

# Growth Performance and Intestinal Villous Morphology Alteration in Pre-Weaning Piglets Fed Spray-Dried Porcine Plasma in Creep Feed

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## ABSTRACT

The effects of creep feed which included spray-dried porcine plasma (SDPP) were investigated on the growth performance and alteration of the intestinal villous morphology in seven-day-old piglets. The piglets were fed 0, 2, 4, and 6% SDPP diets for 24 d. The feed intake and body weight gain were measured during the experimental period. At the end of the experiment, the intestinal villus height, epithelial cell area and number of occurrences of cell mitosis were examined using light microscopy (LM), and the duodenal villus tip surface was observed using scanning electron microscopy (SEM). The results showed there was improved feed efficiency in the SDPP-fed group when compared with the control group. The 4% SDPP-fed group showed the highest improvement, followed by the 6% and 2% SDPP-fed groups, for most of the LM parameters in most intestinal parts. In addition, on the duodenal villus tip surface, the 0, 2, and 4% SDPP-fed groups showed a clearer cell outline, larger cells and protuberance of cells further into the lumen than those of the 6% SDPP-fed group. Thus, the results of this study showed that: the SDPP-fed group achieved improved feed efficiency after the feeding of dietary SDPP; the SDPP could be incorporated into piglet diets up to a level of 4%; and the SDPP might activate the intestinal function at both the villus and cellular levels.

**Keywords:** growth performance, intestinal villous morphology, pre-weaning piglets, spray dried porcine plasma, creep feed

## INTRODUCTION

Weaning is one of the most critical stages in pig production, as the animals are subjected to a combination of stress factors that increase their susceptibility to post-weaning diarrhea (Madec *et al.*, 2000). These factors can be classified into different categories: 1) emotional factors, such as separation from the sow and mixing with pigs from other litters; 2) environmental factors, such as moving to new facilities with different housing

conditions; 3) health factors, such as exposure to new pathogens and loss of maternal passive immunity; and 4) nutritional factors, such as changing from milk to feed that is mainly in a dry form (Torrallardona *et al.*, 2002)

The combination of all these factors usually leads to a severe reduction in feed intake that affects the integrity of the intestinal mucosa and increases animal susceptibility to pathological disorder (Pluske *et al.*, 1997). The intestine of a weaning piglet is a diverse organ that not

only absorbs nutrients and secretes water and electrolytes, but also forms a barrier to pathogenic bacteria and antigens. A strong correlation between dry matter intake and villous height has been reported for pigs fed cow's milk, displaying the immediate need for maintaining feed intake post weaning (Pluske *et al.*, 1996). During a period of post weaning anorexia, the lack of intake reduces the protein mass and DNA content of the small intestine (Burrin and Stoll, 2003), leaving the intestinal lining more penetrable to luminal antigens and pathogens. Any reduction in intestinal integrity will result in, initially, compromised nutrient digestion and absorption, followed by a change to a new environment and then, reduced ability of the pig to resist enteric pathogens. Therefore, pre-weaning diets are formulated not only to provide essential nutrients that result in efficient and rapid weight gain but also to drive rapid feed intake immediately post weaning to prevent the destruction of the small intestinal lining.

Many measures and modifications have been applied to pig feed during the weaning period to decrease weight loss in the post-weaning stage. The use of antimicrobial prophylactic medication at weaning has been advantageous to piglets, but the recent European ban on the use of antimicrobial growth promoters has stimulated the need to investigate new alternatives (Brufau, 2000).

