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Commercial spray-dried porcine plasma does not transmit porcine circovirus type 2 in weaned pigs challenged with porcine reproductive and respiratory syndrome virus

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ABSTRACT

The objective of this study was to evaluate if spray dried porcine plasma (SDPP) containing porcine circovirus type 2 (PCV2) genome supplemented in feed could transmit PCV2 to pigs challenged with porcine reproductive and respiratory syndrome virus (PRRSV). Twenty-three PRRSV-free pigs, non-viraemic for PCV2, were housed in bio-safety level 3 facilities and assigned to four groups in a 2 × 2 factorial design consisting of PRRSV challenge and a negative control. The diet contained 0 or 8 kg SDPP per 100 kg of feed. PRRSV challenge groups were inoculated intranasally with 2 mL of a suspension containing 10⁶ TCID₅₀/mL PRRSV. The SDPP used in the study contained 7.56 × 10⁵ PCV2 genome copies per gram. Dietary treatments were fed from 4 days prior to PRRSV inoculation until 28 days post-inoculation (PI).

All challenged pigs developed PRRSV viraemia by day 3 PI and PRRSV antibodies were detected in sera by day 14 PI, with no difference between diet treatments. Neither PRRSV viraemia nor seroconversion was observed in non-challenged pigs. PCV2 was not detected in the serum of any pigs throughout the experimental period. SDPP containing the PCV2 genome supplemented in feed did not result in PCV2 transmission to either healthy or PRRSV-infected pigs under these experimental conditions.

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Introduction

Spray-dried porcine plasma (SDPP) is a feed ingredient widely used in the diets of weanling pigs to increase growth and feed efficiency (Coffey and Cromwell, 2001; Torrallardona, 2010). The commercial manufacturing process for SDPP starts with blood collected from healthy animals inspected and passed as fit for slaughter for human consumption by mandatory veterinary inspection at authorized abattoirs.

SDPP is prepared immediately after collection when the blood is separated into the plasma and cellular fractions, chilled and stored in an insulated storage tank prior to transportation to a centralized spray drying facility. Alternatively, whole blood can be chilled in the abattoir and transported to the processing plant to be centrifuged and further processed. Individual fractions are then spray-dried (Pujols et al., 2008). Commercial spray-drying conditions involve high inlet air temperature (>200 °C) and outlet air

temperature (>80 °C), as required by the European Directive (EC) 2002/99, which recommends a minimum of 80 °C heat treatment throughout its substances for meat products. Spray-drying significantly reduces the number of viable microorganisms (Lievens, 1991; Polo et al., 2002, 2005; Pujols et al., 2007).

Porcine circovirus type 2 (PCV2) is the essential infectious cause of post-weaning multisystemic wasting syndrome (PMWS), a disease that increases the morbidity and mortality of pigs and causes important economic losses to the pig industry (Segalés et al., 2005). PCV2 is very resistant to a variety of physical and chemical inactivation procedures, such as some disinfectants, organic solvents, and short-term exposure to wet heat (Royer et al., 2001; Welch et al., 2006; Martin et al., 2008). This resistance has raised the concern that PCV2 may not be completely inactivated during the spray-drying process. Pujols et al. (2008) demonstrated that commercially manufactured SDPP containing PCV2 genome did not transmit PCV2 when over-fed to healthy pigs for a period of 45 days. However, using the data from that study it was not possible to differentiate between the PCV2 in the SDPP being non-infectious and healthy immune-competent pigs being

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able to overcome the virus without infection. It is possible that SDPP containing PCV2 could be infectious in pigs affected by concurrent diseases or infections.

Porcine reproductive and respiratory syndrome (PRRS) is considered to be one of the most important diseases of the global pig industry. Importantly, PRRS virus (PRRSV) is an agent that is capable of modulating the immune response of the pig, facilitating its own persistence and transmission and increasing the susceptibility of its host to co-infections (Mateu and Díaz, 2008). The objective of the present study was to evaluate whether commercially manufactured SDPP containing the PCV2 genome (as detected by quantitative real time (qrt)-PCR, could transmit PCV2 to weanling pigs experimentally challenged with PRRSV when included in feed and drinking water.

