Effect of Different Spray Dried Plasmas on Growth, Ileal Digestibility, Nutrient Deposition, Immunity and Health of Early-Weaned Pigs Challenged with *E. coli* K88**

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ABSTRACT : A total of 96 piglets were weaned at 19 and 13 days in Exp. 1 and 2, respectively, and allotted to one of four diets: three with different spray dried plasmas (SPs) and one with hydrolysed casein (HC). SPs were from pigs (SPP), mixed origin (SMP), and mixed origin with standardized level of immunoglobulins (SMPIG). All the diets contained 1.7% total lysine, 25% of the test protein source, 45% corn starch, 15% lactose, 2% sucrose, 7% soybean oil. At d 4 and d 2 in Exp. 1 and 2, respectively, piglets were perorally challenged with 10^{10} CFU *E. coli* K88. Growth performance, immunity, and health condition were measured for 15 days and 14 days in Exp. 1 and 2, respectively. To investigate apparent ileal digestibility and nutrient deposition, all piglets were sacrificed at d 14 in Exp. 2. In Exp. 1, 3 piglets died in HC diet and 1 in SPP diet. HC diet showed higher mortality ($p<0.01$) than other diets. In Exp. 2, no clinical sign of infection was detected, no difference for the content of *E. coli* K88 was found in feces at 4 and 6 days after the infection, and no *E. coli* K88 was found in the jejunum at the end of experiment. In both experiments, feed intake was lower for HC diet and ADG was 96, 106, 122 and 155 for HC, SPP, SMP and SMPIG diet, respectively (HC vs others, $p<0.05$; SMPIG vs other SP, $p<0.01$). Ileal apparent digestibility of nitrogen in sacrificed piglets was higher for HC diet ($p<0.05$). After the challenge, K88-specific IgA titers in saliva (Exp. 1) and in plasma (Exp. 2) were reduced in SMP and SMPIG. The piglets positive to the adhesion of the used *E. coli* strain to the intestinal brush borders had a significantly reduced growth ($p<0.01$) and a higher K88-specific IgA titer in plasma, in comparison with negative ones. This effect was independent of the diet. The data show the relevance of spray dried plasma sources and particularly of SP with standardized level of immunoglobulins for the feeding of early-weaned pigs at the risk of infection by enterotoxigenic bacteria. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 8 : 1138-1143)*

Key Words : Piglet, Spray Dried Plasma, Ileal Digestibility, Nutrient Deposition, Immunity

INTRODUCTION

It is well established that spray dried porcine plasma (SPP) is a valid protein source in the feed of early-weaned pigs and a substitute for spray dried skimmed milk (Thacker, 1999). In very young pigs, many researchers reported that SPP increases ADG and/or feed intake (Ermer et al., 1994; Kats et al., 1994; Coffey and Cromwell, 1995; Chae et al., 1999; Kim et al., 2000)

One explanation of SPP efficacy in the feeding of young pigs deals with its content of immunoglobulins (Ig). It was observed that SPP reduces the occurrence of post-weaning diarrhea (Van der Peet-Schwering et al., 1996) and Gatnau et al. (1989) showed that SPP is a source of absorbable and active Ig in newborn piglets. In performance trials it was demonstrated that, in spray dried plasma, the Ig fraction is the responsible for the positive effect of the product (Gatnau et al., 1995; Owen et al., 1995) or that Ig and albumin fractions could maintain similar performance (Owen et al., 1995). The better performance obtained with the Ig fraction from spray dried plasma can be explained by a protective effect against the adhesion of some pathogens, preventing the colonization of the enterocytes. Deprez et al. (1996) observed in piglets a reduced fecal excretion of a hemolytic *E. coli* strain, even if the SPP was not showing specific antibodies against this strain. This was confirmed by Nollet et al. (1999) and agrees with the *in vivo* inhibition of the adhesion of *E. coli* K99 to enterocytes by means of glycoproteins from bovine plasma (Mouricout et al., 1990). The presence of non-specific mechanism of defense could explain the positive effect of spray dried plasma from different sources on growth performance. Other factors for better performance obtained with spray dried plasma could be its digestibility or its acceptability.

Our goal was to test the effect of commercial spray dried plasmas of different origin or Ig content on growth, ileal digestibility, health and immune function of early-

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weaned pigs challenged with *E. coli* K88.

