



**HAL**  
open science

## Dietary specific antibodies in spray-dried immune plasma prevent enterotoxigenic F4 (EPEC) post weaning diarrhoea in piglets

T.A. Niewold, A.J. van Dijk, P.L. Geenen, H. Roodink, R. Margry, J. van Der Meulen

### ► To cite this version:

T.A. Niewold, A.J. van Dijk, P.L. Geenen, H. Roodink, R. Margry, et al.. Dietary specific antibodies in spray-dried immune plasma prevent enterotoxigenic F4 (EPEC) post weaning diarrhoea in piglets. *Veterinary Microbiology*, 2007, 124 (3-4), pp.362. 10.1016/j.vetmic.2007.04.034 . hal-00532256

**HAL Id: hal-00532256**

**<https://hal.science/hal-00532256>**

Submitted on 4 Nov 2010

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Accepted Manuscript

Title: Dietary specific antibodies in spray-dried immune plasma prevent enterotoxigenic *Escherichia coli* F4 (ETEC) post weaning diarrhoea in piglets

Authors: T.A. Niewold, A.J. van Dijk, P.L. Geenen, H. Roodink, R. Margry, J. van der Meulen



PII: S0378-1135(07)00216-7  
DOI: doi:10.1016/j.vetmic.2007.04.034  
Reference: VETMIC 3676

To appear in: *VETMIC*

Received date: 6-2-2007  
Revised date: 19-4-2007  
Accepted date: 25-4-2007

Please cite this article as: Niewold, T.A., van Dijk, A.J., Geenen, P.L., Roodink, H., Margry, R., van der Meulen, J., Dietary specific antibodies in spray-dried immune plasma prevent enterotoxigenic *Escherichia coli* F4 (ETEC) post weaning diarrhoea in piglets, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.04.034

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 Dietary specific antibodies in spray-dried immune plasma prevent enterotoxigenic  
2 *Escherichia coli* F4 (EPEC) post weaning diarrhoea in piglets.

3

4 Niewold, T.A.<sup>1,4\*</sup>, van Dijk<sup>2</sup>, A.J., Geenen, P.L.<sup>1,5</sup>, Roodink, H.<sup>3</sup>, Margry, R<sup>2</sup>, van der  
5 Meulen, J<sup>1</sup>.

6

7 1. Animal Sciences Group of Wageningen University and Research, P.O. Box 65, Lelystad,  
8 The Netherlands

9 2. CCL-Research, PO Box 107, NL-5460 AC, Veghel, The Netherlands

10 3. Sonac, PO Box 50, NL-7370 AA, Loenen (Gld), The Netherlands

11 4. Present address: Nutrition and Health Unit, Faculty of Bioscience Engineering, Katholieke  
12 Universiteit Leuven, B-3001 Heverlee, Belgium

13 5. Present address: Department of Animal Health Economics, Wageningen University, The  
14 Netherlands

15

16

17

18

19

20 \*: corresponding author

21 Tel: +32 16 321560, Fax: +32 16 321994, e-mail:theo.niewold@biw.kuleuven.be

22

23

24 Keywords: *E. coli*, pig, antibody, spray-dried plasma, F4, heat labile toxin (LT)

25 **Abstract**

26

27 In order to establish the mechanism of spray dried plasma powder (SDPP) in improving  
28 pig health and performance, a diet containing either 8% SDPP, spray dried immune plasma  
29 powder (SDIPP), or control protein (soybean and whey) ration was fed to piglets in an  
30 experimental model of enterotoxigenic *Escherichia coli* F4 (ETEC) post-weaning diarrhoea  
31 (PWD). SDIPP was obtained from pigs immunized with a vaccine containing ETEC fimbrial  
32 subunit F4 and heat-labile toxin (LT), and SDPP from non-immunized controls. Average  
33 daily growth (ADG) was determined, and daily samples of rectal faeces were assessed for  
34 diarrhoea (as percentage of dry matter), and ETEC excretion (in CFU/g).

