

Glutamine modifies immune responses of mice infected with porcine circovirus type 2

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Abstract

The present study was conducted to evaluate the immune-enhancing effects of dietary L-glutamine supplementation in porcine circovirus type 2 (PCV2)-infected mice, and to examine the clearance effects of glutamine against PCV2 in experimentally infected mice. A total of sixty Kunming female mice were infected with PCV2 at a dose of 100 TCID₅₀ (50% tissue culture infection dose) by intraperitoneal injection after 2 weeks of dietary L-glutamine supplementation or L-alanine supplementation (as the control (isonitrogenous) group). The measured variables on 3rd, 5th, 7th, 9th and 11th d post-infection (dpi) included: (1) PCV2 virus loaded in the liver, spleen, heart, lung, kidney, ovary and serum was determined by real-time PCR; (2) IL-2, IL-6, IL-10, interferon (IFN)- α , IFN- γ and C-reactive protein levels in serum were measured by ELISA; (3) serum total superoxide dismutase activity was measured spectrophotometrically at 550 nm absorbance. Dietary L-glutamine supplementation significantly increased serum IL-2 levels on the 3rd ($P < 0.01$), 5th ($P < 0.01$), 7th ($P < 0.05$) and 9th dpi, significantly ($P < 0.05$) increased serum IL-6 levels on 3rd dpi, significantly ($P < 0.05$) increased serum IFN- γ levels on the 9th and 11th dpi and significantly decreased ($P < 0.01$) serum IL-10 levels on the 9th and 11th dpi, compared with those in the control group. Meanwhile, the PCV2 virus genome was detected sporadically throughout the experimental period in both groups. Taken together, the present results suggest that dietary L-glutamine supplementation enhances immune function in PCV2-infected mice.

Key words: L-Glutamine: Porcine circovirus type 2: Amino acids: Antioxidation: Immune function

The Circoviridae family are small, icosahedral, non-enveloped viruses with single-stranded, circular DNA genomes. There are two genera (*Gyrovirus* and *Circovirus*) in the family of Circoviridae that are characterised according to their genome sizes and differences in genome organisation. Porcine circovirus types 1 and 2 (PCV1 and PCV2, respectively) belong to the *Circovirus* genus^(1–3). PCV1 is non-pathogenic and has not been reported to associate with naturally occurring diseases, while PCV2 has been suggested as the essential pathogen for the post-weaning multi-systemic wasting syndrome (PMWS)^(4,5) and porcine circovirus-associated diseases or porcine circovirus diseases^(6,7). The compelling pathological finding is that PCV2 infection damages macrophages,

antigen-presenting cells and other immune-related cells⁽⁸⁾. So the PMWS is considered as an immunosuppressive disease, and the interaction between PCV2 and the immune system is suggested as a determining factor in the pathogenesis of the PMWS⁽⁹⁾.

Glutamine, an immunonutrient and modulator, plays a role in the interaction between the carbon metabolism of carbohydrates and proteins, and has also been found to play an important role in the development of fibroblasts, lymphocytes and enterocytes^(10–12). A large body of evidence shows that glutamine has various beneficial effects on immune and intestinal functions. For example, it was found that glutamine functions as a major energy substrate for cells of the immune

Abbreviations: CRP, C-reactive protein; dpi, days post-infection; IFN, interferon; PCV, porcine circovirus; PMWS, post-weaning multi-systemic wasting syndrome; SOD, superoxide dismutase; TCID₅₀, 50% tissue culture infection dose.

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system^(13–15) and that the provision of glutamine enhances the immunity of the host^(16–19). Moreover, research demonstrated that the ability of lymphocytes to respond to mitogenic stimulation is impaired after failure to supplement culture media with glutamine. Further studies indicated that glutamine is required in terminally differentiated macrophages for the synthesis of mRNA for producing secretory proteins in immune challenge during pinocytosis or phagocytosis⁽²⁰⁾. Other compelling evidence for the physiological functions of glutamine is its relationship with intracellular redox status and oxidative stress. Collectively, accumulating evidence suggests that glutamine plays important roles in promoting immune functions and preventing diseases, especially sub-clinical and immunosuppressive diseases.