MATERIALS AND METHODS

Animals, diets and experimental procedures

Early-weaned pigs were allotted by litter and weight to four diets: based on either 3 different spray dried plasmas (SPs) or on hydrolysed casein (HC). SPs were from pigs (SPP), mixed origin (SMP), and mixed origin with standardized level of immunoglobulins (SMPIG). Two experiments were conducted (48 piglets, 4 treatments, 12 per treatment in each experiment).

The ingredients of the basal diet are presented in table 1. The diets were formulated to contain 1.7% lysine, 0.5% methionine, 1.1% threonine, 0.3% tryptophan and 1.0% isoleucine, and 0.85% calcium and 0.67% phosphorus. Chromic oxide (0.3% wt/wt) was included in the diet as an indigestible marker for determination of ileal digestibility. No anti-microbial additive was added. To prevent possible anti-nutritional or allergic factor from soy-based protein, hydrolysed casein was used in the control diet formulation (Newport, 1980).

Table 1. Composition (%) of experimental diets in Exp. 1 and 2.

	Dietary protein sources[a]	
	HC	SPP, SMP & SMPIG
Ingredients		
Dehydrated casein	25.00	-
Spray dried plasma	-	25.00
Corn starch	42.80	43.56
Lactose	14.00	14.00
Soybean oil	7.00	7.00
Sucrose	2.00	2.00
Dried beet pulp	1.00	1.00
Wheat straw	1.00	1.00
DL-Methionine,	-	0.34
Isoleucine	-	0.35
Lysine-HCL	0.16	-
Tryptophan	0.04	-
Threonine	0.25	-
Calcium carbonate	0.75	1.40
Dicalcium phosphate	3.20	1.50
Chromium oxide	0.30	0.30
Sodium chloride	0.50	-
Magnesium sulphate	0.50	0.45
Potassium carbonate	-	0.60
Vitamin and mineral supplement	1.50	1.50

[a] HC: hydrolysed casein; SPP: spray dried plasma from pigs; SMP: spray dried plasma of mixed origin; SMPIG: spray dried plasma of mixed origin and standardized level of immunoglobulins.

In Exp. 1, piglets were weaned at 19 days and were raised for 15 days. In Exp. 2, pigs were weaned at 13 days and were sacrificed after 14 days. In addition, 4 piglets were sacrificed at the start of the experiment for body composition assessment. In Exp. 1, only growth performance and health were assessed. Piglets were housed in individual pens with mesh floor. The temperature in the nursery was automatically regulated to vary from 30°C at the start to 24°C at the end of the experiments. In the first 3 days, infrared lamps located over the piglets provided the heating zone.

Piglets were challenged per os with 10^{10} CFU of *E. coli* K88 O148, after 4 and 2 days in Exp. 1 and 2, respectively. Fecal samples were collected at 4 and 6 days after the challenge in Exp. 1 and 2, respectively, then, within each diet, feces of 3 piglets were pooled together.

Sample collection, apparent ileal digestibility and nutrient deposition

The piglets to sacrifice were anaesthetized with sodium thiopenthal (10 mg/kg live weight) and then euthanised with a intracardiac injection of Tanax® (A.I.C. Hoechst Roussel, 0.5 ml/kg live weight) within one hour after the last meal in Exp. 2. For microbial content determination a portion of the central jejunum of each subject was isolated, resected, placed in a sterile basin and opened longitudinally. Then two samples from the mucosa of jejunum were obtained by gently scraping and carefully mixed. A second portion from the same intestinal tract was excised to collect villi for the in vitro villous-adhesion assay. The digesta content of ileum was sampled for digestibility measurement. The residual of the digestive tract was emptied and weighted for analysis of the chemical composition of the empty body. The individual samples of the homogenized body and of the ileal chyme were freeze-dried and then analyzed for dry matter, ash, lipid and nitrogen content. The analyses for chemical composition of the diet and the body were carried out according to the official methods of analysis of AOAC International (AOAC, 1995). The crude protein (CP) apparent ileal digestibility was measured by the slaughter method (Donkoh et al., 1994). The chromic oxide contents of diets and ileal digesta were determined by the method of Fenton and Fenton (1979).

Immunity and bacterial counting

Saliva and blood samples were collected for the measure of K88 fimbriae-specific IgA ELISA. The total content of *E. coli* in the chyme was measured as reported by Bosi et al. (1999). For *E. coli* K88 detection, a rabbit immune sera against antigen from a *E. coli* K88 + strain of the bank of Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia was used.