Materials and methods

Animals

Twenty-three 3.5 week-old Landrace piglets (7.6 ± 0.2 kg bodyweight) were obtained from a high health status genetic nucleus selection farm, which was free of PRRSV and other common pathogens (Aujeszky's disease virus, *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Brachyspira hyodysenteriae* and toxigenic *Pasteurella multocida* and *Bordetella bronchiseptica*). Weanling piglets were selected for this experiment because in commercial practice SDPP is used primarily in feed during the initial 2–4 week period after weaning.

Prior to the start of the experiment, candidate piglets were tested and determined to be free of PCV2 and PRRSV by qrt-PCR and RT-PCR methods, respectively, as well as by PRRSV ELISA (HerdChek PRRS 2XR IDEXX Laboratories) (Mateu et al., 2003; Olvera et al., 2004; Hjulsgager et al., 2009). Lack of PCV2 antibodies was not a required selection criterion in the studied animals because presence of maternal antibodies is widespread in commercial weanling piglets (Segalés et al., 2005; Fort et al., 2008).

PCV2 antibody titres were tested by an immunoperoxidase monolayer assay (IPMA) technique (Rodríguez-Arrijoja et al., 2000) and found to be low. Animals were weighed, ear-tagged and randomly distributed in two isolation boxes after matching weights between boxes. Randomly, piglets in one box were inoculated with PRRSV, while piglets in the other box were kept as non-infected controls.

The piglets were reared according to standard procedures developed by the Centre de Recerca en Sanitat Animal (CReSA, Barcelona, Spain) and provided a standard starter feed for weanling piglets during a 4-day acclimatization period.

Dietary treatments

Experimental diets were prepared at the Animal Nutrition Department of IRTA (Institut de Recerca i Tecnologia Agroalimentàries, Reus, Spain). Dietary treatments were formulated to contain similar amounts of crude protein, lysine, and metabolisable energy and were provided in mash form. No growth promoting antibiotics or medications were included in feed or water during the study.

The control diet was devoid of any animal protein derived ingredients, except whey protein. The test diet contained 8 kg SDPP per 100 kg feed and was administered *ad libitum* during the 28 days of the experiment. This time period was established as the appropriate period to observe the clinical effects of the PRRSV infection as well as the period of higher susceptibility of PRRSV infected animals to co-infections (Díaz et al., 2005; Mateu and Díaz, 2008).

The commercially manufactured SDPP (AP820P, APC Europe, S.A., batch number Y836512) used in the test diet contained 7.56×10^5 PCV2 DNA copies per gram when measured by qrt-PCR (Olvera et al., 2004), and had an antibody titre against PCV2 of 1/20480 as measured by IPMA (Rodríguez-Arrijoja et al., 2000). This SDPP batch was chosen to represent the lot with the highest number of PCV2 DNA copies among the analyzed manufacturing lots ($n = 12$) produced in the Spanish manufacturing plant during 2008 (mean and standard deviation: $5.22 \pm 2.0 \times 10^5$ PCV2 DNA copies per gram, with a range between 2.33×10^5 and 7.56×10^5 PCV2 DNA copies per gram).

Spray-dried porcine serum (SDPS, APC Europe, S.A., batch number 09XIP003-4) was added in the drinking water at 2% w/w to further supplement piglets in the event that the PRRSV challenge might reduce feed intake. The SDPS was obtained by defibrination of liquid plasma with CaCl_2 ; the removal of fibrinogen reduces risk of protein precipitation in a liquid state enabling the proteins to be administered in water applications. The SDPS was produced in a pilot plant spray-drier and contained 7.07×10^5 PCV2 DNA copies per gram when measured by qrt-PCR (Olvera et al., 2004).

Animal facilities and monitoring

Animal facilities and monitoring were conducted in bio-safety level 3 facilities at CReSA as previously described by Pujols et al. (2008). Laboratory animal laws and animal experimental procedures previously approved by CReSA Animal Experimentation Ethics Committee were followed.

Virus

A low passage of the wild-type V132 strain of PRRSV retrieved from sera of naturally infected pigs during an outbreak of PRRS was produced on pig alveolar macrophages. The inoculum was tested and found to be free of PCV2, and Torque teno sus viruses 1 and 2 (Olvera et al., 2004; Segalés et al., 2009).