Spray-dried animal plasma (SDPP) is usually of porcine origin and is obtained as a byproduct in slaughter plants. An anticoagulant (usually sodium citrate) is added to the blood from the slaughtered pigs and the erythrocytes are removed by centrifugation. The plasma obtained is subsequently spray dried and used for the production of both human foodstuffs and animal feeds (Gatnau *et al.*, 1989). SDPP has been shown in many studies to improve performance in weaning pigs (Hansen *et al.*, 1993; Kats *et al.*, 1994; Angulo and Cubilo, 1998; van Dijk *et al.*, 2001). With high production, especially in litter sizes having more than 12 piglets per sow, there

is usually a lower weaning weight (Milligan *et al.*, 2002). Thus, SDPP is the one feed additive which may be fed as a supplement to improve performance. At present, there are no available reports on studies involving pre-weaning piglets. Different levels of use and modes of action for SDPP have been proposed (Torrallardona, 2010). However, SDPP can be used as a supplement in creep feed and weaning feed to enhance the growth performance of post-weaning pigs through affecting their intestinal villous morphology. This study aimed to define a satisfactory dose level, and to evaluate the efficiency and impact of the SDPP-fed supplement on the growth performance and the alteration of epithelial cells of the small intestine, with the information obtained being useful for on-farm applications.

## MATERIALS AND METHODS

### Animal and diets

A total of 120 crossbred (25% Large White  $\times$  25% Landrace  $\times$  50% Doroc) piglets were included in four different treatments each involving 30 piglets. All animals were fed with creep feed (protein 23%, calcium 0.9%, available phosphorus 0.49%, lysine 1.55% and fat 6.2%) supplemented with 0%, 2%, 4% and 6% SDPP (composition of SDPP (commercial product) as the percentage fed was 92% dry matter, 78% crude protein, 6.84% lysine, 0.75% methionine, 4.72% threonine and 1.36% tryptophan) from 7 to 31 days of age (DOA). The experiment was carried out under identical conditions for each farrowing pen ( $1.5 \times 2.0 \text{ m}^2$ ) with *ad libitum* feed and water and an initial average body weight of 3 kg at 7 DOA. Weight gain and total feed intake were recorded at 7, 24 and 31 DOA. The sows in each group were removed at day 24 after parturition. At the end of the experiment, three piglets per treatment were slaughtered in the necropsy room (Faculty of Veterinary Medicine, Chiang Mai University), and the duodenum, jejunum and ileum were collected randomly for examination using light microscopy

(LM), and the duodenum was submitted for observation using scanning electron microscopy (SEM). All the experiments were carried out according to the humane care guidelines for the care and use of laboratory animals established by the National Research Council of Thailand (Kunjara and Chatikavanij, 2007) (Reference).

### Tissue sampling

Immediately after slaughtering, the abdominal cavities were opened along the midline, and then the whole intestine and the intestinal organs were excised. Samples of the duodenum were taken at about 20 cm caudal to stomach, of the jejunum at the middle part (about two-fifths of the remaining portion of the small intestine below the duodenum) and of the ileum at about 50 cm proximal to the caecum. In each intestinal segment, both ends of a 5 cm length were tied with a thread, and a fixative (a mixture of 3% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylyte buffer, pH 7.4) was injected without distending. Each intestinal segment was removed from the intestine and kept in the same fixative. Immediately after the fixative step, each segment was prepared for LM and SEM sampling.

### Light microscopy protocol

An  $8 \times 10$  mm of each intestinal segment was taken from a 5 cm length of intestine, fixed with Bouin's fixative solution (Yamauchi *et al.*, 1996) for 1 wk, embedded in paraplast and then cut into 5  $\mu\text{m}$  cross sections. Every fifth section was collected and stained with haematoxylin-eosin (Yamauchi *et al.*, 1996). The villus height was measured from four villi having the lamina propria that were selected from one section. The length from the villus tip to the bottom including no intestinal crypt was measured. Forty villus heights per part of the intestine were measured and the mean for each piglet was determined. To determine the area of one cell in the 5- $\mu\text{m}$  section, the area of the epithelial cell layer was randomly measured at the middle part of the villi and then