The aims of the present study were to evaluate the immune-enhancing effects of dietary L-glutamine supplementation in PCV2-infected mice, and to investigate the potential of clearing PCV2 in experimentally infected mice.

Materials and methods

Animals and feeding

A total of sixty Kunming female mice (body weight 18–22 g) were obtained from the Laboratory Animal Center of Central South University, Hunan, China⁽²¹⁾. Mice were randomly assigned to two treatment groups after 3 d of adaptive feeding: glutamine group (1.0% glutamine + basal diet, *n* 30) and control group (1.22% alanine + basal diet, *n* 30). Glutamine and alanine were purchased from Beijing Chemclin Biotech⁽²²⁾. Amino acid content in the basal diet was measured using an Automatic Amino Acid Analyzer (L-8900; Hitachi)⁽²³⁾. This basal diet contained 1.94% L-glutamate, 1.80% L-glutamine and 0.91% L-alanine. Mice were housed in an environmentally controlled pathogen-free condition. All animals were fed *ad libitum*. The study was carried out in full compliance with the guidelines for animal welfare and was approved by the Animal Care and Use Committee of the Chinese Academy of Sciences (registry no. 011063506)⁽²⁴⁾.

Preparation of porcine circovirus type 2 stock

A PCV2 infectious clone constructed by self-ligation of the PCV2 genome via the *SacII* enzyme site was used to generate the virus stock pools required for experimental infections. Briefly, the continuous porcine kidney cell line PK-15 (gift from Professor Yang, China Agricultural University)⁽²⁵⁾, free of PCV1 and PCV2, was cultured in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 6% (v/v) fetal calf serum. The cell monolayer was dispersed by treating the cells with trypsin-EDTA, and suspended in RPMI-1640 medium supplemented with 6% (v/v) fetal calf serum while cells were infected with the PCV2 infectious clone. After 72 h of incubation, the infected cells were frozen and thawed thrice, and the cell mixture was tested by PCR before storing them at -20°C . PCV2 stocks were titrated on PK-15 cells (gift from Professor Yang, China Agricultural University)⁽²⁵⁾.

Experimental design

Mice were infected with PCV2 at a dose of 100 TCID₅₀ (50% tissue culture infection dose) by intraperitoneal injection after 2 weeks of feeding L-glutamine. On 3rd, 5th, 7th, 9th and 11th d post-infection (dpi), six mice from each group were killed to collect liver, spleen, heart, lung, kidney and ovary, and serum was prepared from blood samples collected from the orbital vein. All the samples were stored at -80°C ^(26,27).

Serum cytokine detection

Serum levels of IL-2, IL-6, IL-10, interferon (IFN)- α , IFN- γ and C-reactive protein (CRP) were measured using ELISA kits in accordance with the manufacturer's instructions (Cusabio Biotech Company Limited)^(28,29). Supplied diluent buffer in the kits was used to dilute standards and serum samples. Next, 100 μl of the sample or standard in duplicate were added to the wells of a microtitre plate pre-coated with antibody. Diluent buffer was used as a negative control. The plate was incubated for 2 h at 37°C . After incubation, 100 μl of biotin antibody were added to each well after removing the liquid from each well and incubated for 1 h at 37°C . The wells were washed thrice with 200 μl volumes of wash buffer. Next, 100 μl horseradish peroxidase-avidin were added to each well for 1 h at 37°C ⁽³⁰⁾. After a final wash, 90 μl of the supplied chromogen were added and incubated for 30 min in the dark at 37°C . The reaction was stopped with 50 μl of the supplied stop solution and absorbance was measured at 450 nm with a spectrophotometer.