Experimental design

A 2×2 factorial arrangement of experimental treatments was used, comprising two groups of PRRSV-challenged piglets fed diets with or without SDPP/SDPS and two groups of non-PRRSV-challenged fed diets with or without SDPP/SDPS. In the challenge box, all allotted piglets were inoculated intranasally with 2 mL of a viral suspension containing 10^6 TCID₅₀ of the PRRSV strain/mL (1 mL per nostril). Piglets from the other box served as controls and were sham inoculated with 2 mL of plain culture medium (MEM, 5%SFT, penicillin 100 UI/mL and streptomycin 100 µg/mL).

The animals in the PRRSV challenged box were divided into two groups: (1) those receiving SDPP/SDPS ($n = 5$; SDPP-PRRSV group) and (2) those that were fed with the control diet ($n = 6$; C-PRRSV group). The animals in the other box were also distributed in two subgroups, which received the SDPP/SDPS ($n = 6$; SDPP-Placebo group) or not ($n = 6$; C-Placebo group). Animals in both SDPP/SDPS groups (SDPP-PRRSV and SDPP-Placebo groups) were fed the diet containing 8 kg SDPP per 100 kg feed and given drinking water containing 2% w/w SDPS starting at 4 days before challenge. The SDPP diet was fed to these groups from 4 days before PRRSV inoculation until 28 days post-inoculation (PI).

The SDPS was included in drinking water from 4 days before PRRSV inoculation until 7 days PI. The SDPS was mechanically stirred into the drinking water daily in amounts to ensure *ad libitum* water consumption. The porcine serum solution was placed in a container that supplied water to the drinkers in the pen. During the first week post-PRRSV inoculation the water consumption was measured daily. Every day the remaining water was weighed and discarded and fresh SDPS solution was prepared again.

Piglets were weighed on days -4, 0, 3, 7, 14, 21 and 28 PI and rectal temperatures were taken daily from day 0 to day 14 PI. Piglets were bled on days 0, 3, 7, 14, 21 and 28 PI. At the end of the study (day 28), piglets were euthanased and necropsied.

Laboratory procedures

Prior to analyses, the SDPP and SDPS powders were reconstituted in sterile distilled water at a concentration of 9% w/v to obtain a similar concentration to that of liquid porcine plasma.

All sera samples collected and the SDPP and SDPS used in the experiment were analyzed for PRRSV genome detection (positive/negative) using a RT-PCR as described by Mateu et al. (2003). The PCV2 genome load was analyzed on the sera of all animals on days 0, 21 and 28 PI by means of the above mentioned qrt-PCR (Olvera et al., 2004).

The presence of antibodies against PRRSV was checked in SDPP, SDPS and sera on days 0, 7, 14, 21 and 28 PI by a commercially available ELISA kit (HerdChek PRRS 2XR, IDEXX Laboratories). According to the manufacturer, sample to positive control ratios (S/P) > 0.4 were considered positive. Antibodies against PCV2 were investigated using IPMA (Rodríguez-Arrijoja et al., 2000) on days 0, 21 and 28 PI.

On day 28 PI, the piglets were euthanased by an overdose of sodium pentobarbital and a complete necropsy was undertaken. Samples from each lung lobe were collected and fixed by immersion in 10% buffered formalin. Tissue samples were subsequently dehydrated through graded alcohols, embedded in paraffin and stained with haematoxylin–eosin stain. An estimated score of the severity of the interstitial pneumonia, based on a previously published scoring system (Halbur et al., 1995), was given as follows: 0 = no microscopic lesions; 1 = mild interstitial pneumonia; 2 = moderate multifocal interstitial pneumonia; 3 = moderate diffuse interstitial pneumonia; and 4 = severe interstitial pneumonia. Necropsies and pathological scoring were performed in a blinded fashion in regards to the treatment groups.

Statistical analysis

Repeated measures of analysis of variance (ANOVA) with Tukey–Kramer multiple comparisons were used to compare rectal temperatures and transformed \log_2 IPMA titres. Average scores of lesions between groups were analyzed by ANOVA-General Linear Model and Tukey–Kramer multiple comparisons (NCSS 2004). The level of significance for all analyses (α) was set to $P < 0.05$.

Results

Starting bodyweights were similar between groups ($P > 0.05$). Nevertheless, by Day 28 PI, average bodyweight in the challenged groups (25.3 ± 2.7 kg for C-PRRSV and 25.0 ± 2.6 kg for SDPP-PRRSV) was lower ($P < 0.05$) than the average body weight of the piglets in the SDPP-Placebo (28.9 ± 3.0 kg) group. The average bodyweight of the remaining group (C-Placebo) was intermediate (27.1 ± 2.1 kg).

