

# Influence of the inclusion of spray-dried animal plasma in weanling pigs' diet on porcine circovirus type 2 (PCV2) loads

## *Influência da inclusão do plasma sanguíneo na dieta de leitões desmamados sobre a carga viral de circovírus suíno tipo 2 (PCV2)*

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### Abstract

Spray-dried animal plasma (SDAP), a natural byproduct of the meatpacking industry, has been shown to have beneficial effects on growth and performance of weaned pigs. Porcine circovirus 2 (PCV2) is an important virus that is disseminated in the pork industry. Regardless of the studies evaluating the possible transmission of PCV2 through SDAP, there is no information about the effects of its inclusion in the PCV2 loads in natural infections. The present investigation evaluated the influence of dietary inclusion levels of SDAP in weanling pigs on PCV2 viremia and humoral immune response. Fifty-six weaned piglets were fed in a 2-period feeding program. Dietary treatments included 0%, 2%, 4% or 6% and 0%, 1%, 2% or 3% of SDAP during period 1 (14 to 28 days old) and 2 (29 to 42-days old), respectively. In period 3 (42 to 56 days old), all piglets received a SDAP-free diet. Serum samples were collected weekly and tested for PCV2 antibodies and DNA load. The results show that the concentration of 6% and 3% of SDAP on feed offered for pigs during period 1 and 2, respectively, may have decreased the PCV2 loads.

**Keywords:** Weaning pigs. Spray-dried animal plasma. Humoral immune response.

### Resumo

O plasma sanguíneo em pó (PSP), produto natural de indústria frigorífica, tem mostrado efeitos benéficos sobre o crescimento e desempenho de leitões desmamados precocemente. Atualmente, embora o circovírus suíno 2 (PCV2) tenha grande importância para a suinocultura, não há informações sobre o impacto do uso de PSP e a resposta imune ao PCV2 em infecções naturais. Este trabalho avaliou diferentes níveis de inclusão de PSP em dietas de leitões e as cargas virais de PCV2 correspondentes. Quatro níveis de inclusão de PSP foram testados em dois períodos consecutivos: 0, 2, 4 ou 6% durante o período 1 (14 aos 28 dias de idade) e 1, 2 ou 3% de PSP durante o período 2 (29 a 42 dias de idade). No período 3 (42 aos 56 dias de idade), todos os leitões foram alimentados com dieta isenta de PSP. Amostras de soro foram coletadas semanalmente e testadas para anticorpos anti-PCV2 e carga de DNA de PCV2. As concentrações de 6% e 3% de PSP fornecidas nas rações durante o período 1 e 2, respectivamente, influenciaram na carga viral de PCV2 de suínos naturalmente infectados.

**Palavras-chave:** Leitões desmamados. Plasma sanguíneo em pó. Resposta imune humoral.

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### Introduction

Spray-dried animal plasma (SDAP) is a natural byproduct of the meatpacking industry. It is commonly included in diets for weanling pigs worldwide and has been shown to have beneficial effects on their growth and performance. SDAP is prepared from blood of healthy animals inspected at a slaughterhouse and suitable

for human consumption. The blood is separated into figurative elements and plasma, the latter is then spray-dried. Spray drying significantly reduces the number of viable microorganisms. The exact mechanism by which SDAP improves nursery performance has not yet been fully understood. Studies suggested that proinflammatory cytokines, released in response to a pathogenic challenge, reduce feed intake and growth, and several studies demonstrate the beneficial effects of SDAP in various disease challenge models (PATTERSON et al., 2010).

Porcine circovirus 2 (PCV2) is one of the most important viruses disseminated in the pork industry today. It has received considerable attention, largely due to its increasing association with various disease conditions in pigs that are collectively denominated Porcine circovirus associated disease (PCVAD) (OPRIESSNIG; HALBUR, 2012).

Although PCV2 DNA has been found in commercially manufactured SDAP, experimental studies have failed to demonstrate its transmission to pigs (SHEN et al., 2011; OPRIESSNIG; HALBUR, 2012). Regardless of the studies evaluating the possible transmission of PCV2 through SDAP, there is no information about the effects of its inclusion to

improve the immune response against PCV2 in natural infections. Therefore, this study evaluated the influence of dietary inclusion levels of SDAP in weaned pigs from 14 to 42 days old on PCV2 viremia and PCV2 humoral immune response.