DNA extraction and porcine circovirus type 2 quantitative PCR

DNA from samples (liver, 10 mg; spleen, 5 mg; heart, 10 mg; lung, 10 mg; kidney, 10 mg; ovary, 10 mg) was extracted using Tissue Genomic DNA Extraction Kits (Betimes Biotechnology Company, Limited) according to the manufacturer's instructions. DNA from the samples was eluted with 80 μl of elution buffer and stored at -20°C . PCV2 in the extracted DNA was quantified using real-time PCR. Before quantification of the PCV2 genome in the collected samples, a PCV2 real-time PCR standard was established. Briefly, the PCV2 genome was cloned in the pMD[®]18-T Vector (TaKaRa) after PCR amplification with primers – forward 5'-CCGCGGGCTG-GCTGAACCTTTTAAAAG-3' and reverse 5'-CCGCGGAAATTC-TGACAAACGTTAC-3' (GenBank accession no. EU095020) – and transformed in TOP10 competent cells (Invitrogen). The plasmid was prepared using a PureLink[™] HiPure Plasmid Midiprep Kit (Invitrogen). The PCV2 plasmid was mixed with mouse DNA extracted from a PCV2 PCR-negative blood sample. Dilutions (10-fold) of this mixture (from 10^{11} to 10^2 PCV2 copy numbers/ μl) were used as a standard for PCV2 quantification. The PCR was performed using a SYBR Green detection kit (Takara), containing MgCl₂, deoxyribonucleoside triphosphate (dNTP) and HotStar Taq polymerase. Then, 1 μl of the template solution was added to a total volume of

10 μ l containing 5 μ l SYBR Green mix (Takara) and 0.2 μ l each of the forward and reverse primers (10 μ M). The PCR was performed under the following conditions: (1) pre-denaturation (30 s at 95°C); (2) amplification and quantification, repeated forty cycles (5 s at 95°C, 34 s at 60°C); (3) melting (60–99°C at a heating rate of 0.1°C/s)⁽³¹⁾.

Total superoxide dismutase activity detection

Total superoxide dismutase (SOD) activity in serum was determined using spectrophotometric kits at 550 nm (Nanjing Jiancheng Biotechnology Institute) according to the manufacturer's instructions. The results were expressed as units/ml serum.

Statistical analysis

All statistical analyses were performed using SPSS 16.0 software (SPSS, Inc.). Group comparisons were performed using Student's *t* test, and data from different time points within the glutamine group were performed using the Student–Newman–Keuls method. Data of the detection rate in each tissue were analysed by Fisher's exact test. Differences were considered significant at *P*<0.05. Data are expressed as means with their standard errors of the mean⁽³²⁾.

Results

In the present study, a series of cytokines related to the pathogenesis of the PMWS were measured to evaluate the immune-enhancing effects of glutamine supplementation. Meanwhile, the total SOD activity in serum was also analysed to profile the redox status in the body. The results showed that dietary L-glutamine supplementation significantly increased serum IL-2 levels on the 3rd (*P*<0.01), 5th (*P*<0.01), 7th (*P*<0.05) and 9th dpi (*P*<0.05), while IL-2 levels on the 7th dpi were much higher (*P*<0.05) than those on the 5th and 11th dpi in the glutamine group (Fig. 1). Serum IL-6 levels in the glutamine group were also significantly (*P*<0.05) higher than those in the control group on the 3rd dpi, while IL-6 levels on the

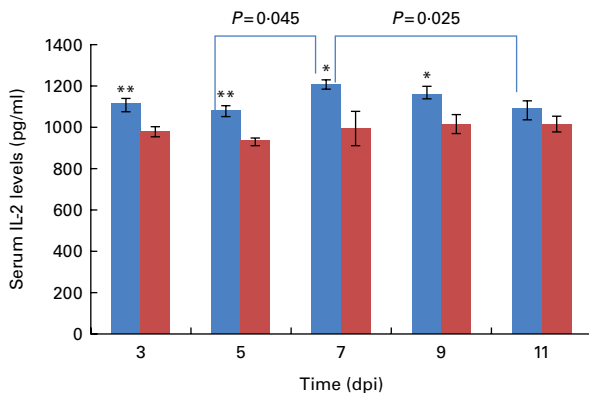


Fig. 1. Serum IL-2 levels in the glutamine (■) and alanine (■) groups. Mice in the glutamine group were fed with the 1.0% glutamine + basal diet, while mice in the alanine group were fed with the 1.22% alanine + basal diet. dpi, Days post-infection. * *P*<0.05; ** *P*<0.01. A colour version of this figure can be found online at journals.cambridge.org/bjn