the number of cell nuclei within this measured epithelial cell layer was counted. Finally, the area of the epithelial cell layer was divided by the number of cell nuclei. This procedure was carried out in two fields of view using LM for the one section, resulting in 30 measurements per intestinal part and the results were then used to calculate the mean cell area in each piglet. The number of occurrences of cell mitosis per crypt was determined from five crypts of similar size within the same microscopic field ( $10 \times 40$  magnification) that were randomly selected, and occurrences of cell mitosis were counted and expressed as the number of cell mitosis occurrences per crypt. One to two measurements were carried out per section using a randomization method which followed previous study. Fifteen measurements of cell mitosis occurrence per crypt were measured per each part of the intestine to calculate the mean occurrence of cell mitosis per crypt in each piglet. These measurements were recorded using an image analyzer (Imaging Software OLYSIA Bioreport; Olympus Optical Co. Ltd.; Tokyo, Japan) in all the intestinal segments.

### Scanning electron microscopic protocol

From 4 cm of each intestinal segment, a  $2 \times 3$  cm block was prepared at the part close to the LM sample by cutting at the non-mesenteric site along its entire length, opening, and washing away the intestinal contents with 0.1 M phosphate-buffered saline (pH 7.4). Finally, blocks were cut into  $3 \times 10$  mm pieces and these tissue samples were pinned flat to prevent curling and fixed vertically with the mucosal face downwards inside a mixture of 3% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylyte buffer (pH 7.4) at room temperature for 1 hr. The pieces were rinsed with 0.1 M sodium cacodylyte buffer (pH 7.4) and post-fixed with 1% osmium tetroxide in ice-cold buffer for 2 hr and then dehydrated with ethanol. The specimens were dried in a critical point drying apparatus using liquid carbon dioxide as the medium. The dried specimens were coated

with platinum (RMC-Eiko RE vacuum coater; Eiko Engineering Co. Ltd.; Tokyo, Japan) and examined using SEM (S-800; Hitachi Ltd.; Tokyo, Japan) at 8 KV. The morphological alterations of the villi tips were compared between the groups.

#### Gross anatomical protocol

After removing the tissue sample for microscopy, the remaining intestine was gross anatomically divided into different intestinal segments according to the surface structural features, with the diameter of the small intestine being smaller than that of the large intestine (colon), which is a spiral feature. Each part was cut and washed with normal saline solution (0.9% NaCl) to remove the intestinal contents. The length and weight of each part were measured after the removal of the intestinal contents with the solution. The internal organs were also weighed.

#### Statistical analysis

All the data measured in the feeding trial and in the LM examination were statistically analyzed using one-way analysis of variance, and the significant differences between the results of the treatments were determined with Duncan's multiple range test using the SPSS program (Version 10.0; SPSS Inc.; Chicago, IL, USA) at the  $P < 0.05$  level of significance.

## RESULTS

#### Growth performance

On average, the final body weights of the 4% and 6% SDPP-fed groups were 7.89 and 8.22 kg, respectively, while those of the 0% and 2% SDPP-fed groups were 7.36 and 7.38 kg, respectively. The body weight gains of the 4% and 6% SDPP-fed groups were 5.28 and 5.17 kg, respectively, whereas for the 0% and 2% SDPP-fed groups, the gains were 4.09 and 4.12 kg, respectively. The average daily gains (ADGs) of the 4% and 6% SDPP-fed groups were 219 and 215 g, respectively, while the average daily gains of the 0% and 2% SDPP-fed groups were 170 and 171 g, respectively. The growth performance of the 4% and 6% SDPP-fed groups significantly ( $P < 0.05$ ) increased the final body weight (BW), BW gain and ADG (Table 1).

#### Gross anatomical observation

No specific differences were found in the weights of the internal organs, with all the experimental groups showing results similar to the control group (Table 2). The control group tended to have a greater intestinal length in the small intestine than did the experimental group.

