.

MATERIALS AND METHODS

Materials

The antimicrobial peptide used in the present study was provided by Rota Bioengineering Co., Ltd. (Sichuan, China). The ABP was composed of swine defensin (DHY -ICAKKGGTNCNFSCPFLNFRIEGTCYGAKCCIR) and a fly antibacterial peptide (ATCDLLSGTGVKHSCAAHCLLRGNRGGYCNGRAICVCRN) at a blending ratio of 50%. Astragalus polysaccharide (AP; net content 65%) was purchased from Centre Biology Co., Ltd. (Beijing, China). All chemicals used were of the highest-purity grade available.

Animals and Experimental Design

Piglets (Landrace × Yorkshire × Duroc; 21 ± 2 d of age) were purchased from Xin Qiao Agricultural Science and Technology Development Co., Ltd. (Chengdu, Sichuan, China) and were acclimated for 5 d before the experiment. Weanling piglets (average BW of 8.24 ± 0.67 kg) were caged in elevated pens with wire flooring and fed a standard diet (Table 1; NRC, 1998). The temperature (26°C to 27°C) and relative humidity (65% to 70%) were kept constant. Food and water were provided ad libitum during the acclimation period and throughout the study. All piglets used in this study were suitably healthy, and all experimental manipulations were undertaken in accordance with the Institutional Guidelines for the Care and Use of Laboratory Animals.

Ninety weanling piglets of both sexes were randomly allotted to 5 treatments in a randomized complete block design for 28 d. Each treatment consisted of 3 replicates with 6 piglets per replicate. Dietary treatments included the negative control (NC; basal diet), positive control (PC; basal diet supplemented with 400 mg/kg AP), and ABP (basal diet supplemented with 250, 500, or 1,000 mg/kg ABP). The NC diet was considered to be a 0 mg/kg ABP treatment. Two piglets from each replicate were selected randomly for blood extraction from the jugular vein to perform peripheral blood T cell subset and T cell proliferation function analyses at 32, 39, 46, and 53 d of age. At 39 and 53 d of age, 2 piglets from each replicate were selected from each treatment group and euthanized for spleen cell cycle and apoptosis determination via flow cytometry (Beckman Coulter Corp., Fullerton, CA).

Lymphocyte Proliferation

At 32, 39, 46, and 53 d of age, 6 piglets from each treatment group were selected, and blood samples were obtained by puncturing the vena cava. Lymphocyte proliferation was measured as described by Fan et al. (2012).

Two milliliters of peripheral blood were collected in 5-mL heparinized vacuum tubes (Vacutainer System, Becton Dickinson, Franklin Lakes, NJ), were mixed with an equal volume of Hanks’ solution (HyClone, Thermo Scientific, Logan, UT), and then were carefully layered on the surface of the lymphocyte separation medium (density of 1.077 ± 0.001 g/mL; Jingyang Co., Tianjin, China). The vacuum tubes were then centrifuged at 3,000 × g for 20 min at room temperature. Mononuclear cells were collected and washed 3 times...
with RPMI 1640 medium (Gibco BRL, Grand Island, NY) without fetal bovine serum. The resulting pellet was resuspended to 2 × 10^6 cells/mL with complete RPMI 1640 medium for proliferation assay.

Suspensions of mononuclear cells (2 × 10^6/well) were incubated in 96-well culture plates at 100 μL per well; each sample was seeded in 6 wells. Exactly 100 μL of concanavalin A (ConA; 10 μg/mL; Sigma Chemical Co., St. Louis, MO) were then added into each well. The plates were incubated in a humid atmosphere of 5% CO_2 for 44 h at 37°C. Then, 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 5 μg/mL; Sigma Chemical Co.) were added to each well, and the plates were reincubated for another 4 h. After incubation, 100 μL of dimethyl sulfoxide (DMSO; Sigma Chemical Co.) were added to each well. The plates were shaken for 10 min to dissolve the precipitate completely and then were placed in an automated ELISA reader (MQX200; BioTek Instruments Inc., Winooski, VT) for absorbance measurement at 570 nm. The stimulation index (SI), which indicates the lymphocyte proliferation activity, was calculated as follows:

\[ SI = \text{OD (optical density)} \text{ value of ConA-stimulating cells/OD value of ConA-free cells.} \]

