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Biotin affects the immune response of piglets inoculated with porcine circovirus type 2

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Abstract: Weanling crossbred pigs were used in a 5-week trial to evaluate the effects of biotin and porcine circovirus type 2 (PCV2) challenge on serum cytokine concentrations and humoral immunoresponse. Although not significant, there was a trend towards reduced interferon-γ concentration in the challenged pigs compared to the controls, especially at 14 and 21 days postinoculation (dpi). Biotin supplementation with 50 or 200 μg/kg biotin improved these levels, with higher doses producing an earlier and stronger response. Although the magnitude of the immune response was small and inconsistent, diet supplementation with 50 or 200 μg/kg biotin appeared to increase serum immunoglobulin G levels. We conclude that PCV2 inoculation depressed, while biotin supplementation increased, the immune response of weanling piglets.

Key words: Weaned pigs, PCV2, biotin, immunity

Introduction

Porcine circovirus type 2 (PCV2) is associated with a number of porcine diseases, the most notable of which is postweaning multisystemic wasting syndrome (PMWS). This disease of weaner and grower pigs is typically seen in 5- to 12-week-old animals, although older pigs may also be affected. Pathologic lesions in this disease are seen most commonly in the lymph nodes, although they may also be found in other lymphoid tissues or organs, including the lungs, kidneys, or liver. The morbidity and mortality associated with PMWS varies depending on the stage of the outbreak and its management within affected units; environmental factors, such as drafts, overcrowding, poor air quality, comingling of age groups, and other stressors may exacerbate the disease severity. In China, approximately 20%-60% of PCV2-infected piglets also reportedly have PMWS (1,2), resulting in a sizable economic loss in pig production.

Immunoglobulin M (IgM) is an important receptor on mature or immature and B prolymphocyte surfaces (3). A previous study found substantially higher PCV2-IgM antibody titers in PCV2-inoculated versus control pigs, with peak concentrations at 14 to 21 days postinoculation (dpi) (4). IgG is the most

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abundant serum Ig class, constituting approximately 80% of the total serum Ig. Its primary function is to activate the complement pathway, and it can bind with macrophages, apocytes, and lymphocytes. The presence of serum IgG antibodies is at an advantage for pigs to increase growth, presumably resulting in healthier and heavier animals.

Immune cells can secrete various cytokines that bind receptors on antigen-activated lymphocytes, triggering processes that are essential for a normal immune response, including intracellular signaling cascades and cell growth, proliferation, and differentiation (5). Interferon (IFN)-γ is a cytokine produced by Th1 and natural killer (NK) cells that activates macrophages and polymorphonuclear cells and induces Th1 cell development, which is critical to cytotoxic T cell responses and IgG production (3). IFN-γ production by NK cells facilitates acute inflammation by activating the adhesive properties of endothelial cells and mediator production by mononuclear phagocytes (6). IFN-γ also mediates antigen presentation and lymphocyte proliferation/differentiation, leading to both immunosuppressive and immunostimulatory effects (3).

The soluble interleukin-2 (IL-2) receptor (sIL-2R) released during chronic T cell activation is the best characterized cytokine inhibitor, binding IL-2 to prevent its interaction with membrane-bound IL-2R. IL-2 is critical in adaptive immunity, mediating T, B, and NK cell proliferation, activation-induced cell death, and NK cell activation. The concentration of IL-2 can determine whether or not a T cell will proliferate and become an armed effector cell (5). sIL-2R is found in the bloodstream in a number of diseases, and it can be used as a clinical marker of chronic T cell activation (3).

In mammals, biotin serves as a coenzyme for 4 carboxylases, which play essential roles in the metabolism of glucose, amino acids, and fatty acids. Biotin deficiency causes decreased rates of cell proliferation (7), impaired immune function, and abnormal fetal development (8), while porcine diet supplementation with 220 or 440 μg/kg biotin can increase the immune response to sheep red blood cells. Peripheral blood mononuclear cells (PBMCs) increase their biotin uptake early in the cell cycle, during cell proliferation (9). In human PBMCs, biotin supplementation increases the expression of genes encoding IFN-γ and IL-1β and decreases the expression of IL-4-encoding genes (10). Adding biotin to a wheat-casein diet in broilers enhances their development, elevates their weight index, improves their antibody titers to Newcastle disease, and significantly enhances their blood B-lymphocyte and splenic T- and B-lymphocyte transformation ratios (11).