35 SDPP and SDIPP ( $p < 0.05$ ) significantly reduced diarrhoea, and SDIPP significantly  
36 reduced ETEC excretion. ADG was not significantly ( $p > 0.05$ ) affected. After the  
37 experiment, 30% of piglets tested F4 receptor positive (F4R+). A significant correlation  
38 between F4R status and morbidity was found. In F4R+ animals, SDIPP significantly  
39 improved diarrhoea and ADG, and decreased ETEC excretion, and SDPP significantly  
40 improved diarrhoea and ADG. Surprisingly, SDPP reduced diarrhoea in F4R+ animals  
41 without significant reduction of ETEC excretion, which is most likely related to the presence  
42 of anti-LT antibodies in SDPP.

43 The results show that oral protection against ETEC by SDPP is attributable to spontaneous  
44 antibodies, in this case anti-LT antibodies. Furthermore, the results indicate that the  
45 combination of anti-LT and anti-F4 antibodies as in SDIPP is most effective in ETEC  
46 prevention. Finally, the F4R distribution in the herd should be taken into account to correctly  
47 assess efficacy.

## 48 1. Introduction

49

50 Spray dried plasma powder (SDPP) is a common protein source in starter diets for weaned  
51 piglets in many countries. It is described to improve growth and health during the first days  
52 after weaning, in particular under high infection pressure (Coffey and Cromwell, 1995,  
53 Bergstrom et al., 1997). Results are varying, which is probably also related to the  
54 susceptibility for one of the most common diseases in the post-weaning period, post weaning  
55 diarrhoea (PWD). Enterotoxigenic *Escherichia coli* (ETEC) induced PWD is an important  
56 cause of morbidity and mortality in neonatal and weaned piglets. In pigs, successful intestinal  
57 colonization of ETEC strains is associated with bacterial adhesion factors (fimbriae), such as  
58 F4 (Gaastra and de Graaf, 1982). Upon colonization ETEC can produce several toxins among  
59 which heat labile toxin (LT), which cause secretory diarrhoea. Immunization of the sow with  
60 ETEC adhesion factors and LT protects suckling piglets against PWD, weaning withdraws  
61 lactogenic protection, and maternal colostrum-derived systemic antibodies in piglets are  
62 ineffective (Francis and Willgohs, 1991). Dietary inclusion of specific antibodies raised  
63 against F4 (Yokoyama et al 1992, de Geus et al., 1998, Owusu-Asiedu et al., 2002, 2003)  
64 protected against *E. coli* F4 ETEC. The mechanism behind SDPP is unclear, transfer of  
65 passive immunity has been proposed (van Dijk et al., 2001), but only in one case specific anti-  
66 F4 antibodies were found in SDPP (Owusu-Asiedu et al., 2002).

67 The objectives of the present study were 1. to establish the effect of addition of SDPP and  
68 spray dried immune plasma (SDIPP) to the weaning diet on prevention of enterotoxigenic *E.*  
69 *coli* F4 post-weaning diarrhoea (PWD), 2. to get insight into the role of specific antibodies in  
70 the pathogenesis of ETEC, and 3. to determine the influence of the F4R status on this process.

71

72

73 **2. Material and methods**

74

75 *2.1. Bacterial strains*

76

77 *E. coli* O149K91 was isolated from a pig farm with post weaning diarrhoea. The ETEC  
78 strain was typed at the Animal Sciences Group in Lelystad, and further characterized as F4  
79 (K88ac), LT+, STb+ (Nabuurs et al., 1993), and designated CVI-1000. As a control in the  
80 brush border adhesion studies CVI-1084 was used, a strain identical to CVI-1000 but without  
81 F4 fimbrial expression (a gift from Dr. F. van Zijderveld, Department of Bacteriology,  
82 Animal Sciences Group, Lelystad, the Netherlands). The strains were grown overnight in  
83 brain heart infusion broth (Difco Laboratories, Detroit, MI, USA), pelleted by centrifugation  
84 and resuspended in PBS (pH 7.2) to an extinction at 600 nm, corresponding to 10<sup>9</sup> CFU/ml.