**Table 1** Growth performance of piglets fed dietary feed supplemented with spray-dried porcine plasma.

Item	Spray-dried porcine plasma			
	0%	2%	4%	6%
Initial body weight (kg)	3.26±0.44	3.26±0.37	2.70±0.87	3.04±0.43
Final body weight (kg)	7.36±1.56 <sup>b</sup>	7.38±0.93 <sup>b</sup>	7.98±1.99 <sup>ab</sup>	8.22±1.26 <sup>a</sup>
Body weight gain (kg)	4.09±1.2 <sup>b</sup>	4.12±0.71 <sup>b</sup>	5.28±1.25 <sup>a</sup>	5.17±0.96 <sup>a</sup>
ADFI (g)	44.51	59.00±11	70.00±22	76.00±8
ADG (g)	170.00±50 <sup>b</sup>	171.00±29 <sup>b</sup>	219.00±52 <sup>a</sup>	215.00±40 <sup>a</sup>
Feed efficiency gain (g per feed kg)	0.37±0.01	0.37±0.06	0.31±0.05	0.37±0.04

ADFI = Average daily feed intake; ADG = Average daily body weight gain.

Values are shown as mean ± SD.

<sup>a,b</sup> = Values in a row with the same lowercase superscript are not significantly ( $P < 0.05$ ) different.

**Table 2** Intestinal length and weight of internal organs in piglets fed with different percentages of spray-dried porcine plasma.

Item	Spray-dried porcine plasma			
	0%	2%	4%	6%
Body weight (kg)	7.36±1.45	7.83±0.2	8.20±1.44	8.66±0.75
Intestinal length (cm)				
Small intestine	818.33±135.3	750.00±245.56	817.66±95.11	665.33±234.61
Colon	97.30±50.7	130.00±10	129.00±3.6	131.00±11
Rectum	17.50±3.5	25.33±10.5	19.66±14.36	17.66±4.93
Internal organ (g)				
Small intestine	626.66±153.73	606.66±135.12	582.00±33.04	531.66±103.72
Colon	268.33±97.51	225.00±18.02	236.66±59.23	293.33±16.07
Rectum	18.00±10.81	17.50±2.5	15.83±1.44	17.16±5
Lung	86.66±15.27	109.16±21.26	103.33±30.55	108.33±20.2
Heart	42.33±11.67	45.00±13.22	51.66±7.63	46.66±2.88
Spleen	18.33±2.88	17.66±4.04	20.00±5	19.16±1.44
Stomach	58.50±13.93	76.66±29.29	65.83±12.33	75.00±25
Kidneys	45.00±8.66	50.00±0	55.00±13.22	45.00±8.66
Liver	193.33±38.83	230.83±13.76	231.66±56.19	210.00±20
Pancreases	12.50±2.5	15.83±3.81	20.83±13.76	16.50±3.77
Urinary bladder	7.16±2.02	7.33±2.51	10.16±2.75	8.33±2.88

Values are shown as mean ± SD.

There were no significant ( $P < 0.05$ ) differences in any item among differing levels of spray-dried porcine plasma supplementation.