**Spleen Cell Cycle**

At 39 and 53 d of age, 6 piglets were euthanized in each group to determine the percentage of apoptotic cells in the spleen, as described by Chen et al. (2013). The cells were consistent with the lymphocyte cycle of the spleen. About 100 μL of the cell suspension were transferred to a centrifuge tube, and about 5 μL of V-FITC (BD Pharmingen, Sparks, Maryland, USA) and 5 μL of propidium iodide (5 μL/mL propidium iodide, 0.5% Triton X-100, 0.5% RNase, PBS) were mixed with this suspension for staining at room temperature for 15 min in the dark. About 400 μL of 1× binding buffer were added to each centrifuge tube, and flow cytometry assay was performed within 1 h.

**T Cell Subsets**

At 32, 39, 46, and 53 d of age, 6 piglets from each treatment group were selected, and blood samples were obtained by puncturing the vena cava. The percentages of CD3^+^, CD3^+CD4^+^, and CD3^+CD8^+^ T cells in the blood were determined via flow cytometry (Beckman Coulter Corp.), as described by Chen et al. (2009).

About 1 mL of peripheral blood was collected in 5-mL heparinized vacuum tubes, was mixed with an equal volume of PBS (0.01 M and pH 7.4), and was carefully layered on the surface of the lymphocyte separation medium. Centrifugation was done at 200 × g for 20 min at room temperature. The lymphocytes were collected, transferred to another centrifuge tube, and then washed with PBS. The resulting pellet was resuspended at a concentration of 1 × 10^6 cells/mL with PBS. About 1 mL of cell suspension was transferred to another tube for centrifugation at 200 × g for 5 min. The supernatant was discarded. The cells were stained with 10 μL of mouse anti-pig CD3 phycoerythrinn (Southern Biotechnology Associates, Birmingham, AL), mouse anti-pig CD4 phycoerythrin (Southern Biotechnology Associates), and mouse anti-pig CD8 FITC (Southern Biotechnology Associates) for 20 min at room temperature and then were washed with PBS. The supernatant was discarded, and cells were resuspended in 0.5 mL of PBS and analyzed by flow cytometry.

**Data Analysis**

Results are reported as mean ± SD. Statistical analysis was performed by 1-way ANOVA using SPSS 19.0 software (International Business Machines Corporation, Armonk, NY). Duncan’s test for multiple comparisons was done, and P < 0.05 was considered statistically significant.
RESULTS

Peripheral Lymphocyte Proliferation Assay

During the entire experimental period, the SI of peripheral blood T cells was greater \( (P < 0.05; \text{Table 2}) \) in piglets fed the PC and the 250, 500, and 1,000 mg/kg ABP diets than in the piglets fed the NC diet. Piglets given the 250 mg/kg ABP diet on d 46 and 53 and the 500 and 1,000 mg/kg ABP diets on d 32, 39, 46, and 53 had greater \( (P < 0.05) \) SI values for peripheral blood T cells than piglets given the PC diet. Moreover, the SI of peripheral blood T cells linearly improved \( (P < 0.05) \) as the level of dietary ABP increased.

Cell Cycle of Spleen

On d 39 and 53, the \( G_0/G_1 \) phase distributions of spleen cells were lower \( (P < 0.05; \text{Table 3 and Fig. 1}) \) in piglets fed the PC and 250, 500, and 1,000 mg/kg ABP diets than in piglets fed the NC diet. The S and \( G_2 + M \) phase cell distributions as well as PI gain were greater \( (P < 0.05) \) in the spleen cells of piglets fed the PC and 250, 500, and 1,000 mg/kg ABP diets than in piglets fed the NC diet. Moreover, the SI of peripheral blood T cells linearly improved \( (P < 0.05) \) as the level of dietary ABP increased.