Herein, we evaluate the influence of supplemental biotin on the immune response of weanling pigs challenged with PCV2. Weanling piglets fed diets supplemented with different concentrations of biotin were inoculated with PCV2, and humoral immunoresponses in these animals, as well as their serum cytokine levels, were tested. Improving the immune response of young pigs to the dangerous infection could reduce the losses farmers suffer, thereby increasing the productivity of these farms.

Materials and methods

Experimental design and sample collection

The experiments presented here were conducted according to protocols approved by the Sichuan Agricultural University Animal Care and Use Committee. An animal feeding trial to determine the effect of supplemental biotin on the immune response of PCV-challenged weaning pigs was performed using 48 crossbred (Duroc × Landrace × Yorkshire) animals (initial body weight: 6.8 kg). The pigs were weaned at 28 days, and their PCV2 antibody levels were tested using a PCV2-specific enzyme-linked immunosorbent assay (ELISA). A ratio of the sample mean / (positive – negative means) (S/P) of less than 0.15 was considered negative for maternal antibodies (12).

A 2 × 3 factorial design was employed using 3 levels of biotin (0, 50, and 200 μg/kg) and 2 levels of PCV2 (unchallenged and challenged). Forty-eight piglets weaned at 28 days were divided into 6 groups of 8 pigs each (Table 1) and penned individually in 2 temperature-controlled nursery buildings (25-28 °C). The basal diets were formulated to meet or exceed National Research Council nutrient

Table 1. Experimental design.

Group	PCV2	Biotin (μg/kg)	Pigs
A–	Unchallenged	0	8
B–	Unchallenged	50	8
C–	Unchallenged	200	8
A+	Challenged	0	8
B+	Challenged	50	8
C+	Challenged	200	8

The basal diet was supplemented with 0.00 mg/kg (Groups A– and A+), 0.05 mg/kg (Groups B– and B+), or 0.20 mg/kg (Groups C– and C+) biotin. Groups marked with – or + were uninoculated or inoculated with PCV2, respectively.

requirements (13) and contained 180 μg/kg biotin. The experimental design is shown in Table 1. Dietary treatment involved the adding of 0, 50, or 200 μg biotin/kg to the basal diet. Biotin was administered from days 1 to 35 of the experiment. One room of 24 piglets, fed the 3 different diets, was inoculated with PCV2, while pigs in the other room were inoculated with sodium chloride through the nasal cavity.

All of the piglets were bled weekly by puncturing the vena cava. A blood sample was collected in a 5-mL tube and centrifuged (3500 × *g* for 5 min) to collect serum, which was stored at –20 °C until analysis for PCV2 antibodies, IgG, IgM, IFN-γ, IL-2, and sIL-2R levels. The slowest growing pig under each treatment was sacrificed and necropsied at 7, 14, 21, 28, and 35 dpi.

Viral challenge

On day 1 of the experiment, the pigs of the challenged groups were inoculated with PCV2-infected PK-15 cell lysates [1 mL of a PCV2 pool containing $1.0 \times 10^{5\text{-}6}$ tissue culture infective dose 50 ($TCID_{50}$)/mL] through the nasal cavity. Unchallenged pigs were mock-inoculated with 1 mL of a saline solution. Biosecurity measures were implemented to prevent contamination of the uninfected room with PCV2.

PCV2-infected PK-15 cell lysates were a gift from Dr Zhiwen Xu (Animal Biotechnology Center, Sichuan Agricultural University, P.R. China).

Assays of indexes

Tissues samples were fixed by immersion in 10% neutral buffered formalin immediately after collection. Fixed tissues were processed, embedded, and sectioned following routine procedure histopathological examination and then stained with hematoxylin and eosin.