The *in vitro* villous-adhesion assay was done with the method proposed by Van den Broek et al. (1999) and according to the response to the test, pigs were classified as K88 positive-receptor pigs ($K88R^+$) or K88 negative-receptor pigs ($K88R^-$), that is pigs susceptible or not were made as reported by Van den Broek et al. (1999).

Statistical analysis

For Exp. 1, the data were analyzed by means of a linear model considering the effect of the diet and litter. In case of bacterial counts, litter was not considered, but the day of sampling was added. For Exp. 2, effects of the sensibility to *E. coli* K88 adhesion and the interaction with diet were added to the basal model. The analyses were performed by SAS (1996), with dietary treatment least squares means adjusted for the other effects. Three orthogonal contrasts were evaluated: HC vs others, SPP + SMP vs SMPIG and SMP vs SMPIG.

RESULTS

Experiment 1

In Exp. 1, the feeding with SPs did not significantly improve daily live weight gain, but there was a trend of increase of daily feed intake (11%, p=0.08, table 2). The use of SMPIG as protein source improved growth compared with SPP (69%, p<0.05), but not with SMP. However, some piglets (4 and 1 piglets in HC and SPP diet, respectively) were excluded from these comparisons because of death during Exp. 1 (table 2). In all the dead pigs, *E. coli* K88 was detected and some signs of enterotoxaemia were found. The mortality was significantly higher in the HC group. However, diet did not change the number of subjects in therapy.

Effects of the protein source on K88-specific IgA titers in saliva at 10 days after the challenge are shown in table 2. A trend of decrease of IgA titer in saliva was observed for all the diets with spray dried plasmas (p=0.09). This susceptible, respectively, to *E. coli* infection. The isolation of K88 fimbriae and the detection of K88-specific IgA antibodies was due to the very low level observed for SMP and SMPIG.

Experiment 2

In Exp. 2, the dietary substitution of the spray dried plasmas for hydrolysed casein improved ADG (p=0.08) and ADFI (p=0.07, table 3). The positive effect of SPs on growth was mainly due to the high growth of the group fed SMPIG. This group had a 25% higher growth compared with others SPs (p<0.05). CP apparent ileal digestibility was higher for the group fed hydrolysed casein (p<0.01). No significant difference was observed between the different SPs. No significant interaction between diet and K88 receptor type on growth performance and CP apparent ileal digestibility was found. The effect of K88 receptor type on growth performance and CP apparent ileal digestibility is presented in table 4. Piglets with $K88R^+$ had a 31% reduced growth (p<0.01) and an impaired food conversion (p<0.05). Receptor type did not affect CP apparent ileal digestibility.

Body composition (table 3) was not affected by the protein source, except for ash body content that was reduced in piglets fed SMPIG (p<0.01, vs others SPs). Feeding SPs increased daily body protein (p=0.08) and body fat (p<0.05) gain. Compared with other SPs, SMPIG increased body protein growth only as a tendency (p=0.07).

Effects of the dietary protein source and K88 receptor type on K88-specific IgA titer in plasma are presented in table 5. The K88-specific IgA titer was reduced in SMPIG group compared with HC group (p<0.07) and SPP group

Table 2. Effects of protein sources on growth performance, health condition and K88-specific IgA titer in saliva of piglets in Exp. 1

	Protein sources[a]				SE	HC vs others	SPP+ SMP vs SMPIG	SMP vs SMPIG
	HC	SPP	SMP	SMPIG				
Growth performance								
Initial live weight, kg	5.02	4.93	4.92	4.78	0.21	NS	NS	NS
Final live weight, kg	6.53	6.43	7.00	7.32	0.36	NS	NS	NS
ADG,g	101	100	139	169	23.0	NS	0.09	NS
ADFI, g	226	251	251	252	11.3	0.08	NS	NS
F/G	2.25	2.52	1.80	1.49			ND[c]	
Health condition, %								
Pigs in therapy	4.2	8.3	8.3	4.2				
Pigs dead	25.0	8.3	0	0				
K88-specific IgA titer[b]	0.47	0.51	0.04	0.11	0.13	0.09	NS	NS

[a] See note in table 1. [b] At 10 days after challenge, √(arbitrary units/ml). [c] Not determined, due to some negative values. NS: not significant, p>0.1.