85

86 *2.2. Post weaning diarrhoea (PWD) animal model*

87

88 The protocol was approved by the local Ethics Committee for Animal Experiments in  
89 Lelystad. PWD is induced in piglets weaned at 3 weeks of age. The induction protocol  
90 involves cold stress, fasting, treatment with colistin to disturb intestinal ecology, inoculation  
91 with rotavirus, and inoculation with ETEC. Animals (96 from 16 different litters, 3 wk of age)  
92 were purchased from a commercial piggery, and transported to our facilities on the day of  
93 weaning. Piglets were housed in 4 multipurpose boxes, each containing 3 pens, separated by  
94 closed planking, containing 8 piglets each. Piglets were divided over the pens, taking litter,  
95 weight, and sex into account. Piglets were kept at a 16 h light, 8 h dark cycle, at 24°C .  
96 Animals were fasted on day 1 and 2. Water was given ad lib throughout the experiment.  
97 Colistin sulphate (Eurovet, Bladel, The Netherlands)(60 mg/L) was added to the drinking

98 water on the first 5 days. From day three on, animals had ad lib access to one of the three diets  
99 (containing SDPP, SDIPP, or control protein). On day 5, all piglets were orally inoculated by  
100 syringe with 2 ml ( $10^6$  particles/ml) rotavirus strain RV277 (Debouck and Pensaert, 1979)  
101 suspension. On day 6 and 7, all animals received 5 ml ETEC suspension of  $10^9$  CFU/ml. From  
102 day 2 on, each day the animals were weighed, their health was assessed, the total food intake  
103 per pen was determined for determination of average daily food intake (ADFI) and feed  
104 conversion ratio (FCR). Daily, a rectal content sample was taken for determination of the %  
105 dry matter (%DM), and for determination of ETEC counts. On day 15, the animals were  
106 euthanized by Nembutal, bled, and heparin plasma was taken. Gross pathology was  
107 performed, and a jejunal sample was taken for determination of F4R status by brush border  
108 adhesion assay. On animals found dead gross pathology was performed and, if possible, a  
109 plasma and a jejunal sample were collected.

110

### 111 *2.3. Determination of the percentage dry matter (%DM) of faeces*

112

113 Faeces (0.5-3 g) was weighed into aluminium trays. Samples were desiccated for 20 h in  
114 an incubator at 80 °C, and weighed again to determine lost water.

115

### 116 *2.4. Quantification of ETEC excretion*

117

118 One gram of faeces was weighed into 10 ml tubes, and 9 ml of 0.9% NaCl was added, and  
119 vortexed. Dilutions were plated on sheep blood agar plates containing 5% sheep blood,  
120 streptomycin 50 µg/ml, tetracycline 25 µg/ml, vancomycin 50 µg/ml (Biotrading, The  
121 Netherlands). Haemolytic colonies with the typical appearance of *E. coli* were counted. In  
122 cases of ambiguous colony morphology, identity was confirmed by slide agglutination.