Apoptosis of Spleen Cells

On d 39 and 53, piglets given the PC and 250, 500, and 1,000 mg/kg ABP diets had lower \( (P < 0.05; \text{Table 4 and Fig. 2}) \) percentages of apoptotic spleen cells than piglets given the NC diet. However, piglets given the 1,000 mg/kg ABP diet had fewer \( (P < 0.05) \) \( G_0/G_1 \) phases, \( G_2 + M \) phases, S phase cell distributions, and PI in spleen cells than piglets given the PC diet. However, piglets given the 1,000 mg/kg ABP diet showed better \( (P < 0.05) \) overall \( G_0/G_1 \) phases, S phase cell distributions, and PI in spleen cells than piglets given the PC diet on d 39. On d 53, piglets given the 250, 500, and 1,000 mg/kg ABP diets showed better \( (P < 0.05) \) \( G_0/G_1 \) phases, \( G_2 + M \) phases, S phase cell distributions, and PI in spleen cells than piglets given the PC diet. However, piglets given the 1,000 mg/kg ABP diet had fewer \( (P < 0.05) \) \( G_0/G_1 \) phases, \( G_2 + M \) phases, and S phase cell distributions and a lower PI in spleen cells than piglets given the 250 and 500 mg/kg ABP diets.

Table 2. Changes in the stimulation index of peripheral blood T cells\(^{1, 2}\)

<table>
<thead>
<tr>
<th>Day</th>
<th>PC</th>
<th>0 (NC)</th>
<th>250</th>
<th>500</th>
<th>1,000</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>1.35 ± 0.04(^c)</td>
<td>1.23 ± 0.06(^d)</td>
<td>1.31 ± 0.07(^c)</td>
<td>1.51 ± 0.03(^b)</td>
<td>1.62 ± 0.04(^a)</td>
<td>0.39</td>
</tr>
<tr>
<td>39</td>
<td>1.43 ± 0.04(^c)</td>
<td>1.25 ± 0.05(^d)</td>
<td>1.40 ± 0.07(^c)</td>
<td>1.62 ± 0.03(^b)</td>
<td>1.73 ± 0.05(^a)</td>
<td>0.57</td>
</tr>
<tr>
<td>46</td>
<td>1.48 ± 0.05(^d)</td>
<td>1.30 ± 0.09(^e)</td>
<td>1.54 ± 0.01(^c)</td>
<td>1.65 ± 0.04(^b)</td>
<td>1.81 ± 0.05(^a)</td>
<td>0.06</td>
</tr>
<tr>
<td>53</td>
<td>1.51 ± 0.02(^d)</td>
<td>1.27 ± 0.04(^e)</td>
<td>1.58 ± 0.03(^c)</td>
<td>1.71 ± 0.06(^b)</td>
<td>1.88 ± 0.06(^a)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

\(^{a–e}\)Means in the same row with different superscripts differ \( (P < 0.05) \).

\(^1\)Dietary treatments: NC = negative control (basal diet without any antimicrobials), PC = positive control (basal diet mixed with 400 mg/kg Astragalus polysaccharide), and antibacterial peptide (ABP) = basal diet supplemented with 250, 500, or 1,000 mg/kg diet ABP. NC (diet without antimicrobials) was considered to be 0 mg/kg diet ABP. Data are presented as mean ± SD.

\(^2\)Each number represents the mean of 6 piglets.