Table 3. Effects of protein sources on growth performance, apparent ileal digestibility, body composition and daily nutı means)

Item	Protein sources[a]				SE	HC vs others	SPP+ SMP vs SMPIG	SMP vs SMPIG
	HC	SPP	SMP	SMPIG				
Growth performance								
Initial live weight, kg	4.14	4.25	4.24	4.22	0.13	NS	NS	NS
ADG,g	100	118	107	140	10.6	0.08	*	*
ADFI, g	197	208	208	215	6.5	0.07	NS	NS
F/G	1.96	1.78	1.96	1.53	0.41	NS	NS	NS
Apparent ileal digestibility, %								
Crude protein	84.3	72.1	70.6	72.4	4.2	**	NS	NS
Chemical composition, %								
Dry matter	27.6	28.9	28.6	28.5	0.66	NS	NS	NS
Crude protein	14.3	14.2	14.3	14.4	0.18	NS	NS	NS
Fat	8.3	9.6	9.0	9.3	0.53	0.10	NS	NS
Ash	3.3	3.2	3.5	3.0	0.09	NS	**	**
Daily nutrient gain, g								
Crude protein	15.6	17.9	17.9	21.9	1.40	0.08	0.07	NS
Fat	2.9	10.0	6.8	10.5	2.10	*	NS	NS
Ash	3.7	4.2	5.0	4.0	0.42	NS	NS	0.08

[a] See note in table 1. NS: not significant, p>0.1; * p<0.05; ** p<0.01.

Table 4. Effects of K88 receptor type on growth performance and apparent ileal digestibility of piglets in Exp. 2 (least square means)

	K88 receptor type[a]		SE	Statistical significance
	$K88R^+$	$K88R^-$		
Growth performance				
Initial live weight, kg	4.32	4.10	0.10	NS
Final live weight, kg	5.62	6.06	0.15	0.08
ADG,g	95	138	8.2	**
ADFI, g	200	214	5.2	0.09
F/G	2.92	1.35	0.51	*
Apparentileal digestibility, %				
Crude protein	76.8	72.7	4.0	NS

[a] R^+: positive-receptor pigs, R^-: negative-receptor pigs
NS: not significant, p>0.1; * p<0.05; ** p<0.01.

(p<0.05). Piglets with the receptors for *E. coli* K88 in the jejunal brush border membranes showed higher IgA titer than those without (p<0.01).

Diets did not change significantly total *E. coli* and *E. coli* K88 contents in feces (table 6). However, *E. coli* K88 was not detected in the sample from jejunum at the end of Exp. 2.

DISCUSSION

Effects on growth performance

In our study, piglets challenged will E. coli K88 were only observed for a short-term period after weaning because health and immunity are highly sensitive to diet and environment in this period. A limited significance for ADG was observed between HC diet and other SPs because of high mortality in HC diet and different ADG among different source of SPs. In detail, growth performance was improved by feeding SPs in Exp. 2 only, but it is noticeable that in Exp. 1, piglets with low performance were excluded as death in HC group. In addition, a trend of increase of body protein growth was observed. All the whole SPs are better protein sources than hydrolysed casein for the early-weaned pig in our study. However, casein is not considered as a best ingredient for early-weaned pigs because it reduced ADFI compared with other protein sources like isolated soybean protein, fish meal, soybean meal, or blended protein (Leibholz, 1982; Hansen et al., 1990). The better performance was permitted by the higher feed intake observed on average for SP groups in both trials. This agrees with the dietary preference for spray dried porcine plasma compared to dried-skim milk observed in piglets by Ermer et. al. (1994). In our experiments, piglets fed spray dried plasma of mixed origin and with standardized level of immunoglobulins showed higher ADG than those fed other SPs. If we pool the values of both experiments, ADG were 106, 122 and 155 for SPP, SMP and SMPIG, respectively (SMPIG vs other SPs, p<0.01; SMPIG vs SMP, p<0.07). However, the use of a SP with a standardized level of immunoglobulins does not seem to improve further the feed intake compared with normal SPs.

The better results obtained with SPs are not related to CP apparent ileal digestibility, as HC presented a higher value than those of all the SPs. The average values for HC and for

Table 5. Effect of protein source and K88 receptor type on K88-specific IgA titer in plasma of piglets at the end of Exp. 2

Item	Protein sources[a]				SE	K88 receptor type[b]		SE
	HC	SPP	SMP	SMPIG		K88 R^+	K88 R^-	
K88-specific IgA titer[cd]	2.85	3.37	1.38	0.67	0.97	3.49	0.65	0.66

[a] See note in table 1. [b] R^+: positive-receptor pigs, R^-: negative-receptor pigs. [c] √(arbitrary units/ml). [d] SMPIG vs SPP: $p<0.05$, SMPIG vs HC: $p<0.07$, effect of K88 receptor type: $p<0.01$. No interaction between protein sources and receptor types.