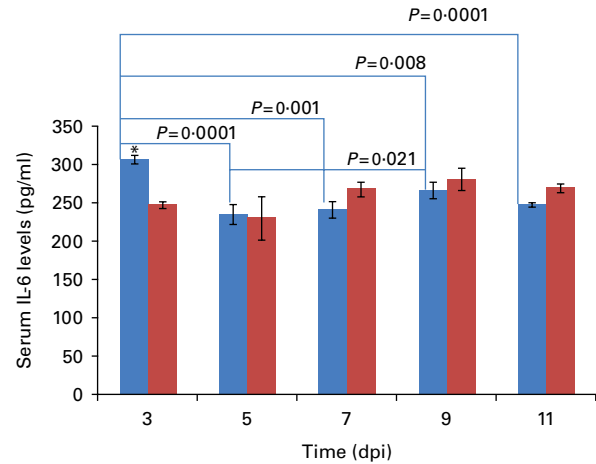


Fig. 2. Serum IL-6 levels in the glutamine (■) and alanine (■) groups. Mice in the glutamine group were fed with the 1.0% glutamine + basal diet, while mice in the alanine group were fed with the 1.22% alanine + basal diet. dpi, Days post-infection. * *P*<0.05. A colour version of this figure can be found online at journals.cambridge.org/bjn

3rd dpi were much higher (*P*<0.01) than those on the 5th, 7th, 9th and 11th dpi in the glutamine group (Fig. 2). Furthermore, dietary glutamine supplementation significantly increased (*P*<0.05) serum IFN- γ levels on the 9th and 11th dpi, while IFN- γ levels on the 9th and 11th dpi were much higher than those on the 3rd, 5th and 7th dpi in the glutamine group (Fig. 5). Serum IL-10 levels in the alanine group were much higher than those in the glutamine group on the 9th and 11th dpi. IL-10 levels on the 3rd dpi were significantly higher (*P*<0.05) than those on the 7th, 9th and 11th dpi in the glutamine group (Fig. 3). No significant difference was observed for serum IFN- α levels (Fig. 4), CRP levels (Fig. 6) and total-SOD activity (Fig. 7) between the glutamine and alanine groups, while, in the glutamine group, IFN- α levels significantly (*P*<0.05) decreased on the 5th dpi when compared with those on the 3rd, 9th and 11th dpi (Fig. 4). Meanwhile, CRP levels significantly (*P*<0.01) increased on the 9th and 11th dpi compared with those on the 3rd and 5th dpi (Fig. 6).

In the present study, to explore the clearance effects of glutamine against PCV2 in the mouse model, PCR and quantitative PCR were used to detect the PCV2 virus load in liver, spleen, heart, lung, kidney and ovary tissue, and serum on the 3rd, 5th, 7th, 9th and 11th dpi. For this purpose, first, PCV2 real-time PCR standard and the DNA template from each sample were prepared. The PCR was then used to screen the template, which showed that most of the samples were negative. Furthermore, using quantitative real-time PCR, we detected the PCV2 virus genome sporadically in liver, spleen, heart, lung, kidney and ovary tissue, and serum on the 3rd, 5th, 7th, 9th and 11th dpi (Table 1 shows the data from the 5th, 7th, 9th and 11th dpi).

Discussion

Glutamine is generally considered as a non-essential amino acid, which is synthesised mainly in the muscle from non-essential amino acids and glucose. However, in a situation

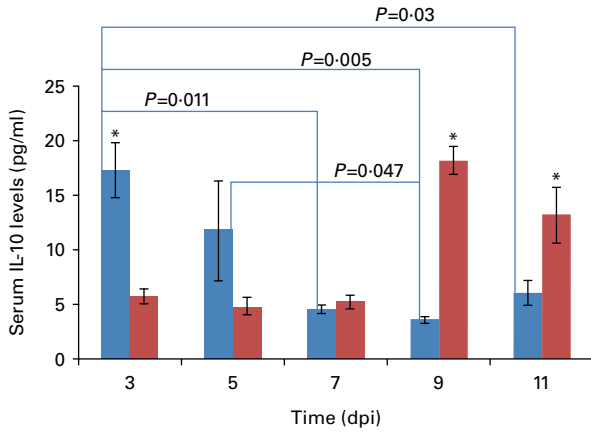


Fig. 3. Serum IL-10 levels in the glutamine (■) and alanine (■) groups. Mice in the glutamine group were fed with the 1.0% glutamine + basal diet, while mice in the alanine group were fed with the 1.22% alanine + basal diet. dpi, Days post-infection. * $P < 0.01$. A colour version of this figure can be found online at journals.cambridge.org/bjn