To measure the humoral immune response in the pigs, PCV2 serum antibody levels were assayed by ELISA using an immunoassay kit (Beijing Anheal Laboratories, China), the optical density values of the PCV2 serum antibody levels were read, and the S/P ratio was calculated. Serum samples were also tested for IgM and IgG isotype-specific antibodies by immunoturbidimetry using an IgG/IgM assay kit (Sichuan Maker Science Technology, China).

The levels of serum IFN-γ, IL-2, and sIL-2R were determined by ELISA using an immunoassay kit with a goat antipig coating antibody (RapidBio Lab, USA). The sensitivities of these assays were 1.0 pg/mL, 10 pg/mL, and 10 pg/mL, respectively.

Statistical analyses

Data were analyzed using a general linear model function in SPSS 13.0. The biotin level, presence/absence of PCV2 challenge, and 2-way interactions were included in all of the analyses. B, V, and B&V represent the P-values of the main effects of the biotin, PCV2 inoculation, and the interaction between the PCV2 inoculation and the biotin treatment, respectively. Statistical significance was determined using Duncan's multiple range test with $P < 0.05$ considered as significant. Data are presented as mean ± standard error of the mean (SEM).

Results

Pathological evaluation

Gross and histopathological lesions, typical of PMWS, were seen in this experiment (see Figures 1A-1F). The results of the postmortem examinations revealed that there were gross pathological changes in samples from the lung, spleen, thymus, kidney, inguinal lymph nodes, and mandibular lymph node in the challenged pigs. In contrast, no visible or mild lesions were observed in the unchallenged pigs. More severe changes (especially in the lung, spleen, stomach, and mesenteric lymph nodes) were seen in A6+, B5+, and C2+ pigs, dead at 12, 8, and 9 dpi in the A+, B+, and C+ group, respectively. Pathological slides were not obtained from several of the pigs in groups C– and C+, but were obtained from all of the pigs in groups A–, A+, B–, and B+ (Table 2).

The pathological assays revealed mild, moderate, and severe pathological changes (see Table 2 and Figure 1). These changes included histiocytic infiltration of the lymphoid tissues, suppurative bronchopneumonia and interstitial pneumonia, interstitial nephritis, histiocytic infiltration of the lymphoid sheaths in the spleen and in the inguinal lymph nodes, as well as periportal inflammation in the liver, with obvious necrosis of the hepatocytes.

Humoral immune response

The PCV2 serum antibody titers of the pigs were taken at different time points (Table 3). All of the pigs tested negative for the PCV2-specific antibody on the day of inoculation, and the unchallenged pigs remained negative throughout the entire experiment. The PCV2 antibody titers of the challenged pigs increased from 14 dpi, with all of the challenged piglets being PCV2-positive by 21 dpi. This confirmed that the challenged pigs were infected with PCV2 after inoculation. The antibody titer of the challenged groups peaked at 4-5 weeks postinoculation.

We also tested serum immunoglobulin levels (Table 4). Serum IgM levels in the challenged pigs peaked first at 7 dpi for animals fed diets supplemented with 200 or 50 μg/kg biotin. This first peak was delayed by 1 week in the challenged pigs fed nonsupplemented diets. The serum IgM levels of the challenged groups were markedly reduced at 21 dpi compared with those of the unchallenged groups.

Serum IgG concentrations were markedly depressed at 7, 14, 21, and 35 dpi for pigs challenged with PCV2 (Table 4), and the level of IgG was greater at 7 and 14 dpi for pigs supplemented with 50 or 200 μg/kg biotin than for the nonsupplemented pigs. Serum IgG concentrations were the highest for pigs fed 200 μg/kg biotin, particularly at 35 dpi.

Cytokine response

The serum IFN-γ levels of the challenged pigs were significantly decreased at 14 and 21 dpi, and were greater for the 200 μg/kg biotin groups than for the 50 or 0 μg/kg biotin groups at 7 dpi (Table 5). This concentration was also higher at 21 dpi for pigs fed any biotin compared to the 0 μg/kg group. The virus × biotin interaction was significant for the serum IFN-γ concentration at 7 dpi, with higher IFN-γ

Table 2. Statistical analysis of the number of pathological slides obtained during the study.