Table 6. Effects of protein sources on least square means of *E. coli* K88 and total *E. coli* contents in feces at 4 and 6 days after challenge in Exp. 1 and Exp. 2, respectively.

Item	Protein sources[a]				SE
	HC	SPP	SMP	SMPIG	
E. coli K88, ln CFU[bc]	9.8	14.1	13.0	12.2	2.5
Total *E. coli*, ln CFU	22.3	17.9	17.1	18.0	2.7

[a] See note in table 1. [b] Colony forming unit.
[c] At 12 days after the challenge, *E. coli* K88 was not detected by the scrapings from jejunum mucosa of piglets.

the SPs were similar with the values for CP true ileal digestibility reported by NRC (1998) and Chae et al. (1999). Recently, Jiang et al. (2000a) also reported that supplementing early-weaned pig diets with animal plasma increased the efficiency of dietary protein use for lean tissue growth in early-weaned pigs and that this response is mediated in part by decreased amino acid catabolism.

Effects on Immunity and Pathology

Cain and Zimmerman (1997) observed that SP does not reduce fecal shedding of haemolytic *E. coli* in a segregated early-weaned environment. In Exp. 2, SPs did not reduce *E. coli* K88 content in feces, while in Exp. 1, SPs could protect better against the development of enterotoxaemia. Deprez et al. (1996) and Nollet et al. (1999) observed a reduced fecal excretion of the challenge haemolytic *E. coli* strain and protection against clinical symptoms even if the SPP was not showing specific antibodies against this strain.

Improved ADG in SMPIG group agrees with other researches showing that the immunoglobulin fraction is the main component responsible of the effect of SPs. However, due to a similar excretion of *E. coli* K88 in feces and a reduced mortality in all the SP groups, we could not verify if the immunoglobulin fraction was the responsible of a reduced invasive effect of *E. coli* K88. In both experiments, the reduced K88-specific IgA titers in plasma and in saliva indirectly support this hypothesis. It is speculated that the stimulation of the local immune system is reduced when feed immunoglobulins aggregate the challenged *E. coli*. Furthermore, Godfredson-Kisic et al. (1999) showed that in phase 1 diets of early weaned pigs, 2% of porcine globulin proteins could maintain the same performance as 8% of SPP. Then, Jiang et al. (2000b) observed that at the same feed intake as control, dietary plasma protein significantly reduced the intravillous lamina propria cell density in the proximal jejunum. This supports again the hypothesis of a reduced local proinflammatory response and a reduced leukocytic infiltration.

The differences in growth and K88-specific IgA titer between different pig types for intestinal receptors clearly indicate that the adhesion of *E. coli* K88 to small intestinal villous brush borders is required for the production of IgA against the K88 fimbriae. This agrees with the absence of immune response to vaccination with K88 fimbriae in case of pigs not-susceptible to *E. coli* infection, observed by Van den Broek et al. (1999). Nevertheless, in our case, even in some not-susceptible pigs, a little K88-specific IgA titer was observed. This could be due to some episodic adherence not detected at the adhesion test or to the translocation of some bacteria throw the epithelial wall with the consequent contact to the lamina propria and the stimulation of the immune system. After *E. coli* K88 challenge, in gnotobiotic not-susceptible piglets, Berberov et al. (1999) detected some bacteria in mesenteric lymph nodes, but not in blood.

Concerning the dietary effect on K88-specific IgA titer, the low values observed in saliva and plasma for the SMPIG diet can be explained by a reduced contact with the challenged *E. coli* strain in the susceptible piglets.

CONCLUSIONS

In early-weaned pigs orally challenged with *E. coli* K88 the substitution of spray dried plasma for hydrolysed casein improves growth, protein and lipid deposition and resistance to enterotoxaemia. Spray dried plasma of mixed origin with standardized level of immunoglobulins provides better performance than those from swine or from mixed origin without Ig standardized level. The low K88-specific IgA titers observed in plasma and saliva of susceptible subjects fed plasma with Ig standardised level are indicators of a better protection against the challenged *E. coli* strain. The production of IgA against *E. coli* K88 is linked to the presence of receptors for this microbial strain in the jejunum.

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