Rectal temperature measured daily until Day 14 PI showed no statistical differences between groups. However, two fever peaks (above 40°C as average) in groups SDPP-PRRSV (days 2 and 8 PI) and C-PRRSV (days 4 and 11 PI) were observed.

Sera from the non-inoculated piglets (C-Placebo and SDPP-Placebo groups) were free of PRRSV viraemia during the entire experiment. All inoculated animals had PRRSV viraemia by day 3 PI. PRRSV viraemia from day 0 to 21 PI was not different between diets in the challenged groups (SDPP-PRRSV and C-PRRSV groups). However, on day 28 PI the inoculated group fed the diet with SDPP tended ($P = 0.07$) to have fewer viraemic piglets (2/5) than the inoculated group fed the control feed (5/6).

SDPP and SDPS contained high ELISA S/P ratios of antibodies against PRRSV (mean S/P ratio of 2.39 ± 0.1 and 2.31 ± 0.06 , respectively). All PRRSV challenged animals seroconverted starting at day 14 PI (Fig. 1). In the challenged groups (C-PRRSV and SDPP-PRRSV) mean S/P ratio values against PRRSV were not different between dietary treatments. Seroconversion against PRRSV was not observed during the entire experiment for the piglets in the non-inoculated groups (C-Placebo and SDPP-Placebo groups).

Mean individual interstitial pneumonia lesion scores by lobe and by lung on day 29 PI were numerically higher in the C-PRRSV group compared to the SDPP-PRRSV group, although no statistical differences ($P < 0.05$) were observed (Table 1). The PRRSV-challenged groups had significantly higher ($P < 0.05$) mean individual interstitial pneumonia lesion scores either by lobe or by lung than non-challenged piglets. No difference between the two groups of non-challenged piglets in interstitial pneumonia lesion score was observed.

None of the piglets from either challenged or non-challenged groups fed SDPP or control feed had PCV2 viraemia at any stage. At the beginning of the study, all piglets from the four experimental

Table 1

Microscopic interstitial pneumonic lesions on day 29 in each lobe of lungs classified using a 0–4 scale (Halbur et al., 1995): 0 (negative), +1 (slight), +2 (mild), +3 (intense) and +4 (severe).^A

Lobe region	C-PRRSV	SDPP-PRRSV	C-Placebo	SDPP-Placebo
Apical	1.0	1.2	0.0	0.0
Mediastinic	1.3	1.0	0.0	0.3
Diaphragmatic	1.8	1.4	0.0	0.5
Accessory	1.7	1.2	0.0	0.0
Mean by lobe	1.5 ± 0.37^a	1.2 ± 0.16^a	0.0 ± 0.0^b	0.2 ± 0.25^b
Mean by lung	5.8 ± 2.14^a	4.8 ± 2.28^a	0.0 ± 0.0^b	0.8 ± 0.98^b

C-PRRSV, control diet with PRRSV challenge; SDPP-PRRSV, plasma diet (8%) with PRRSV challenge; C-Placebo, control diet without PRRSV challenge; SDPP-Placebo, plasma diet (8%) without PRRSV challenge.

^a Values are expressed as Mean \pm SD. Values on the same row with different superscript were significantly different ($P < 0.05$) by ANOVA-General Linear Model and Tukey–Kramer multiple comparisons.

^A Values within lobe region represent the mean of lesions scores 0–4 for all pigs used within group ($n = 5$ –6 pigs/group).

Table 2

Immunoperoxidase monolayer assay (IPMA) antibody titres to PCV2 at all sampling days (expressed as \log_2 of the reciprocal of the serum dilution \pm standard deviation) for all the experimental groups. Statistical differences between groups were not detected, but overall mean titres on day 0 > day 21 and day 28; $P < 0.05$.

Groups	IPMA titre		
	Day 0	Day 21	Day 28
C-PRRSV	8.32 ± 1.79	5.64 ± 1.97	4.81 ± 2.17
SDPP-PRRSV	7.12 ± 2.28	5.93 ± 2.51	5.52 ± 2.16
C-Placebo	7.66 ± 1.03	5.64 ± 1.03	5.0 ± 1.37
SDPP-Placebo	7.99 ± 2.33	5.83 ± 2.74	4.32 ± 1.55
Mean by day	7.80 ± 1.83^a	5.76 ± 1.99^b	$4.80 \pm 1.73^{\text{sxsb}}$

C-PRRSV, control diet with PRRSV challenge; SDPP-PRRSV, plasma diet (8%) with PRRSV challenge; C-Placebo, control diet without PRRSV challenge; SDPP-Placebo, plasma diet (8%) without PRRSV challenge.