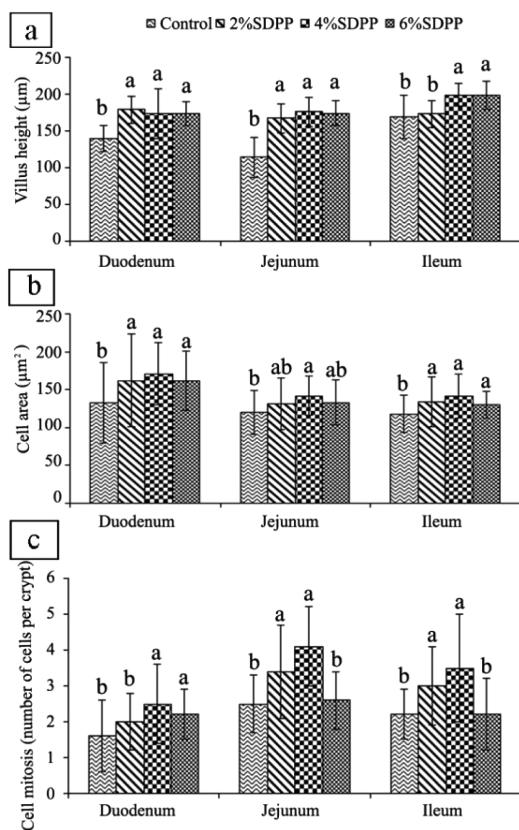
### Light microscopy observations

Inspection by LM of the intestinal villous morphology showed alterations in the villous height, cell area and cell mitosis number. For the duodenum and jejunum, the villous heights in the 2%, 4%, and 6% SDPP-fed groups were significantly higher than those in the 0% SDPP-fed group. For the ileum, the villous heights in the 4% and 6% SDPP-fed groups were significantly higher than those of the 0% and 2% SDPP-fed groups. For the duodenum and ileum, the epithelial cell areas in the 0%, 4% and 6% SDPP-fed groups were significantly different from the 2% SDPP-fed group, while the epithelial cell area in the jejunum of the 4% SDPP-fed group was significantly different from that of the 0% SDPP-fed group. For the duodenum, the occurrences of cell mitosis in the 4% and 6% SDPP-fed groups were significantly higher than those in the 0%

and 2% SDPP-fed groups. For the jejunum, the occurrences of cell mitosis in the 2% and 4% SDPP-fed groups were significantly higher than those of the 0% and 6% SDPP-fed groups. Finally, for the ileum, the occurrences of cell mitosis in the 2% and 4% SDPP-fed groups were higher than those in the 0% and 6% SDPP-fed groups (Figure 1).

### Scanning electron microscopy observations

On the duodenal villi tip surfaces of the control, cell outlines were found between the different each epithelial cells (Figure 2a). For the SDPP-fed groups, a clearer cell outline appeared in the 2% and 4% SDPP-fed groups (Figure 2b, 2c). However, these SEM features become faint and the cell size became small and smooth-surfaced in the 6% SDPP-fed group (Figure 2d).



**Figure 1** (a) Intestinal villus height; (b) cell area; and (c) cell mitosis in the duodenum, jejunum and ileum of piglets fed 0%, 2%, 4% and 6% dietary spray-dried porcine plasma. Vertical error bars show mean  $\pm$  SD. Bars with the same lowercase letters above them within each organ are not significantly ( $P < 0.05$ ) different.