Group	Numbers of killed[#] piglets	Numbers of dead[†] piglets	Total sets of slides	Numbers of pathological slides (set)	Ratio of pathological to normal slides
A–	5	0	5	2	40
B–	5	0	5	2	40
C–	5	0	5	1	20
A+	5	1	6 (killed and dead)	6	100
B+	5	1	6 (killed and dead)	6	100
C+	5	1	6 (killed and dead)	3	50

Each set of slides for a pig contained slices of the ileum, lung, spleen, thymus, and inguinal lymph nodes. Killed[#] piglets refers to those that did not die during the course of the experiment but were killed at the end of the study. Dead[†] piglets refers to those that died through the course of the experiment, presumably as a result of inoculation.

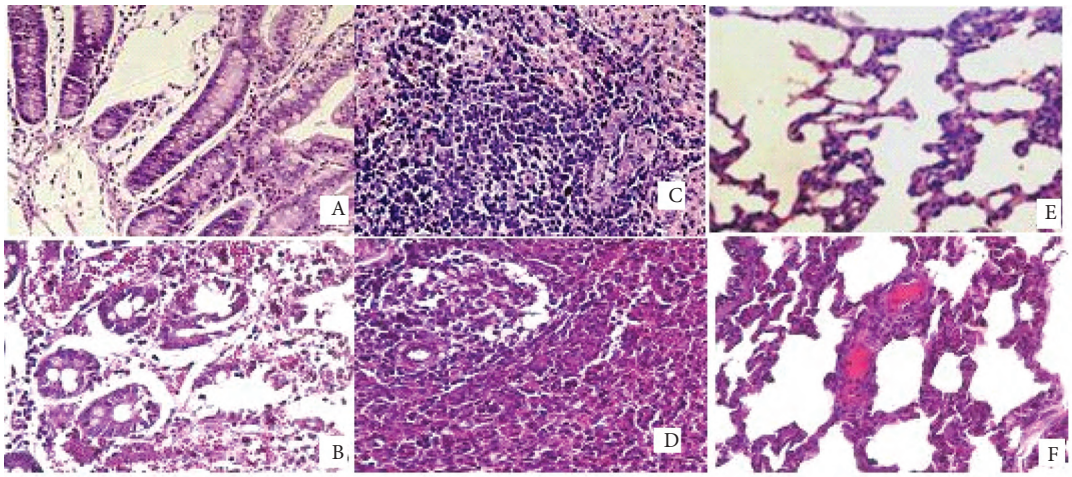

Figure 1. Pathological changes in pigs inoculated with PCV2 (400×). A, C, and E: normal ileum, inguinal lymph nodes, and lung tissue slices; B, D, and F: abnormal ileum, inguinal lymph nodes, and lung tissue slices; B: necrosis, denaturation of the intestinal epithelium cells and intestinal gland cells, exposed lamina propria, edema of the submucous membrane, rarefaction between muscles, and an increased number of plasmocytes in the interstitium; D: noticeable reduction and sparseness of lymphocytes in the white pulp and hyperemia in the red pulp; F: thickening of the alveolar wall and inflammatory cell infiltrates in the lung, hyperemia, hemorrhage, interstitial pneumonia, and compensating emphysema.

levels in the inoculated pigs fed 200 μg/kg biotin than in the inoculated pigs fed lower concentrations.

No significant differences were observed in the IL-2 or sIL-2R concentrations in any of the groups at any time during the study period.

Discussion

Here, we used weanling pigs to evaluate the effect of biotin on the immunoresponse of PCV2-infected pigs. IFN-γ concentrations, but not IL-2 or sIL-2R levels, were reduced in the challenged pigs after PCV2 inoculation, especially at 14 and 21 dpi. Diet supplementation with low or high doses of biotin improved these levels, with higher doses producing an earlier and stronger response. Although the magnitude of the immune response was small and inconsistent, diet supplementation with 50 or 200 μg/kg biotin increased some indexes of the humoral and cellular immune responses.