Table 3. Changes in the cycle of spleen cells\(^{1, 2}\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Day</th>
<th>PC, %</th>
<th>0 (NC)</th>
<th>250</th>
<th>500</th>
<th>1,000</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( G_0/G_1 )</td>
<td>39</td>
<td>87.17 ± 0.33(^d)</td>
<td>93.35 ± 0.38(^a)</td>
<td>90.48 ± 0.62(^b)</td>
<td>87.94 ± 0.50(^c)</td>
<td>84.74 ± 0.58(^e)</td>
<td>0.39</td>
</tr>
<tr>
<td>53</td>
<td>90.06 ± 0.61(^b)</td>
<td>94.16 ± 0.60(^a)</td>
<td>82.26 ± 0.36(^d)</td>
<td>81.81 ± 0.28(^d)</td>
<td>84.57 ± 0.53(^c)</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>39</td>
<td>6.02 ± 0.42(^c)</td>
<td>4.40 ± 0.38(^b)</td>
<td>5.20 ± 0.49(^d)</td>
<td>7.87 ± 0.22(^b)</td>
<td>9.22 ± 0.63(^a)</td>
<td>0.57</td>
</tr>
<tr>
<td>53</td>
<td>6.21 ± 0.33(^d)</td>
<td>3.83 ± 0.40(^d)</td>
<td>9.48 ± 0.28(^b)</td>
<td>10.09 ± 0.29(^a)</td>
<td>8.41 ± 0.66(^d)</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>( G_2 + M )</td>
<td>39</td>
<td>6.81 ± 0.31(^a)</td>
<td>2.65 ± 0.13(^d)</td>
<td>4.32 ± 0.70(^c)</td>
<td>4.19 ± 0.69(^b)</td>
<td>6.05 ± 0.50(^c)</td>
<td>0.03</td>
</tr>
<tr>
<td>53</td>
<td>3.73 ± 0.39(^b)</td>
<td>2.00 ± 0.76(^a)</td>
<td>8.26 ± 0.36(^d)</td>
<td>8.10 ± 0.14(^a)</td>
<td>7.02 ± 0.45(^a)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>39</td>
<td>12.83 ± 0.33(^b)</td>
<td>6.65 ± 0.38(^d)</td>
<td>9.52 ± 0.62(^d)</td>
<td>12.05 ± 0.50(^c)</td>
<td>15.26 ± 0.58(^b)</td>
<td>0.39</td>
</tr>
<tr>
<td>53</td>
<td>9.94 ± 0.61(^a)</td>
<td>5.83 ± 0.62(^d)</td>
<td>17.74 ± 0.36(^a)</td>
<td>18.19 ± 0.28(^b)</td>
<td>15.43 ± 0.53(^b)</td>
<td>0.25</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a–e}\)Means in the same row with different superscripts differ \( (P < 0.05) \).

\(^1\)Dietary treatments: NC = negative control (basal diet without any antimicrobials), PC = positive control (basal diet mixed with 400 mg/kg Astragalus polysaccharide), and antibacterial peptide (ABP) = basal diet supplemented with 250, 500, or 1,000 mg/kg diet antibacterial peptide. NC (diet without antimicrobials) was considered to be 0 mg/kg diet antibacterial peptide. Data are presented as mean ± SD.

\(^2\)Each number represents the mean of 6 piglets.
Antibacterial peptide for weaned piglets

The percentages of CD3+ and CD3+CD4+ ratios (d 39, 46, and 53) and CD4+CD8+ ratios (d 32, 39, 46, and 53) were greater \( (P < 0.05; \text{Table 5}) \) in piglets given the 250, 500, and 1,000 mg/kg ABP diets than in piglets fed the NC diet. The percentages of CD3+, CD3+CD4+, and CD4+CD8+ ratios (d 32, 39, 46, and 53) and CD3+CD8+ ratios (d 46 and 53) were greater in piglets given the 500 mg/kg ABP diet than in piglets given the PC diet. The percentages of CD3+, CD3+CD4+, CD3+CD8+, and CD4+CD8+ ratios linearly improved \( (P < 0.05) \) as the level of dietary ABP increased.