## DISCUSSION

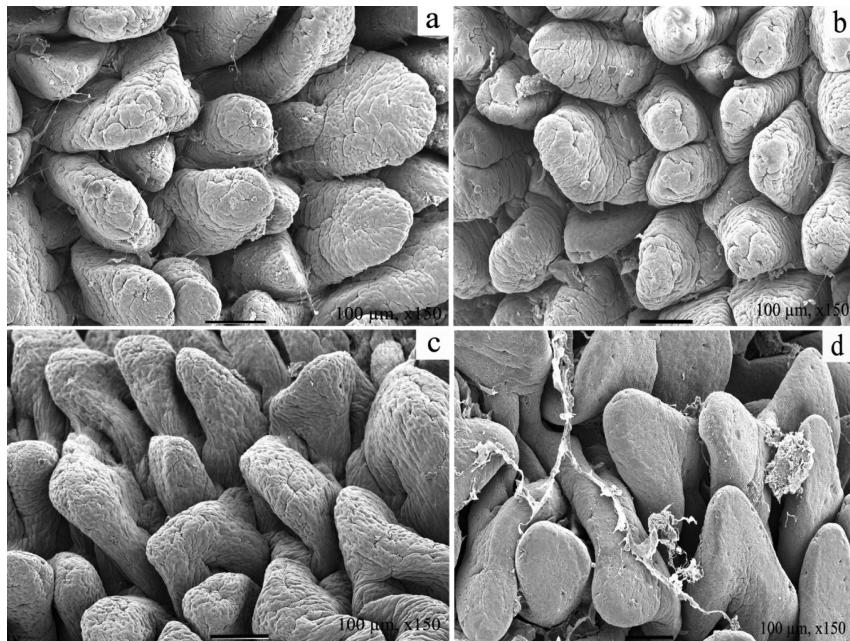
### Growth performance

Spray-dried porcine plasma can be used as a feed additive for swine (Torraldardona, 2010). It has effects on the ADG, the average dairy feed intake (ADFI) and the feed efficiency in piglets ( $P < 0.05$ ). Many researchers reported that SDPP can be added to improve feed palatability and enhance piglet performance

by improving immunocompetence by way of the immunoglobulins present in the SDPP (van Dijk *et al.*, 2001; Pujols *et al.*, 2008). Ermer *et al.* (1994) performed a preference test in which weaning piglets had a choice of diets containing either SDPP or dried skim milk. The ADFI was found to be higher in the piglets which opted for the feed containing SDPP and, thus, it has been suggested that the higher intake was associated with a greater palatability. As for the effects on the ADG, ADFI, and feed efficiency, Hansen *et al.* (1993) found lower dry matter and nitrogen digestibility in piglets consuming diets containing SDPP instead of daily proteins. Knabe *et al.* (1989) found lower apparent ileal amino acid digestibility for SDPP than blood meal. However, the impact of the lower digestibility of SDPP must be small because the feeding of diets with up to 6% SDPP actually improves the ADG and feed conversion ratio. However, the results of this study did not talk about feed cost per gain which can have an economic impact on the user. It is important to find the equilibrium point between cost and profit, known as the break event point which determines the optimum level of SDPP to use.

### Light microscopic and scanning electron microscopic alterations

The intestine is the most important site of nutrient absorption, as it has been suggested that the prevalence of long villi results in an increased surface area capable of greater absorption of available nutrients (Casparty, 1992), and in the current study, a greater villus height and numerous occurrences of cell mitosis in the intestine are indicators that the function of the intestinal villi is activated. In fact, intestinal morphology is markedly affected by the diets fed to animals (Langhout *et al.*, 1999), and in the current study, long villi were observed in the pigs showing an increased body weight gain. Yamauchi *et al.* (1996) suggested that the villus height, the cell area and the number of occurrences of cell mitosis were also related to functional alterations of the



**Figure 2** Duodenal villi tip surfaces of the piglets fed dietary spray-dried porcine plasma at levels of: (a) 0%; (b), 2%; (c); and (d) 6% . The rough surface and the largest cells are in the 2% SDPP-fed group, followed by the 4% SDPP-fed group, in contrast to the faint and smooth surface in the 6% SDPP-fed group.

villus. From the current study, the highest values of most LM parameters in the 4% SDPP-fed group might emphasize the hypertrophied villi, followed by the 6% and 2% SDPP-fed groups. However, the lower values of LM parameters in the 6% SDPP-fed group suggest no activation of the villus function.

The duodenum is an important organ that absorbs nutrients and is easily destroyed by other microorganisms and chyme as was observed by SEM. In the SEM observations, very large cells and a rough surface were found on the duodenal tip surface in the 0%, 2%, and 4% SDPP-fed groups. However, such morphological features become faint in the 6% SDPP-fed group. The result for the 6% SDPP-fed group is not clear and might have been a result of a poor sampling protocol.

## CONCLUSION

It can be inferred that SDPP could be incorporated into piglet diets up to the 4% and 6% levels, and that this might activate the intestinal function at both the villus and cellular levels, resulting in an improvement in feed efficiency. The 4% SDPP level in the diet was the optimum level for improving growth performance and for alteration to the intestinal villous morphology. In addition, although the economic impact was not considered, the level of 4% SDPP appeared to be a better level than 6% SDPP.

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