of abnormal muscle protein metabolism, the production of endogenous glutamine is probably impaired and becomes a conditionally essential amino acid in disease conditions such as major trauma, major surgery, sepsis, bone marrow transplantation, intense chemotherapy and radiotherapy⁽¹²⁾. So we hypothesised that glutamine content in the feed is not enough for the pig suffering from a viral or bacterial infection similar to arginine^(33,34), and dietary L-glutamine supplementation performs various beneficial effects on a PCV2-infected mouse model.

The mechanisms of immunosuppression in PCV2 infection are described by lymphocyte depletion, interfering with antigen presentation and the induction of apoptosis in the immune system for its main target cells that are considered to be monocyte/macrophage lineage cells and other antigen-presenting cells⁽³⁵⁾. Meanwhile, recent studies have suggested that lymphocyte-like cells may be important cell populations that support early PCV2 replication, whereas monocytes may be the site for PCV2 persistence in the infected host⁽³⁶⁾. So PCV2 infection could down-regulate IL-2 expression, but up-regulate other pro-inflammatory mediators such as IL-8, IL-1 and IL-6⁽³⁷⁾. Other compelling research has indicated that PCV2 infection could impair natural IFN-producing cell activity^(38,39). Thus, PCV2 infection may decrease IFN- α levels. The number of IL-10-producing cells is higher in all PMWS animals compared with control pigs not infected with PCV2⁽⁴⁰⁾. This could explain why IL-10 is increased in PCV2 infection.

IL-2 is secreted by activated T lymphocytes, especially by activated CD4⁺ T-helper cells and CD8⁺ T-helper cells⁽⁴¹⁾, and named T-cell growth factor as it stimulates T cell proliferation and differentiation^(42,43). IL-2 could promote the activity of immune cells to kill cancer cells, abnormal cells infected by virus and bacteria^(44,45). In the present study, dietary L-glutamine supplementation significantly increased serum IL-2 levels on the 3rd, 5th, 7th and 11th dpi. IL-6 plays a very complex role in biological events, including immune responses, haematopoiesis, and regulation of the endocrine and nervous systems^(46,47).

Here, we found that serum IL-6 levels in the glutamine group was significantly higher than those in the control group on the 3rd dpi. The prime function of IL-10 is to inhibit many functions of natural killer cells, T cells, and macrophage and dendritic cells, and to reduce the production of inflammatory cytokines^(48,49). There is an indication of a transient correlation between IL-10 levels and the viral load of PCV2 in pigs⁽⁵⁰⁾. In the present study, serum IL-10 levels were significantly decreased ($P < 0.01$) in the glutamine group on the 9th and 11th dpi. IFN- γ is a multifunctional protein first observed as an anti-viral activity in cultures of Sindbis virus-infected human leucocytes stimulated by phytohaemagglutinin (PHA). Later work indicated that it induces anti-viral, anti-proliferative and immunomodulatory effects on target cells⁽⁵¹⁾. A significantly higher serum IFN- γ level was observed in the glutamine group on the 9th and 11th dpi. IFN- α is a member of the type I IFN family, which is active as an anti-viral and immunomodulatory cytokine. CRP is a product of hepatocytes as a non-specific response to tissue damage. However, no significant difference in serum IFN- α and CRP levels was found between the glutamine and control groups. SOD is an important antioxidant enzyme that could increase the clearance of the superoxide radical. It has already been reported that administration of SOD is effective in decreasing tissue inflammation and injury in experimental models of ischaemia and reperfusion, chronic gut inflammation, arthritis and immune complex-induced pulmonary disease⁽⁵²⁾. Evidence indicates that glutamine supplementation increases antioxidant defence ability as glutamine up-regulates the synthesis of glutathione and antioxidative gene expression^(53,54). Unfortunately, no significant difference was observed for total-SOD activity between the glutamine and alanine groups.