^{a,b} Values are expressed as Mean \pm SD. Values on the same row with different superscript were significantly different ($P < 0.05$) by ANOVA-General Linear Model and Tukey–Kramer multiple comparisons.

groups had varying levels of PCV2 antibody titres, ranging from $4.32 \log_2$ to $10.32 \log_2$ (Table 2). No statistical differences between the experimental groups were observed. PCV2 antibody titres

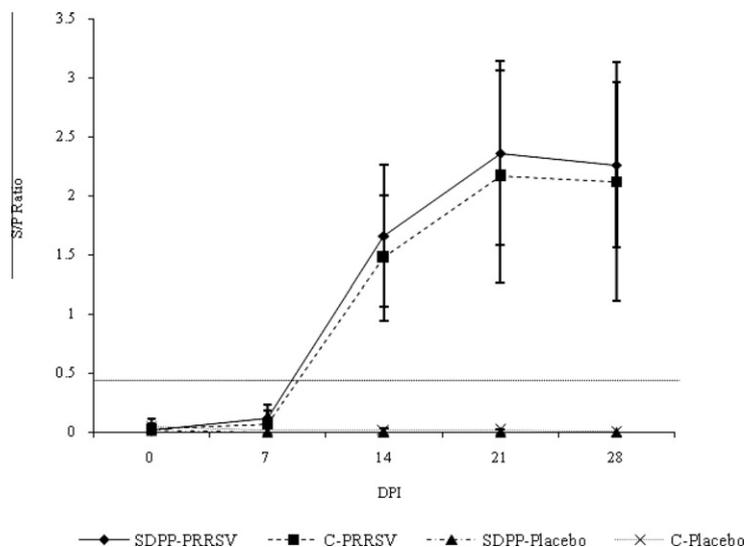


Fig. 1. ELISA antibody S/P ratio evolution of PRRSV-challenged groups (C-PRRSV and SDPP-PRRSV) and unchallenged groups (C-Placebo and SDPP-Placebo). Sample to positive control ratio (S/P) > 0.4 were considered positive. SDPP-PRRSV, plasma diet (8%) with PRRSV challenge; C-PRRSV, control diet with PRRSV challenge; SDPP-Placebo, plasma diet (8%) without PRRSV challenge; C-Placebo, control diet without PRRSV challenge. DPI, days post infection.

decreased in all experimental groups over time; reaching negative/low or moderate titres by day 28 PI (range 4.32 log₂–8.32 log₂).

Discussion

The results indicated that PRRSV-infected piglets fed commercially produced SDPP/SDPS with PCR quantified PCV2 DNA did not become infected with PCV2 and did not seroconvert against PCV2 virus during the study period. On the other hand, the lower number of PRRSV-viraemic piglets and the numerically less severe interstitial pneumonia lesions in the lungs of the challenged group consuming SDPP compared with the C-PRRSV group suggest that SDPP may be beneficial in reducing the negative impact of PRRSV on swine productivity. However, such effects must be demonstrated by means of studies with higher number of animals per group.

All piglets in this study had low to moderate PCV2 antibody titres according to Rodríguez-Arrijoja et al. (2000), Vincente et al. (2004) and Fort et al. (2008), which represents the usual situation of nursery piglets under field conditions. These PCV2 antibody titres were considered to be of maternal immunity origin since they decreased in all experimental groups over time, reaching negative/low or moderate titres by day 28 PI. The reduction profile for the different groups was within the expected range under a non-viraemic situation as described elsewhere (Fort et al., 2008, 2009). Therefore, the serological results, together with those from qrt-PCR, indicate that the studied animals were not exposed to active PCV2 infection.

The presence of maternal antibodies against PCV2 is considered to play an important role in preventing the development of PMWS, but they cannot prevent the establishment of subclinical PCV2 infection or seroconversion. Several studies (McKeown et al., 2005; Meerts et al., 2006; Fort et al., 2007) have shown that piglets with similar maternal antibody titres to those observed at day 0 of the present experiment were unable to prevent PCV2 infection or seroconversion upon experimental or natural infection with this virus.