123

## 124 2.5. Source of plasma, and immune plasma powder

125

126 At a large commercial breeder and fattener, animals (n=320) were immunized (i.m. in the  
127 neck) with 2 ml of Porcilis coli (Intervet International BV, Boxmeer, The Netherlands)  
128 containing adhesion factors K88ab (F4), K88ac (F4), K99 (F5), 987P (F6), and heat labile  
129 toxin (LT). Pigs were immunized at 6, 4, and 2 weeks before slaughter (at ca. 115 kg live  
130 weight). The non-immunized control group (n= 320) was from the same farm, and from the  
131 same period. At the slaughterhouse of the farm, blood was collected separately per animal  
132 into citrated bags. After post mortem inspection, blood was pooled per group (ca 1000 L),  
133 transported to the plasma protein factory (Sonac), plasma was separated by centrifugation, the  
134 pH increased to 10 using a 25% ammonia solution, and stored overnight at 2°C. The next day,  
135 plasma was two-fold concentrated by ultra filtration (membrane cut-off 9 kD, MSD3 Ultra  
136 filtration unit, Stork, The Netherlands), and spray dried in an Anhydro Lab S1 spray dryer  
137 (Anhydro, Copenhagen, Denmark), at an outlet temperature of 80-83 °C. Since antibiotics  
138 possess growth promoting and antibacterial activity, anti-microbial drug residue was  
139 determined by the well diffusion method, using 10% SDPP and SDIPP in PBS in 9 mm  
140 diameter wells in 5 different 2 mm agar plates seeded with *Micrococcus luteus* at pH 6 or pH  
141 8, and *Bacillus subtilis* at pH 6 or pH 8 with or without Trimethoprim. After overnight  
142 incubation at 37°C the inhibition zone was measured. An inhibition zone < 3 mm is  
143 considered to be negative. Microbiological quality was determined according to the  
144 International Standard, for total bacterial count (ISO 4833:1991(E), for *Enterobacteriaceae*  
145 (ISO 7402:1993(E), and *Salmonella spp* (ISO 6579:1993(E). The percentage dry matter was  
146 determined by drying at 103 °C, and the soluble fraction in water was determined  
147 gravimetrically.

148

149 *2.6. Production of pelleted feed*

150

151 The composition of the three experimental diets is shown in Table 1. The diets contained  
152 either soybean (meal) and whey powder, or SDPP, or SDIPP as main protein source. The diets  
153 were pelleted at 61 °C, 66 °C and 67 °C, respectively and were formulated to contain 1.03 %  
154 apparent ileal digestible lysine, 2352 kcal NE/kg and 4.7 % lactose (van Dijk et al., 2002).  
155 Analysis of feed was performed as described before (van Dijk et al., 2002). The total  
156 immunoglobulin content of SDPP, SDIPP, whey powder and the diets was determined by  
157 protein-G affinity chromatography with UV detection (Pharmacia/LKB, Uppsala, Sweden).

158

159 *2.7. Immunological analysis*

160

161 Pelleted samples were first ground up to powder. Dry powder samples were incubated  
162 overnight 10% w/v in PBS at 4°C. Samples were then centrifuged and the supernatant  
163 analyzed. Samples were analyzed by ELISA for the presence of specific antibodies. Pooled  
164 plasma from specific pathogen free (SPF) piglets from the Lelystad facilities served as  
165 negative control. Indirect ELISA for determination of anti-F4 and anti-LT titre was performed  
166 according to van Zijderveld et al. (1990), and Lauterslager et al. (2001) respectively, using  
167 96-wells microtiter plates coated with 0.5 µg purified F4ac/well or 0.05 µg LT (Sigma, St.  
168 Louis, Missouri, USA)/well. Samples were serially diluted, incubated, washed, and bound  
169 antibodies were detected with HRPO-labelled rabbit anti-swine immunoglobulins (Dakopatts,  
170 Copenhagen, Denmark). Titres were expressed as EC50.

171

172 *2.8. Determination of piglet F4R status by brush border adhesion (BBA) assay*

173

174 At necropsy, 5-10 cm of jejunal mucosa was scraped off, and epithelial brush borders were  
175 prepared, and the F4R status determined using the BBA method modified after Sellwood et al.  
176 (1975). The number of bacteria attached to 50-100 cells with well-defined brush borders were  
177 counted by phase contrast microscopy (magnification, x 400). Animals with no or an average  
178 of 1-2 bacteria/brush border were considered F4R-, samples exceeding this are judged F4R+.  
179 In case of ambiguity, the test was repeated.

180

### 181 *2.9. Brush border adhesion (BBA) inhibition assay*

182

183 Functionality of plasma was determined by the BBA assay described above, modified as  
184 described (Harmsen et al., 2005). Briefly, serial dilutions of plasma preparations were added  
185 to brush border preparations. Then the bacterial suspension was added, and bacterial adhesion  
186 was determined. Titres were expressed as the reciprocal highest dilution showing complete  
187 inhibition of BBA.