Table 3. Effects of biotin supplementation on PCV2 antibody titer (S/P).

PCV2	Non-PCV2-inoculated				PCV2-inoculated				SEM	P-value		
	n	0.00 μg/kg	50 μg/kg	200 μg/kg	n	0.00 μg/kg	50 μg/kg	200 μg/kg		B	V	B × V
0 dpi	8	0.055 ± 0.02	0.047 ± 0.01	0.045 ± 0.02	8	0.077 ± 0.02	0.071 ± 0.02	0.060 ± 0.01	0.007	0.73	0.14	0.97
7 dpi	8	0.048 ± 0.01	0.051 ± 0.01	0.061 ± 0.01	7	0.077 ± 0.02	0.107 ± 0.05	0.048 ± 0.01	0.065	0.511	0.17	0.27
14 dpi	7	0.069 ± 0.01^{C}	0.084 ± 0.01^{BC}	0.074 ± 0.01^{C}	6	0.115 ± 0.02^{ABC}	0.149 ± 0.03^{A}	0.132 ± 0.32^{AB}	0.007	0.37	0.01	0.87
21 dpi	6	0.083 ± 0.01^{B}	0.102 ± 0.01^{B}	0.097 ± 0.02^{B}	5	0.158 ± 0.03^{B}	0.169 ± 0.02^{B}	0.295 ± 0.08^{A}	0.013	0.06	0.01	0.09
28 dpi	5	0.088 ± 0.01^{B}	0.111 ± 0.01^{B}	0.110 ± 0.02^{B}	4	0.252 ± 0.05^{A}	0.243 ± 0.06^{A}	0.334 ± 0.05^{A}	0.014	0.30	0.01	0.43
35 dpi	4	0.092 ± 0.01^{B}	0.104 ± 0.01^{B}	0.120 ± 0.01^{B}	3	0.318 ± 0.08^{A}	0.361 ± 0.05^{A}	0.289 ± 0.01^{A}	0.013	0.64	0.01	0.42

The P-values represent the main effect of biotin (B), PCV2 inoculation (V), or the interaction between the PCV2 inoculation and the biotin treatment (B × V). Means on the same line without a common lowercase superscript differ at $P < 0.05$. Those without a common uppercase superscript differ significantly at $P < 0.01$. The same conventions apply for Tables 4 and 5.

Table 4. Effects of biotin supplementation on piglet Ig levels.