Our results are in agreement with previous studies in which no seroconversion to PCV2 was observed when pigs were fed diets containing commercial SDPP (Nofrarías et al., 2006; Pujols et al., 2008). It is important to note that non-inoculated control pigs used in several PCV2 challenge studies undertaken at Iowa State University were routinely fed commercial feed that contained SDPP during the first 1–4 weeks of the experiments and remained free of PCV-2 infection (Opriessnig et al., 2006). These challenge studies involved more than 1,000 pigs tested weekly for the presence of PCV2 antibodies and viral load in tissues and serum. Our results are in agreement with these experiments.

However, Patterson et al. (2010) recently reported PCV-2 transmission in naïve pigs given an oral gavage of experimentally produced SDPP. The experimental SDPP used by Patterson et al. (2010) was produced using a laboratory scale spray-dryer from the blood of a single infected pig showing clinical evidence of PMWS. Significant differences exist between the experimentally produced SDPP used by Patterson et al. (2010) and the commercially produced SDPP used in studies reported by Pujols et al. (2008), Nofrarías et al. (2006), Opriessnig et al. (2006) and the current study. Patterson et al. (2010) used experimentally inoculated viraemic, diseased pigs to produce the SDPP, while the protein used in the present study and above mentioned studies was of commercial origin.

Commercial production of SDPP involves sourcing plasma from pigs which have been officially declared as healthy. The pooling of material from numerous slaughter weight pigs with the inherent presence of neutralizing antibodies consequently reduces the

PCV2 DNA load compared to experimentally inoculated pigs. Additionally, processing conditions used in the commercial SDPP manufacturing process are much more complex than laboratory-produced SDPP, involving the use of higher pressure and a higher drying temperature with an extended period of dwell time than the process used by Patterson et al. (2010). It is thus likely that differences in source material and manufacturing processes explain the conflicting results obtained by Patterson et al. (2010) and studies that used commercially produced SDPP.

The presence of the PCV2 genome in SDPP/SDPS was not unexpected since Carasova et al. (2007) reported the presence of PCV2 genome (around 10⁵ copies/mL) in the blood of 19–25 week-old pigs, which is the usual slaughter age in Spain. In fact, these PCV2 values were fairly similar to the PCV2 loads in SDPP used in a previous experiment (Pujols et al., 2008). However, it must be emphasized that qrt-PCR does not distinguish between infective and non-infective virus particles. Therefore, the lack of PCV2 infectivity in the studied piglets fed SDPP could be partially attributed to virus inactivation by neutralizing antibodies to PCV2 in the pooled liquid plasma before it was spray-dried.

The neutralization potential of contaminating viruses has been suggested in previous research by Solheim et al. (2008) and Williams and Khan (2010). Another important factor for the inactivation of PCV2 involves the commercial manufacturing process. Past research (Polo et al., 2005; Pujols et al., 2007) reported that spray-drying can inactivate several logarithm titres of other swine viruses (PRRSV, Aujeszky's disease virus and swine vesicular disease virus).

Messier et al. (2007) reported that the inclusion of SDPP in diets reduced the mortality and medication cost related with porcine circovirus associated disease (PCVAD) in a commercial grow-finish farm in Quebec, Canada. The presence of antibodies against PCV2 (1/1250–1/2560 titres measured by an immunofluorescent antibody test) in the SDPP used in the mentioned study might partially explain the beneficial effects of feeding SDPP in reducing the severity of PMWS, although it could also be partially attributed to the known effect of SDPP on intestinal inflammation and mucosal barrier function (Nofrarías et al., 2006; Moretó and Pérez-Bosque, 2009; Peace et al., 2010).

Conclusions

To the authors' knowledge, this is the first work in which the safety of commercially produced SDPP/SDPS regarding PCV2 transmission in piglets previously infected with an immunomodulatory pathogen (PRRSV) has been studied. Oral feeding of PCV2 qrt-PCR positive commercial SDPP for 28 days to PRRSV infected piglets did not result in transmission or seroconversion to PCV2 under the conditions of this study.

Conflict of interest statement

L.E. Russell, J.M. Campbell and J.D. Crenshaw are employed by APC Inc., Ankeny, IA, USA; J. Polo is employed by APC Europe, S.A.

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