188

### 189 *2.10. LT Vero-cell assay*

190

191 Functionality of anti-LT antibodies was assayed in Vero cells (Speirs et al., 1977). Pooled  
192 plasma from SPF-piglets from the Lelystad facilities served as negative control.

193

### 194 *2.11. Statistics*

195

196 The piglet was the experimental unit. A four factorial analysis on the effects of the  
197 different feeds, the two F4 receptor types, box and pen was carried out. There were no main

198 and interaction effects of box and pen and consequently these factors were omitted from  
199 further analysis. A two factorial ANOVA with the factors FEED (control, SDPP, SDIPP) and  
200 F4Receptor (F4R+, F4R-) was performed (GLM procedure, SAS version 9.1, SAS Institute,  
201 Cary, NC). In case of significant FEED by F4R interactions one factorial ANOVA was  
202 performed with the factor Group (representing all 6 combinations of FEED and F4R)  
203 supplemented by Fisher's least significant difference (LSD) post hoc comparisons. Statistical  
204 significance was predefined at  $p < 0.05$ . Data are presented as mean  $\pm$  sem.

205

### 206 **3. Results**

207

#### 208 *3.1. Feed analysis*

209

210 Analysis of pelleted feed showed that the analyzed values for feed composition were in  
211 line with those calculated (Table 1). The immunoglobulin content of control feed was below  
212 the detection limit, in undiluted preparations the immunoglobulin content was 1.3 % in whey  
213 powder, 23.7 % in SDPP, and 25.7 % in SDIPP.

214

#### 215 *3.2. Production of porcine plasma powder, analysis of SDPP and SDIPP, and survival of* 216 *antibody titres in the production process*

217

218 From 320 animals, a little over 20 kg spray dried plasma powder was obtained (Table 2a).  
219 Microbial quality was within the industrial norm. Antimicrobial drug residue activity was  
220 below the detection limit (Table 2b). Immune plasma did shown high anti-F4 titres, whereas  
221 normal plasma did not. Immune plasma showed BBAI reciprocal titre of  $>100$ , normal  
222 plasma, and SPF plasma  $<3$ . Survival of anti-F4 titres in SDIPP was 70% after spray drying,

223 no further reduction was found at pelleting (Table 2c). Further testing for anti-LT  
224 immunoreactivity showed that immune plasma had a titre of  $976 \pm 241$  (n=2), and normal  
225 plasma  $140 \pm 25$  (n=2). In the Vero cell bio-assay, at a dilution of 1:50, both normal plasma  
226 and immune plasma completely inhibited LT cytotoxicity, whereas SPF plasma did not.

227

### 228 *3.3. The effect of diet composition on PWD ignoring the F4R status*

229

230 Five animals out of 96 (5.2 %) were found dead during the experiment. One animal died at  
231 day 5, due to hernia diafragmatica. The other 4 deaths were associated with PWD, and were  
232 found in the control group (1) at day 8, 13, 14, and one in the SDIPP group at day 12. Weight  
233 of the animals at the start of the experiment was  $8.04 \pm 0.16$  kg (n = 91), and no significant  
234 differences were found concerning box or diet. Faecal %DM of all three diets was  $29.0 \pm 0.8$   
235 pre-infection (day 1-6). ADG did not significantly differ between diets on days 1-6.

236 After inoculation with ETEC, control animals (n = 29) excreted high numbers of ETEC (Fig  
237 1). Feeding SDPP (n = 32) did lower ETEC excretion, but not significantly. SDIPP (n = 30)  
238 did lower faecal ETEC counts compared to both control and SDPP. Concerning %DM, the  
239 lowest % was found in the control group. Feeding SDPP improved this significantly, and  
240 SDIPP improved it even further, being significantly different from both control and the SDPP  
241 group. Concerning ADG no significant differences ( $p > 0.05$ ) were found between the diets,  
242 nor for ADFI ( $0.278 \pm 0.023$  kg/day in the control group, increasing to 0.316 kg/day in SDPP,  
243 and 0.326 kg/day in SDIPP). FCR did not differ between diets during the entire experiment  
244 (results not shown).