IgM (mg/mL)	Non-PCV2-inoculated				PCV2-inoculated				SEM	P-value		
	n	0.00 μg/kg	50 μg/kg	200 μg/kg	n	0.00 μg/kg	50 μg/kg	200 μg/kg		B	V	B × V
0 dpi	8	0.128 ± 0.014	0.134 ± 0.015	0.137 ± 0.016	8	0.118 ± 0.016	0.112 ± 0.012	0.124 ± 0.014	0.01	0.83	0.23	0.92
7 dpi	8	0.122 ± 0.010	0.121 ± 0.007	0.116 ± 0.013	7	0.122 ± 0.015	0.148 ± 0.018	0.142 ± 0.026	0.01	0.72	0.20	0.64
14 dpi	7	0.120 ± 0.011	0.097 ± 0.011	0.122 ± 0.013	6	0.125 ± 0.020	0.106 ± 0.009	0.125 ± 0.015	0.01	0.18	0.59	0.97
21 dpi	6	0.105 ± 0.008^{BC}	0.156 ± 0.025^{A}	0.136 ± 0.011^{AB}	5	0.095 ± 0.013^{BC}	0.085 ± 0.011^{C}	0.106 ± 0.015^{BC}	0.01	0.25	0.01	0.12
28 dpi	5	0.115 ± 0.007	0.110 ± 0.007	0.111 ± 0.010	4	0.114 ± 0.024	0.128 ± 0.008	0.115 ± 0.013	0.01	0.86	0.43	0.69
35 dpi	4	0.155 ± 0.041	0.126 ± 0.002	0.126 ± 0.018	3	0.125 ± 0.026	0.129 ± 0.016	0.107 ± 0.016	0.01	0.60	0.43	0.77
IgG (mg/mL)												
0 dpi	8	0.989 ± 0.043	1.003 ± 0.036	1.026 ± 0.044	8	0.953 ± 0.038	1.066 ± 0.041	1.073 ± 0.0538	0.02	0.16	0.48	0.47
7 dpi	8	0.895 ± 0.022^{B}	0.962 ± 0.024^{AB}	0.990 ± 0.032^{A}	7	0.598 ± 0.016^{D}	0.716 ± 0.050^{C}	0.632 ± 0.031^{DC}	0.01	0.02	0.01	0.21
14 dpi	7	0.701 ± 0.029^{AB}	0.744 ± 0.032^{A}	0.759 ± 0.024^{A}	6	0.532 ± 0.038^{C}	0.636 ± 0.016^{B}	0.682 ± 0.024^{AB}	0.01	0.01	0.01	0.26
21 dpi	6	0.973 ± 0.052^{AB}	1.072 ± 0.055^{A}	0.943 ± 0.026^{B}	5	0.594 ± 0.029^{C}	0.639 ± 0.020^{C}	0.589 ± 0.035^{C}	0.02	0.08	0.01	0.61
28 dpi	5	0.862 ± 0.039	0.887 ± 0.036	0.976 ± 0.017	4	0.855 ± 0.058	0.867 ± 0.045	0.892 ± 0.019	0.02	0.14	0.24	0.55
35 dpi	4	0.986 ± 0.061^{A}	0.906 ± 0.039^{A}	0.999 ± 0.029^{A}	3	0.609 ± 0.040^{B}	0.689 ± 0.036^{B}	0.886 ± 0.098^{A}	0.02	0.02	0.01	0.08

We obtained slightly fewer slides of lymphatic organ pathological lesions from pigs in the 0.20 mg/kg biotin supplementation group than from pigs in the 0 or 0.05 mg/kg biotin supplementation groups.

Seroconversion to PCV2 emerged in our study at 14 dpi, consistent with the time frame of PCV2 seroconversion in previous reports of weanling or colostrum-deprived piglets (14-19). These previous studies reveal that numerous factors influence PCV2 antibody production, including the pig model used (i.e. conventional, gnotobiotic, caesarean-delivered, or colostrum-deprived), the PCV2 strain or inoculum used (i.e. infected tissue homogenate or cell culture fluid), the inoculation route (i.e. nasal aspiration or subcutaneous or abdominal cavity injection), viral dosage, rearing management of the pigs, environmental conditions, the animal's age, and so forth. However, all of the available research has indicated that serum PCV2 antibodies emerge at 14-21 dpi and increase stably for about 3 weeks thereafter.

IgM is an early antibody and its concentration in pigs was higher at 7 dpi than at 0 dpi, with an upward trend in concentration indicating that PCV2 inoculation induced a first-time humoral immunoresponse in the infected pigs. However, this immune response was weak; no significant differences were observed between the challenged and unchallenged groups at 7 dpi. Since there was no clear association between biotin dose and IgM levels in our experiments, we conclude that biotin doses do not affect IgM levels.

Previous studies have indicated that serum Ig is consumed by PCV2 infection, thereby suppressing the humoral immune response (6,19,20). Consistent with this, the serum IgG concentrations of the challenged pigs in our study decreased until 28 dpi (Table 4). The challenged pigs experienced 1 peak in IgG levels, while the unchallenged groups experienced 2. Compared with the unchallenged groups, the first peak of serum IgG in the challenged animals was delayed for 1 week in the biotin-supplemented groups and was delayed for 2 weeks in the nonsupplemented pigs. These results, together with Table 6, suggest that supplementation with biotin improved the immune response of the challenged animals at 28 dpi. If the

Table 5. Effects of biotin supplementation on levels of serum cytokine.