## Materials and Methods

### Experimental design and sampling

The experimental protocol was approved by the Institute of Animal Science – Institutional Animal Care and Use Committee. The experiment was carried out in the swine facilities of the Animal Science Institute in Nova Odessa, São Paulo. Fifty-six 14-day-old newly weaned piglets of similar weight ( $3.9 \pm 0.65$  kg) were obtained from six PCV2-vaccinated sows from a commercial herd. The same number of male and female piglets were randomly housed in one of the four pens ( $n = 14$  pigs/pen). The animals had free access to water and one of four experimental nursery diets (T1 to T4) containing 0%, 2%, 4% or 6% SDAP in period 1 (14 to 28 days old) or 0%, 1%, 2%, 3% SDAP in period 2 (29 to 42 days old) (Table 1). In period 3, all the animals received SDAP-free diets.

Table 1 – Composition of experimental diets offered to the piglets in period 1 (14 to 28 old days) and 2 (29 to 42 old days) for the different treatments (T1, T2, T3 and T4) – Nova Odessa – SP – 2012

	Percentage in diet for period 1 (period 2) <sup>1*</sup>			
	0 (0)	2 (1)	4 (2)	6 (3)
<b>Spray-dried plasma (%)</b>	0 (0)	2 (1)	4 (2)	6 (3)
<b>Digestible Energy (Mcal/kg)</b>	3,50 (3,51)	3,50 (3,51)	3,51 (3,51)	3,51 (3,51)
<b>Phosphorus (%)</b>	0,57 (0,45)	0,57 (0,45)	0,57 (0,45)	0,57 (0,45)
<b>Calcium (%)</b>	0,90 (0,82)	0,90 (0,82)	0,90 (0,82)	0,90 (0,82)
<b>Fat (%)</b>	2,74 (4,02)	2,44 (4,03)	2,18 (4,05)	2,20 (4,06)
<b>Crude protein (%)</b>	20,87 (22,21)	20,88 (22,24)	20,86 (22,28)	20,94 (22,31)
<b>Lysine (%)</b>	1,47 (1,65)	1,48 (1,66)	1,48 (1,66)	1,53 (1,67)
<b>Sodium (%)</b>	0,29 (0,23)	0,28 (0,23)	0,28 (0,23)	0,28 (0,23)
<b>Lactose (%)</b>	15 (5)	15 (5)	15 (5)	15 (5)

<sup>1</sup> Supplied per kilogram of complete diet: 5.0000 U.I of vitamin A; 1000 U.I of vitamin D<sub>3</sub>; 15 mg vitamin E; 2 mg vitamin K<sub>3</sub>; 3.6 mg of vitamin B<sub>2</sub>; 14 µg of vitamin B<sub>12</sub>; calcium 6 mg of calcium pantothenate; 20 mg of niacin; 1 mg of biotin; 100 mg of choline; 50 mg of antioxidant; 80 mg of Fe; 70 mg of Cu; 40 mg of Mn; 80 mg of Zn; 720 µg of Co; 1.68 mg of I and 240 µg of Se

\*Data from period 2 are in parenthesis

The diets were formulated to contain identical levels of metabolized energy and essential amino acids and to meet the nutrient requirements (Table 1), thus mimicking the concentrations offered by swine farmers in fields. Five animals of each treatment ( $n = 20$ ) were randomly chosen on the first day of the trial and bled at

the jugular vein on days 14, 21, 28, 35, and 42. The blood was stored in sterile tubes, centrifuged at 2000 g for three minutes, and the serum obtained was stored at -20°C until polymerase chain reaction (PCR). Additionally, ten aliquots of SDAP used on the diet were taken overtime to quantify the amount of PCV2 DNA. To evaluate the

maternal antibodies, one sample from each sow ( $n = 10$ ) whose piglets were used to compose the trial was also tested for anti-PCV2 antibodies.

### Quantitative polymerase chain reaction

The samples were refrigerated during transport, DNA was extracted using phenol-chloroform and proteinase K protocols, and stored at  $-20^{\circ}\text{C}$  (CHOMCZYNSKI, 1993). The extracts were subsequently used to quantify PCV2 DNA copies using a SYBR Green qPCR assay, with the primer pair SybPCV2F (5'ATA ACC CAG CCC TTC TCC TAC C 3') and SybPCV2R (5' GGC CTA CGT GGT CTA CAT TTC C 3') to amplify a 145 nt fragment (YANG et al., 2007). The quantitative polymerase chain reaction (qPCR) contained 10  $\mu\text{L}$  of PCR SYBR<sup>®</sup> Green Master Mix (Fermentas Thermo Scientific, Canada), 4  $\mu\text{L}$  of extracted DNA, 0.2  $\mu\text{M}$  of each primer, and sterile water to 25  $\mu\text{L}$ . The PCR conditions were 10 minutes at  $95^{\circ}\text{C}$  and 1 minute at  $60^{\circ}\text{C}$ , followed by 40 cycles of 15 seconds at  $95^{\circ}\text{C}$ , 1 minute at  $60^{\circ}\text{C}$ , and a melting curve analysis. The reactions were performed using the StepOne<sup>™</sup> Real-Time PCR System (Life Technologies Inc., USA). The number of viral DNA copies was determined in comparison with a standard curve, and the viral concentration was expressed as  $\log_{10}$  PCV2 DNA per milliliter (mL) of serum or per gram (g) of SDAP. Extracted samples were also tested for  $\beta$ -actin by a qualitative polymerase chain reaction (PCR), as previously described (HUI et al., 2004), to identify