245

### 246 *3.4. The effect of diet composition on PWD taking the F4R status into account*

247

248 In the brush border adhesion assay, 30% tested F4R+, no ambiguous results were seen. The  
249 F4R distribution varied between the different treatments, F4R+ vs F4R- was in the control  
250 group 10 vs 19, in the SDPP group 10 vs 22, and in the SDIPP group 8 vs 22. There was a  
251 significant interaction of FEED x F4R. No significant differences between F4R- animals were  
252 found in all three treatments. In the control animals, F4R status dependent significant  
253 differences were found for all three parameters, F4R- animals excreted less *E. coli*, had a  
254 higher faecal %DM, and a higher ADG. Concerning *E. coli* excretion, feeding SDPP did not  
255 have a significant effect on the difference between F4R+ and F4R- animals. Feeding SDIPP  
256 significantly lowered *E. coli* excretion in F4R+ animals compared to F4R+ animals in both  
257 the control and the SDPP group. Concerning faecal %DM, in F4R- animals, feeding SDPP  
258 gave a significant improvement compared to controls, and SDIPP was significantly better  
259 than both the control and SDPP. Both SDPP and SDIPP significantly improved ADG in F4R+  
260 animals compared to the controls, no significant difference was found between SDPP and  
261 SDIPP. ADFI and FCR did not differ between diets during the entire experiment (results not  
262 shown).

263

### 264 3.5. *Anti-F4 and anti-LT antibodies in piglet plasma*

265

266 Plasma of all piglets contained antibodies to both F4 and LT, in varying titres. No significant  
267 relationship was found with diet, box, pen, litter, *E. coli* excretion, faecal %DM, ADFI, FCR  
268 or F4R status (results not shown), consistent with maternal colostrum derived antibodies.

269

## 270 **Discussion**

271

272 In the present study, we set out to determine whether SDPP has indeed beneficial  
273 properties, with particular attention to the role of specific antibodies. We have used a  
274 relatively cheap and readily available source of SDPP, and compared it with immune plasma  
275 SDIPP, in our model of post-weaning diarrhoea (PWD). Both SDPP and SDIPP were  
276 included in pelleted feed in order to work as realistic as possible. Processing may seriously  
277 affect bioactivity. As judged by anti-F4 titre in SDIPP, the most critical step in the production  
278 process is spray-drying at which not more than 30 % of the activity is lost. Further processing  
279 to pellets does not lead to appreciable losses.

280 We have demonstrated that both SDPP and SDIPP do improve performance and health,  
281 similar to what has been shown in neonatal diarrhoea using egg yolk or SDPP derived  
282 antibodies (Yokoyama et al., 1992, Owusu-Asiedu et al., 2002). We also show that this  
283 happens in a F4R status dependent way. SDIPP protected against PWD, and this could be  
284 attributed to the presence of both anti-LT and anti-F4 antibodies. SDPP protected only  
285 significantly against diarrhea, which is consistent with the presence of anti-LT antibodies  
286 only. The unanticipated presence of anti-LT antibodies in SDPP probably reflects natural  
287 exposure to pathogens, similar to what has been found in normal bovine colostrum (Rump et  
288 al., 1992). The absence of anti-F4 antibodies is consistent with the absence of significant  
289 colonization inhibition by SDPP as is particularly evident in F4R+ piglets. Our SDPP did not  
290 contain anti-F4 activity as opposed to the commercial preparation used by Owusu-Asiedu et  
291 al., 2002. It is reasonable to assume that batch differences in natural antibodies is one of the  
292 possible causes for variation in results obtained with SDPP. Another source of variation could  
293 be the differences in prevalence of F4R-status of the herds used in the various experiments. In  
294 most studies, piglet F4R status is not mentioned. Here we found that *E. coli* F4 can maintain  
295 itself at relatively high levels (up to  $10^5$ /g faeces on a given day) in F4R- animals in an  
296 apparent F4R independent manner. This level is affected by the presence of sufficient number

297 of F4R+ animals which maintain a high infection pressure by excreting high levels of bacteria  
298 (Geenen et al., 2005).