DISCUSSION

Lymphocyte proliferation is an indicator of the state of cellular immunity. T and B lymphocytes play an important role in enhancing the immune functions of various organisms (Minato et al., 2004). In our study, supplementation with AP and ABP enhanced the proliferation of T lymphocytes, although ABP showed better effects than AP. This result is consistent with Fan et al.'s (2012) study, in which 4.0 mg/mL AP was administered to weaned piglets. In line with the results of the present study, Wang and Li (2007) reported that oral administration of 300 and 600 mg/kg ABP diet to broilers could promote \( (P < 0.05) \) proliferation of T lymphocytes in peripheral blood. Similarly, weaned piglets fed diets supplemented with 1,000 mg/kg ABP (lactoferrin) were reported to have greater \( (P < 0.05) \) phytohemagglutinin (PHA)-stimulated peripheral lymphocyte proliferation (Shan et al., 2007). Increased \( (P < 0.05) \) ANAE+ (acidalpha naphthyl acetate esterase) percentages of T lymphocytes of the thymus, spleen, and bursa of Fabricius were also reported in chickens receiving drinking water supplemented with 1 μg/mL ABP that was isolated from African ostrich skin (Yang et al., 2009). In contrast to the results of the present study, Yang et al. (2006) reported no significant differences in B lymphocyte proliferation in chickens receiving drinking water supplemented with chicken intestinal antimicrobial peptides (1 mg/mL) right after hatching. This discrepancy in the results may be attributed to variations in the type of ABP used, the level of dietary supplementation, and the mode of action of the ABP.

Four major phases of the eukaryotic cell cycle have been described: the G1 phase, which occurs before DNA replication; the S phase, which describes periods of DNA synthesis; the G2 phase, which occurs before cell division; and the M phase, which describes actual cell division (Pines, 1995). To the best of our knowledge, no reports on the effects of ABP on the spleen cell cycle of weanling piglets are yet available. In our study, supplementation of AP and ABP in piglets caused decreases in G1 phase cells, which corresponds to increases in S and G2 + M phase cells and PI in the spleen. Also, ABP showed better effects than AP. Results showed that AP and ABP sufficiency can cause developmental progression of the spleen because of cell growth promotion in piglets. Shan et al. (2007) reported that supplementing 1,000 mg/kg antimicrobial peptide (lactoferrin) remarkably increases \( (P < 0.05) \) ConA and PHA-induced spleen lymphocyte proliferation in weanling piglets. The mechanism of ABP in lymphocyte proliferation is not clear. Interleukin-2 is believed to play a central role in regulating host responses to pathogenic challenges and is known as the principal cytokine responsible for the progression of T cells from the G1 phase to the S phase of the cell cycle (Bonham et al., 2002). Therefore, we suppose that ABP could promote lymphocyte proliferation by improving production levels of the cytokine IL-2.
Apoptosis is a greatly regulated process used to eliminate dysplastic or damaged cells from multicellular organisms (King and Cidlowski, 1995; Bortner and Cidlowski, 2004). To the best of our knowledge, no report on the effect of ABP on apoptotic spleen cells in the spleen of weanling piglets has yet been published. In our study, AP and ABP were added to the diets of weanling piglets, and the percentages of apoptotic spleen cells significantly decreased. The effect of ABP was better than that of AP. The mechanism of ABP in apoptotic spleen cells is not yet clear, but an association with reactive oxygen radical production and proapoptotic protein overexpression is suggested (Cryns and Yuan, 1998); these oxidation products could impair DNA and induce apoptosis (Fisher, 2001). Our studies indicate that ABP could decrease the levels of malonaldehyde (MDA) and improve the inhibition capacity of hydroxy free radicals (OH\(^{-}\)), total antioxidative capacity (T-AOC), glutathione peroxidase (GSH-Px) activity, superoxide dismutase (SOD), and catalase (CAT) of the spleen. These results indicate that ABP could remove free radicals and reduce spleen damage. As such, we hypothesize that ABP reduces apoptosis in spleen cells by improving the antioxidant function of the spleen.