Collectively, dietary 1.0% L-glutamine supplementation had a beneficial effect on the cytokine profile in the PCV2-infected mouse model. Cytokines are a large family of proteins and important players in innate and adaptive immune systems, which are produced by leucocytes and other cells. Glutamine

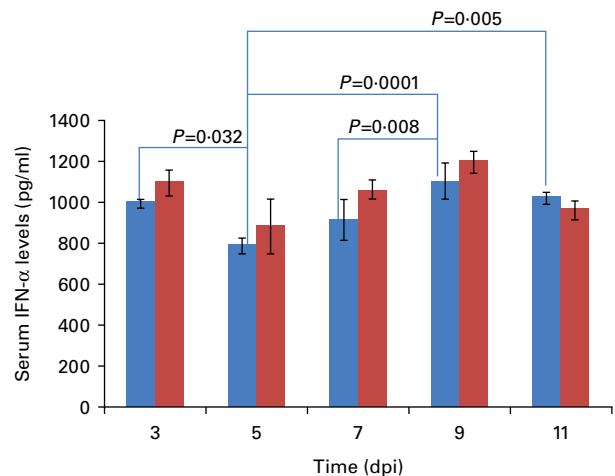


Fig. 4. Serum interferon (IFN)- α levels in the glutamine (■) and alanine (■) groups. Mice in the glutamine group were fed with the 1.0% glutamine + basal diet, while mice in the alanine group were fed with the 1.22% alanine + basal diet. dpi, Days post-infection. A colour version of this figure can be found online at journals.cambridge.org/bjn

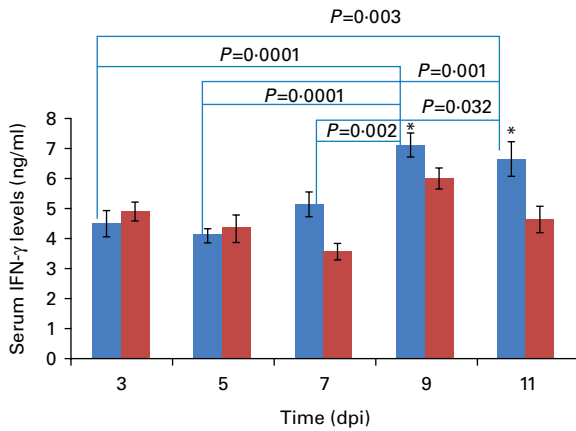


Fig. 5. Serum interferon (IFN)- γ levels in the glutamine (■) and alanine (■) groups. Mice in the glutamine group were fed with the 1.0% glutamine + basal diet, while mice in the alanine group were fed with the 1.22% alanine + basal diet. dpi, Days post-infection. * $P < 0.05$. A colour version of this figure can be found online at journals.cambridge.org/bjn

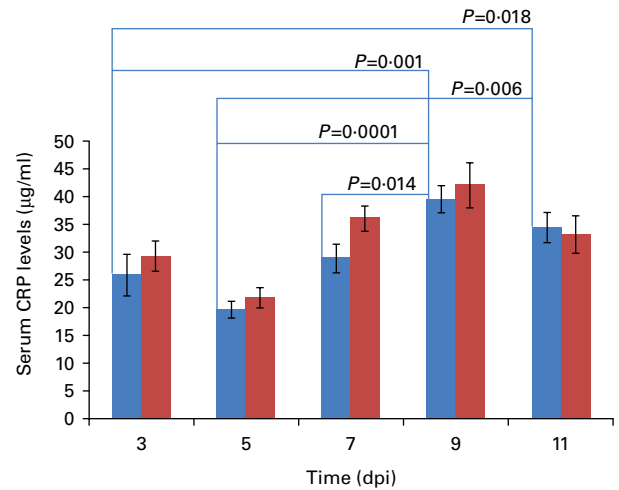


Fig. 6. Serum C-reactive protein (CRP) levels in the glutamine (■) and alanine (■) groups. Mice in the glutamine group were fed with the 1.0% glutamine + basal diet, while mice in the alanine group were fed with the 1.22% alanine + basal diet. dpi, Days post-infection. A colour version of this figure can be found online at journals.cambridge.org/bjn