299 Our findings confirm that SDPP reduces PWD because it contains specific antibodies, in  
300 our case anti-LT antibodies. Comparing SDPP and SDIPP it is evident that the latter not only  
301 protects against diarrhoea, but also reduces ETEC excretion, which will reduce transmission  
302 within a herd (Geenen et al., 2005). Finally, these positive results were obtained in a herd in  
303 which a relatively low percentage (30%) of pigs was F4R+. It is clear that a much larger  
304 effect can be expected in higher F4R+ prevalence herds.

305

### 306 **Acknowledgements**

307

308 This study was supported by the Dutch Ministry of Economic Affairs project number  
309 BTS97014. The technical assistance of Gert Jan de Graaf, Arie Hoogendoorn, Suzan van der  
310 Hoven, and Daphne Oostenrijk is greatly appreciated. We thank Dr. Frans Josef van der Staay  
311 for his expert assistance with the statistical analysis.

312

### 313 **References**

314

- 315 Bergstrom, J.R., Nelssen, J.L., Tokach, M.D., Goodband, R.D., Dritz, S.S., Owen, K.Q.,  
316 Nessmith, W.B. 1997. Evaluation of spray-dried animal plasma and select menhaden fish  
317 meal in transition diets of pigs weaned at 12 to 14 days of age and reared in different  
318 production systems. *J. Anim. Sci.* 75: 3004-3009.
- 319 Coffey, R.D., Cromwell, G.L. 1995. The impact of environment and antimicrobial agents on  
320 the growth response of early-weaned pigs to spray-dried porcine plasma. *J. Anim. Sci.* 73:  
321 2532-2539.

- 322 Debouck P., Pensaert, M. 1979. Experimental infection of pigs with Belgian isolates of the  
323 porcine rotavirus. Zbl. Vet. Med. B. 26: 517-526.
- 324 De Geus B., Harmsen M., van Zijderveld, F. 1998. Prevention of diarrhoea using pathogen  
325 specific monoclonal antibodies in an experimental enterotoxigenic *Escherichia coli*  
326 infection in germfree piglets. Vet. Quart. 20: S87-S89.
- 327 Francis, D.H., Willgoths, J.A. 1991. Evaluation of a live avirulent *Escherichia coli* vaccine  
328 for K88+, LT+ enterotoxigenic colibacillosis in weaned pigs. Am. J. Vet. Res. 52: 1051-  
329 1055.
- 330 Gaastra W., de Graaf, F.K. 1982. Host-specific fimbrial adhesins of noninvasive  
331 enterotoxigenic *Escherichia coli* strains. Microbiol. Rev. 46:129-61.
- 332 Geenen, P.L., Döpfer, D., Van der Meulen, J., De Jong, M.C.M. 2005. Transmission of F4+  
333 *E.coli* in groups of early weaned piglets. Epidem. Infect. 133: 459-468..
- 334 Harmsen, M.M., van Solt, C.B., Hoogendoorn, A., van Zijderveld, F.G., Niewold, T.A., van  
335 der Meulen, J. 2005. *Escherichia coli* F4 fimbriae specific llama single-domain antibody  
336 fragments effectively inhibit bacterial adhesion in vitro but poorly protect against  
337 diarrhoea. Vet. Microbiol. 111: 89-98.
- 338 Lauterslager, T.G.M., Florack, D.E.A., van der Wal, T.J., Molthoff, J.W., Langeveld, J.P.M.,  
339 Bosch, D., Boersma, W.J.A., Hilgers, L.A.T. 2001. Oral immunisation of naive and primed  
340 animals with transgenic potato tubers expressing LT-B. Vaccine 19: 2749-2755.
- 341 Nabuurs, M.J.A., Hoogendoorn, A., van Zijderveld, F.G., van der Klis, J.D. 1993. A long-  
342 term perfusion test to measure net absorption in the small intestine of weaned pigs.  
343 Res.Vet. Sci. 55: 108-14.
- 344 Owusu-Asiedu, A., Baidoo, S.K., Nyachoti, C.M., Marquardt, R.R. 2002. Response of early-  
345 weaned pigs to spray-dried porcine plasma-based diets supplemented with egg-yolk  
346 antibodies against enterotoxigenic *Escherichia coli*. J. Anim. Sci. 80: 2895-2903.