potential false-negatives resulting from failures in DNA extraction or from the PCR.

### Serology

PCV2 IgG antibody titers were tested by an immunoperoxidase monolayer assay (GERBER et al., 2009). The total IgG antibody concentration was expressed as the average  $\log_2$  titer, which corresponded to the following dilutions: 20 = 4.32; 80 = 6.32; 320 = 8.32; 1280 = 10.32; and 5120 = 12.32. PCV2 IgM was tested using a commercial ELISA (INGEZIM Circovirus IgG/IgM, Ingenasa, Spain), according to the manufacturers' instructions. Results were given as corrected optical density (ODc) = [OD sample / (OD positive control - OD negative control)].

### Statistical Analysis

The Shapiro-Wilk test was used to evaluate the normality of data distribution in the examined variables. The nonparametric Kruskal-Wallis test was used to analyze the PCV2 viral load, PCV2-antibody titers, and PCV2 IgM S/P ratios between treatments. The results were considered significant when  $p < 0.05$ .

### Results

PCV2 loads and anti-PCV2 IgG titers in serum from the piglets are shown in Table 2. The ELISA anti-PCV2 IgG ODc of the PCV2-vaccinated sows from a commercial herd ranged from 0.7 to 0.9.

Table 2 – PCV2 viral load and anti-PCV2 IgG titers during periods 1 and 2 on treatment T1 to T4. – Nova Odessa – SP – 2013

Treatment	PCV2 DNA <sup>a,b</sup>		Anti-PCV2 IgG <sup>c</sup>	
	Period 1	Period 2	Period 1	Period 2
T1	3.03 ± 1.39 <sup>ab</sup>	3.85 ± 0.73	9.56 ± 2.09 <sup>a</sup>	8.27 ± 3.65 <sup>a</sup>
T2	4.31 ± 1.39 <sup>a</sup>	4.43 ± 1.71	6.17 ± 3.00 <sup>b</sup>	5.53 ± 3.06 <sup>b</sup>
T3	3.47 ± 0.92 <sup>ab</sup>	3.61 ± 0.57	9.42 ± 3.30 <sup>a</sup>	7.75 ± 3.74 <sup>a</sup>
T4	2.21 ± 1.32 <sup>b</sup>	2.47 ± 1.48	7.38 ± 2.66 <sup>ab</sup>	5.32 ± 4.15 <sup>ab</sup>

<sup>a</sup>  $\log_{10}$  transformed titer (mean ± sd)

<sup>b</sup> Different superscripts within the column indicate significant differences ( $p < 0.05$ ) between the treatments

<sup>c</sup>  $\log_2$  transformed titer (mean ± sd)

T1=0% for period 1 and 2; T2 = 2% and 1 % for period 1 and 2, respectively; T3 = 4% and 2 % for period 1 and 2, respectively; T4 = 3% and 6 % for period 1 and 2, respectively

Animals from the T4 group presented an overall lower PCV2 viral load in serum compared to animals from the T1 and T2 groups (mean ± average  $\log_{10}$  PCV2 copies/mL 2.25 ± 1.31 versus 3.38 ± 1.23 and 4.11 ± 1.38). Animals from the T3 group presented a similar PCV2 viral load to the other groups (3.50 ± 0.79  $\log_{10}$ ).

The average PCV2 DNA load per gram of spray dried plasma was  $1.7 \times 10^4 \pm 6$ . This corresponds to an intake of nearly  $1.7 \times 10^6$  to  $2.09 \times 10^6$ ,  $3.4 \times 10^6$  to  $4.08 \times 10^6$  and  $5.1 \times 10^6$  to  $6.2 \times 10^6$  during period 1 (14 to 28-days old) or period 2 (29 to 42-days old) by the animals from T2, T3, and T4, respectively.