displays a number of pharmacological actions and is a fuel for all rapidly differentiated and/or activated cells, including phagocytes, lymphocytes and other immune-related cells^(55,56). Thus, it is not unexpected that serum cytokine levels would not be ameliorated after glutamine supplementation. In fact, another interesting experiment showed that maximal IL-1 and TNF- α production by cultured murine macrophages, and maximal production of IL-6 and IL-8 by cultured human monocytes require a sufficient supply of glutamine⁽⁵⁷⁾. However, the other mechanisms by which glutamine regulates the cytokine profile need further study.

In the present study, to explore the clearance effects of glutamine against PCV2 in a mouse model, PCR and quantitative PCR were applied to detect the PCV2 virus load in liver, spleen, heart, lung, kidney and ovary tissue, and serum on the 3rd, 5th, 7th, 9th and 11th dpi. Unfortunately, the PCV2 virus genome was detected sporadically. In fact, this irregularity also existed in other experiments using PCV2 experimentally infected mice⁽⁵⁸⁾. Although there has been a promising progress in PCV2 research, little information is known about PCV2 performance in species other than swine. There are only a few studies about PCV2 performance in mice, but their results are mixed. Kiupel *et al.*^(59,60) showed that PCV2 replicates in 8-week-old BALB/c mice inoculated with PCV2, and PCV2 is detected by *in situ* hybridisation and PCR in mice on 7, 14, 28 and 42 dpi. In agreement with Kiupel, Li *et al.*⁽⁶¹⁾ also found that PCV2 replication, seroconversion and microscopic lesions are found in inoculated Kunming mice, and that Kunming mice could be infected by the PCV2 virus and used as a PCV2-infected experimental model. However, Quintana *et al.*⁽⁶²⁾ indicated that porcine circoviruses do not cause any disease or microscopic lesions in inoculated mice during the experimental period, and intraperitoneally inoculated mice might have harboured PCV2 in the circulation without the evidence of viral replication. Meanwhile, Opriessnig *et al.*⁽⁶³⁾ also reported that the PCV2 DNA is detected by PCR in 93% (100/108) of tissues and 42.6% (46 out of 108) of serum samples from PCV2-inoculated mice from days 12 to 37 and the mouse model probably has

limited utility to advance the understanding of the pathogenesis of PCV2-associated lesions, but mice could be potentially important in the epidemiology of PCV2. The main reason is that the virus stock (i.e. passage, origin and dose) used in these studies differed.

The speculative reasons that the PCV2 virus genome was detected sporadically are as follows. (1) Although the virus stock is different, the dose that we used was not enough. Li *et al.*⁽⁶¹⁾ inoculated mice orally and intramuscularly with 0.1 ml PCV2 (106.2 TCID₅₀/ml). (2) The time limited our observation. Bolin *et al.*⁽⁶⁴⁾ reported that PCV2 DNA could be detected by PCR from brain, lymphoid tissue, bone marrow, kidney, ileum, liver, heart, spleen, thymus, tonsil and pancreas from 20 to 28 dpi in caesarean-derived, colostrum-deprived pigs inoculated with PCV2 intranasally and subcutaneously. In fact, we found that PCV2 DNA could be detected in serum from 14 dpi in our other studies when we incubated mice with 100 TCID₅₀. (3) Amino acid supplementation in the present