IFN-γ (pg/mL)	Non-PCV2-inoculated				PCV2-inoculated				SEM	P-value		
	n	0.00 μg/kg	50 μg/kg	200 μg/kg	n	0.00 μg/kg	50 μg/kg	200 μg/kg		B	V	B × V
0 dpi	8	60.06 ± 12.36	62.81 ± 7.20	73.81 ± 12.90	8	81.45 ± 23.63	91.20 ± 12.52	85.69 ± 20.27	6.28	0.85	0.11	0.86
7 dpi	8	73.38 ± 10.35^{ABC}	79.65 ± 9.02^{AB}	65.52 ± 5.73^{CD}	7	35.41 ± 10.32^{CD}	35.29 ± 4.96^{D}	102.46 ± 20.27^{A}	4.61	0.02	0.11	0.01
14 dpi	7	110.29 ± 18.49^{A}	80.129 ± 3.22^{AB}	77.479 ± 3.99^{B}	6	58.12 ± 12.72^{B}	60.23 ± 10.54^{B}	59.87 ± 4.80^{B}	4.36	0.29	0.01	0.21
21 dpi	6	66.54 ± 5.20^{BC}	96.06 ± 11.13^{A}	81.21 ± 7.40^{AB}	5	46.04 ± 11.77^{C}	60.04 ± 9.46^{BC}	77.54 ± 8.02^{AB}	3.69	0.03	0.01	0.22
28 dpi	5	60.15 ± 9.01	121.60 ± 30.61	149.14 ± 96.75	4	208.74 ± 92.41	188.37 ± 62.55	111.06 ± 35.55	25.84	0.92	0.27	0.35
35 dpi	4	167.66 ± 35.76	224.87 ± 33.03	189.87 ± 38.84	3	91.12 ± 21.27	232.37 ± 17.31	203. 41 ± 59.91	18.45	0.60	0.60	0.99

The P-values represent the main effect of biotin (B), PCV2 inoculation (V), or PCV2 interaction between the inoculation and the biotin treatment (B × V).

biotin was given prior to the PCV2 challenge, we suspect that the immune response of the challenged pigs would be better than that of the pigs receiving biotin and PCV2 simultaneously.

Prior infection of cell cultures with PCV2 significantly reduces IL-2 or IFN-γ release after stimulation with phytohemagglutinin or staphylococcal enterotoxin B, regardless of whether the cells are from PMWS or healthy pigs (21). The IFN-γ mRNA levels in the PBMCs of PMWS or healthy piglets significantly decrease after PCV2 inoculation, as do levels in the inguinal or bronchial lymph nodes, spleens, and tonsils (22). Moreover, IFN-γ added to the culture medium of porcine kidney or monocytic cells before, during, or after inoculation increases the number of PCV2 antigen-positive cells in a dose-dependent manner that can be blocked with IFN-γ-neutralizing antibodies (23). Although previous cell culture studies have shown that PCV2 replication is enhanced with increased IFN-γ levels, the immune response can differ significantly due to individual differences in immune status, nutritional status, and other factors. In this study, compared to the unchallenged groups, the serum IFN-γ concentrations of the challenged pigs (except for the 200 μg/kg biotin group) were reduced following PCV2 inoculation, particularly at 7 to 21 dpi. This is consistent with previous in vitro research showing that IFN-γ is inhibited after PCV2 infection (24). Furthermore, our results suggest that biotin supplementation can improve serum IFN-γ concentrations for challenged pigs, consistent with abundant research indicating that biotin has a potentially important role in lymphocyte maturation, responsiveness to stimulation, and antigenic response (10).

We have shown that PCV2 inoculation alters the concentrations of IgG and IFN-γ in weanling piglets. Furthermore, diet supplementation with biotin, particularly at 200 μg/kg, could improve lymphocyte maturation and responsiveness to stimulation, and, consequently, the capacity of the immune system to respond to an antigenic challenge. Water-soluble vitamins were excreted by the kidneys after achieving steady-state levels in cells, resulting in nondose-dependent changes between the supplemented dose and some indexes, such as IgG. The appropriate supplementation level of biotin needs to be performed.

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