The percentage of T cell subsets is an important indicator of the composition of mature T cells in the body. The biological function of T cells and the cellular immune function of the body are dependent on the composition of mature T cells. As surface markers of mature T cells, CD\(_{3}^{+}\) molecules reflect the mature T cell population. Similar to other mammalian species, the mature T cells of piglets may be further classified according to the presence of CD\(_{4}^{+}\) and CD\(_{8}^{+}\) proteins (Janeway et al., 1999). In piglets, most CD\(_{4}^{+}\) T cells are helper/inflammatory T cells responding to exogenous antigens in association with major histocompatibility complex (MHC) class II molecules. CD\(_{8}^{+}\) T cells respond to endogenous antigens in association with MHC class I molecules and generally function as cytotoxic T cells (Chan et al., 1988). In our study, the percentages of CD\(_{3}^{+}\), CD\(_{3}^{+}\)CD\(_{4}^{+}\), and CD\(_{3}^{+}\)CD\(_{8}^{+}\) increased after ABP and AP intake, which demonstrates that the population and biological function of mature T cells were positively affected. The effects of ABP were better than those of AP. Previous research indicates that weaned piglets fed 324 to 563 mg/kg AP show enhanced percentages of CD\(_{4}^{+}\) and CD\(_{8}^{+}\) T cells (Yuan et al., 2006). Mice gavage fed a daily dosage of 0.2 mL of 7.5 g/L ABP isolated from bovine neutrophils also show significantly improved (\(P < 0.05\)) percentages of CD\(_{3}^{+}\), CD\(_{4}^{+}\), and CD\(_{8}^{+}\) T cells (Jin et al., 2009). The CD\(_{4}^{+}/\)CD\(_{8}^{+}\) ratio is also used as an end point in assessing the state of an immune system.

### Table 4. Changes in apoptotic spleen cells\(^{1,2}\)

<table>
<thead>
<tr>
<th>Day</th>
<th>PC, %</th>
<th>0 (NC)</th>
<th>250</th>
<th>500</th>
<th>1,000</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>18.01 ± 0.17(^{d})</td>
<td>21.83 ± 0.12(^{a})</td>
<td>20.45 ± 0.26(^{b})</td>
<td>18.41 ± 0.25(^{c})</td>
<td>16.16 ± 0.14(^{e})</td>
<td>0.59</td>
</tr>
<tr>
<td>53</td>
<td>17.23 ± 0.17(^{b})</td>
<td>23.13 ± 0.16(^{a})</td>
<td>14.50 ± 0.24(^{d})</td>
<td>13.97 ± 0.26(^{e})</td>
<td>15.68 ± 0.30(^{c})</td>
<td>0.68</td>
</tr>
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</table>

\(^{a}\)–\(^{e}\)Means in the same row with different superscripts differ (\(P < 0.05\)).

\(^{1}\)Dietary treatments: NC = negative control (basal diet without any antimicrobials), PC = positive control (basal diet mixed with 400 mg/kg *Astragalus* polysaccharide), and antibacterial peptide (ABP) = basal diet supplemented with 250, 500, or 1,000 mg/kg diet antibacterial peptide. NC (diet without antimicrobials) was considered to be 0 mg/kg diet antibacterial peptide. Data are presented as mean ± SD.

\(^{2}\)Each number represents the mean of 6 piglets.