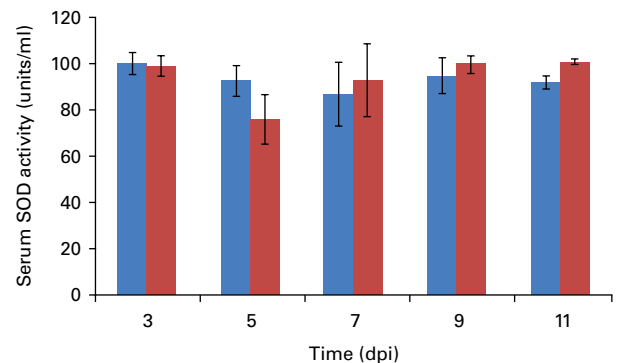


Fig. 7. Serum total-superoxide dismutase (SOD) activity in the glutamine (■) and alanine (■) groups. Mice in the glutamine group were fed with the 1.0% glutamine + basal diet, while mice in the alanine group were fed with the 1.22% alanine + basal diet. dpi, Days post-infection. A colour version of this figure can be found online at journals.cambridge.org/bjn

Table 1. Porcine circovirus type 2 (PCV2) virus load in the liver, spleen, heart, lung, kidney and ovary tissue, and serum of control and glutamine-supplemented mice (Mean values with their standard errors for PCV2 log₁₀ genomic copies/g sample or ml serum)

	5th dpi			7th dpi			9th dpi			11th dpi		
	Number of positive mice/number of tested mice	Mean	SEM	Number of positive mice/number of tested mice	Mean	SEM	Number of positive mice/number of tested mice	Mean	SEM	Number of positive mice/number of tested mice	Mean	SEM
Liver												
1.0% Gln	0/6	0.00	0.00	0/6	0.00	0.00	0/6	0.00	0.00	1/6	1.07	0.00
1.22% Ala	0/6	0.00	0.00	1/6	2.31	0.00	0/6	0.00	0.00	0/6	0.00	0.00
Spleen												
1.0% Gln	0/6	0.00	0.00	1/6	2.48	0.00	2/6	2.79	0.00	0/6	0.00	0.00
1.22% Ala	0/6	0.00	0.00	0/6	0.00	0.00	1/6	1.74	0.00	1/6	3.06	0.00
Heart												
1.0% Gln	0/6	0.00	0.00	0/6	0.00	0.00	0/6	0.00	0.00	0/6	0.00	0.00
1.22% Ala	0/6	0.00	0.00	0/6	0.00	0.00	0/6	0.00	0.00	0/6	0.00	0.00
Lung												
1.0% Gln	0/6	0.00	0.00	1/6	1.95	0.00	0/6	0.00	0.00	2/6	3.41	0.00
1.22% Ala	0/6	0.00	0.00	2/6	1.83	0.00	1/6	0.00	0.00	2/6	3.62	0.00
Kidney												
1.0% Gln	0/6	0.00	0.00	0/6	0.00	0.00	0/6	0.00	0.00	0/6	0.00	0.00
1.22% Ala	0/6	0.00	0.00	1/6	2.41	0.00	0/6	0.00	0.00	2/6	4.02	0.00
Ovary												
1.0% Gln	0/6	0.00	0.00	0/6	0.00	0.00	1/6	3.59	0.00	0/6	0.00	0.00
1.22% Ala	0/6	0.00	0.00	0/6	0.00	0.00	0/6	0.00	0.00	0/6	0.00	0.00
Serum												
1.0% Gln	2/6	2.94	0.00	0/6	0.00	0.00	1/6	3.71	0.00	0/6	0.00	0.00
1.22% Ala	1/6	2.08	0.00	0/6	0.00	0.00	0/6	0.00	0.00	2/6	6.08	0.00

dpi, Days post-infection; 1.0% Gln, 1.0% glutamine + gestation diet; 1.22% Ala, 1.22% alanine + gestation diet.

study could be sufficient to clear the virus. Questions about this phenomenon also existing in the control group of the present study will be generated. In the present study, alanine was chosen as a N control in such a model; however, research has found that alanine also had some effect on immune function⁽⁶⁵⁾. Meanwhile, alanine also plays a role in the inhibition of pyruvate kinase and hepatic autophagy, gluconeogenesis, transamination and the glucose–alanine cycle^(66,67).

In conclusion, this is the first report that dietary L-glutamine supplementation enhances immune function in PCV2-infected mice, and may clear PCV2 in experimentally infected mice; however, its effect on swine and its mechanism need further investigation.

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