Animals from T1 presented the overall highest anti-PCV2 IgG titers (mean  $\pm$  sd  $7.75 \pm 3.50 \log_2$ ), while animals from T4 presented the lowest overall anti-PCV2 IgG titers (mean  $\pm$  sd  $4.88 \pm 4.24$ ). Interestingly, T4 low anti-PCV2 IgG titers were accompanied by the lowest number of viral DNA copies/mL observed during the whole experiment.

## Discussion

Over the years, weaning age has been dramatically reduced, resulting in smaller and less mature immune and digestive systems as depriving the piglets of their mothers' milk makes them more susceptible to problems in the early post-weaning period. The addition of SDAP to the first diet after weaning has improved feed intake and growth rates (TOUCHETTE et al., 2002). Use of SDAP is a common procedure of Brazilian pig farmers in order to reduce PCV2 associated losses. The exact mechanism by which SDAP improves the performance of the animals in PCV2-affected herds has not yet been fully elucidated. The hypothesis is the ability of SDAP to modulate the immune system (SHEN et al., 2011).

As the intention herein was to mimic the procedure used by Brazilian swine farmers, the experimental design did not allow further statistical analysis. However, the results show some tendencies that can be used directly for further studies or to partially explain what has been seen in the field. The pigs from the T4 group, which received the highest spray-dried diet inclusion, showed an overall lower PCV2 viral load in serum compared to the groups with lower or no inclusion. These results suggest that SDAP consumption could have a role in ameliorating the immune response against PCV2 infection during the nursing period in which animals are changing from a passive to an active immune response.

A delay in seroconversion has been observed in animals with high passive immunity against PCV2 under field conditions (GERBER et al., 2009). The animals used herein were selected from PCV2-vaccinated sows with high titers of anti-PCV2 IgG (ELISA ODC ranged from 0.7 to 0.9), which may have also played a role in protecting the pigs against PCV2 infection and viral load during the nursery period. Considering Opriessnig et al.'s (2004) experiment, based on the titer of anti-PCV2 IgG of the animals by the beginning of the current experiment the seroconversion should have occurred between the 8th and 11th weeks of age. Thus, the absence of seroconversion

herein could be a consequence of the highly passive maternal antibodies.

In order to make SDAP, the plasma is separated from the cellular fraction immediately after collection, chilled, and stored in insulated tanks. Thus, plasma from a single pig is pooled with those from other pigs, resulting in a mixture of antibodies from all pathogens circulating in the population at any point in time (PATTERSON et al., 2010). Pooling of plasma containing antibodies for different pathogens is recognized as a way to ensure neutralization of potentially contaminating viruses, especially stable viruses that are difficult to inactivate by other methods (OPRIESSNIG et al., 2004). The anti-PCV2 antibody titers were measured in fresh and spray-dried plasma by Shen et al. (2011), and a decrease in anti-PCV2 antibodies concentration was found in the spray-dried plasma when compared to the fresh liquid plasma. The titer of anti-PCV2 IgG was not measured herein and the neutralizing capacity of IgG could have influenced the result of this study, especially regarding differences in PCV2 load in serum between groups. However, this does not explain the low anti-PCV2 IgG and PCV2 DNA loads in the animals from T4, which received higher percentages of SDAP in their diets. It is generally assumed that the main basis for PCV2 protection relies on anti-PCV2 antibodies. However, low antibody responses as well as lack of antibodies do not always correlate with lack of protection. Cell mediated immunity has been proposed to be important for protective immune defense (FENAUX et al., 2004; PÉREZ-MARTÍN et al., 2010). Considering the results observed herein, other components present in SDAP, as well as the anti-PCV2 antibody titers, may have contributed to the lower PCV2 load in the T4 group.

It is well known that other agents can trigger PCV2-associated diseases (OPRIESSNIG; HALBUR, 2012). The diversity and quantity of antibodies for other infectious agents, not measured herein and in other studies, could also interfere in the results obtained with SDAP diet inclusion. Indeed, Van Dijk et al. (2001) suggested that the response to inclusion of SDAP in feed depends on the health and hygiene status of the herd.

Additionally, management factors can also increase the risk of PCVAD, such as the presence of large pens, proximity to other pig farms, high level of cross-fostering, and continuous flow through (GRAU-ROMA et al., 2011), commonly observed under field conditions in Brazilian swine herds. Therefore, the beneficial

result of SDAP inclusion on nursery pigs' diets should be considered with caution, since pigs from this study where kept under experimental design in a controlled environment. In conclusion, considering the conditions of the present study, the inclusion of 6% and 3% of SDAP on feed offered for pigs during period 1 (14 to 28 old days) and 2 (29 to 42 old days), respectively, may have decreased the PCV2 load in serum of naturally infected animals.

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## Conflict of Interest

The authors declare that they have no competing interests.

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