![Figure 2](image-url) Scattergram of apoptotic splenocytes at 53 d of age. Cells stained positively for Annexin V-FITC and negatively for propidium iodide (PI) staining solution demonstrate apoptosis. Cells stained positively for both Annexin V-FITC and PI are either at the end stage of apoptosis and undergoing necrosis or already dead. The quadrantal diagrams show that the percentages of cells are lower in samples with (b) 250 and (c) 1,000 mg/kg antibacterial peptide than in (a) the negative control.
Table 5. Changes in peripheral blood T cell subsets in piglets\(^1,2\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Day</th>
<th>PC (0 (NC))</th>
<th>250</th>
<th>500</th>
<th>1,000</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD(4^+)/CD(8^+)</td>
<td>32</td>
<td>0.72 ± 0.04(\text{bc})</td>
<td>0.65 ± 0.04(\text{d})</td>
<td>0.69 ± 0.03(\text{e})</td>
<td>0.75 ± 0.03(\text{b})</td>
<td>0.77 ± 0.03(\text{a})</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>0.78 ± 0.02(\text{b})</td>
<td>0.64 ± 0.02(\text{d})</td>
<td>0.74 ± 0.03(\text{c})</td>
<td>0.81 ± 0.04(\text{e})</td>
<td>0.84 ± 0.03(\text{a})</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>0.75 ± 0.02(\text{a})</td>
<td>0.62 ± 0.03(\text{b})</td>
<td>0.76 ± 0.05(\text{a})</td>
<td>0.77 ± 0.04(\text{a})</td>
<td>0.79 ± 0.04(\text{a})</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>0.73 ± 0.02(\text{b})</td>
<td>0.64 ± 0.02(\text{c})</td>
<td>0.75 ± 0.03(\text{a})</td>
<td>0.76 ± 0.04(\text{a})</td>
<td>0.75 ± 0.03(\text{a})</td>
</tr>
</tbody>
</table>

\(a\)–\(d\)Means in the same row with different superscripts differ \((P < 0.05)\).

\(^1\)Dietary treatments: NC = negative control (basal diet without any antimicrobials), PC = positive control (basal diet mixed with 400 mg/kg Astragalus polysaccharide), and antibacterial peptide (ABP) = basal diet supplemented with 250, 500, or 1,000 mg/kg diet antibacterial peptide. NC (diet without antimicrobials) was considered to be 0 mg/kg diet antibacterial peptide. Data are presented as mean ± SD.

\(^2\)Each number represents the mean of 6 piglets.

The individual’s immune system. In our study, the CD\(4^+\)/CD\(8^+\) ratio increased after ABP and AP intake; ABP consistently showed better effects than AP. In line with the results of the present study, Qiu et al. (2007) reported the positive effects of dietary supplementation with 2 mg/mL AP on CD\(4^+\)/CD\(8^+\) ratios in chickens. Geng et al. (2011) also reported that mice gavaged fed a daily dosage of 0.2 mL of 15.0 g/L ABP isolated from Yingtaogu duck blood showed markedly improved \((P < 0.05)\) CD\(4^+\)/CD\(8^+\) ratios. The SI of peripheral blood T cells under ABP sufficiency also markedly increased over the entire experiment period. These results indicate that ABP sufficiency could affect T cell proliferation, relative proportions of T cells subsets, and the immune function. The increase in T cell proliferation response shows that ABP sufficiency may affect T cells by promoting the production of the proteins responsible for proliferation, such as IL-2.

Piglets given the 1,000 mg/kg APB diet showed lower \((P < 0.05)\) G\(_{0}/\)G\(_1\) phases, G\(_2\) + M phases, S phase cell distributions, PI, and percentages of apoptotic cells in the spleen than piglets given the 250 and 500 mg/kg APB diets on d 53. These results indicate that the addition of a large dose (1,000 mg/kg) of ABP for 4 wk may inhibit lymphocyte proliferation in the spleen and may enhance the percentage of apoptotic spleen cells. Thus, we suggest optimum dosages of 500 mg/kg ABP for 4 wk and 1000 mg/kg ABP for 2 wk.

This study showed that ABP sufficiency could increase the T cell population as well as the proliferation function of T cells and could induce decreases in the percentage of apoptotic cells. The cellular immune function was evidently improved in weaned piglets. We suggested that the optimum supplement dosage is 500 mg/kg ABP for 4 wk or 1,000 mg/kg ABP for 2 wk.

LITERATURE CITED