- 347 Owusu-Asiedu, A., Nyachoti, C.M., Baidoo., S.K., Marquardt, R.R., Yang, X. 2003.  
348 Response of early-weaned pigs to an enterotoxigenic *Escherichia coli* (K88) challenge  
349 when fed diets containing spray-dried porcine plasma or pea protein isolate plus egg yolk  
350 antibody. *J. Anim. Sci.* 81: 1781-1789.
- 351 Rump, J.A., Arndt, R., Arnold, A., Bendick, C., Dichtelmuller, H., Franke, M., Helm, E;B.,  
352 Jager, H., Kampmann, B., Kolb P. 1992. Treatment of diarrhoea in human  
353 immunodeficiency virus-infected patients with immunoglobulins from bovine colostrum.  
354 *Clin. Investig.* 70: 588-594.
- 355 Sellwood, R., Gibbons, R.A., Jones, G.W., Rutter, J.M. 1975. Adhesion of enteropathogenic  
356 *Escherichia coli* to pig intestinal brush borders: the existence of two pig phenotypes. *J.*  
357 *Med. Microbiol.* 8: 405-411.
- 358 Speirs, J.I., Stavric S., Konowalchuk, J. 1977. Assay of *Escherichia coli* heat-labile toxin with  
359 Vero cells. *Inf. Immun.* 16: 617-622.
- 360 Van Dijk, A.J., Everts, H., Nabuurs, M.J.A., Margry, R.J.C.F., Beynen, A.C., 2001. Growth  
361 performance of weanling pigs fed spray-dried animal plasma: a review. *Livest. Prod. Sci.*  
362 68: 263-274.
- 363 Van Dijk, A.J., Enthoven, P.M., Van den Hoven, S.G., Van Laarhoven, M.M., Niewold, T.A.,  
364 Nabuurs, M.J., Beynen, A.C. 2002. The effect of dietary spray-dried porcine plasma on  
365 clinical response in weaned piglets challenged with a pathogenic *Escherichia coli*. *Vet.*  
366 *Microbiol.* 84: 207-18.
- 367 Van Zijderveld, F.G., Anakotta, J., Brouwers, R.A.M., Van Zijderveld, A.M., Bakker, D., de  
368 Graaf, F.K. 1990. Epitope analysis of the F4 (K88) fimbrial antigen complex of  
369 enterotoxigenic *Escherichia coli* by using monoclonal antibodies. *Inf. Immun.* 58: 1870-  
370 1878.

371 Yokoyama, H., Peralta, R.C. Diaz R, Sendo S, Ikemori Y, Kodama Y. 1992. Passive  
372 protective effect of chicken egg yolk immunoglobulins against experimental  
373 enterotoxigenic *Escherichia coli* infection in neonatal piglets. Inf. Immun. 60: 998-1007.

374

375 Figure 1. The effect of SDPP and SDIPP on ETEC excretion, (in log CFU/g faeces),  
376 diarrhoea as % DM, and performance (ADG). Left panel with shaded bars represent results  
377 obtained without taking the F4R status into account. In the right panel, the results per  
378 treatment for F4R+ (open bars), and F4R- (black bars), respectively are shown.

379 Data are mean  $\pm$  s.e.m. Significant differences ( $p < 0.05$ ) between groups/treatments are  
380 indicated with lines and asterisks.

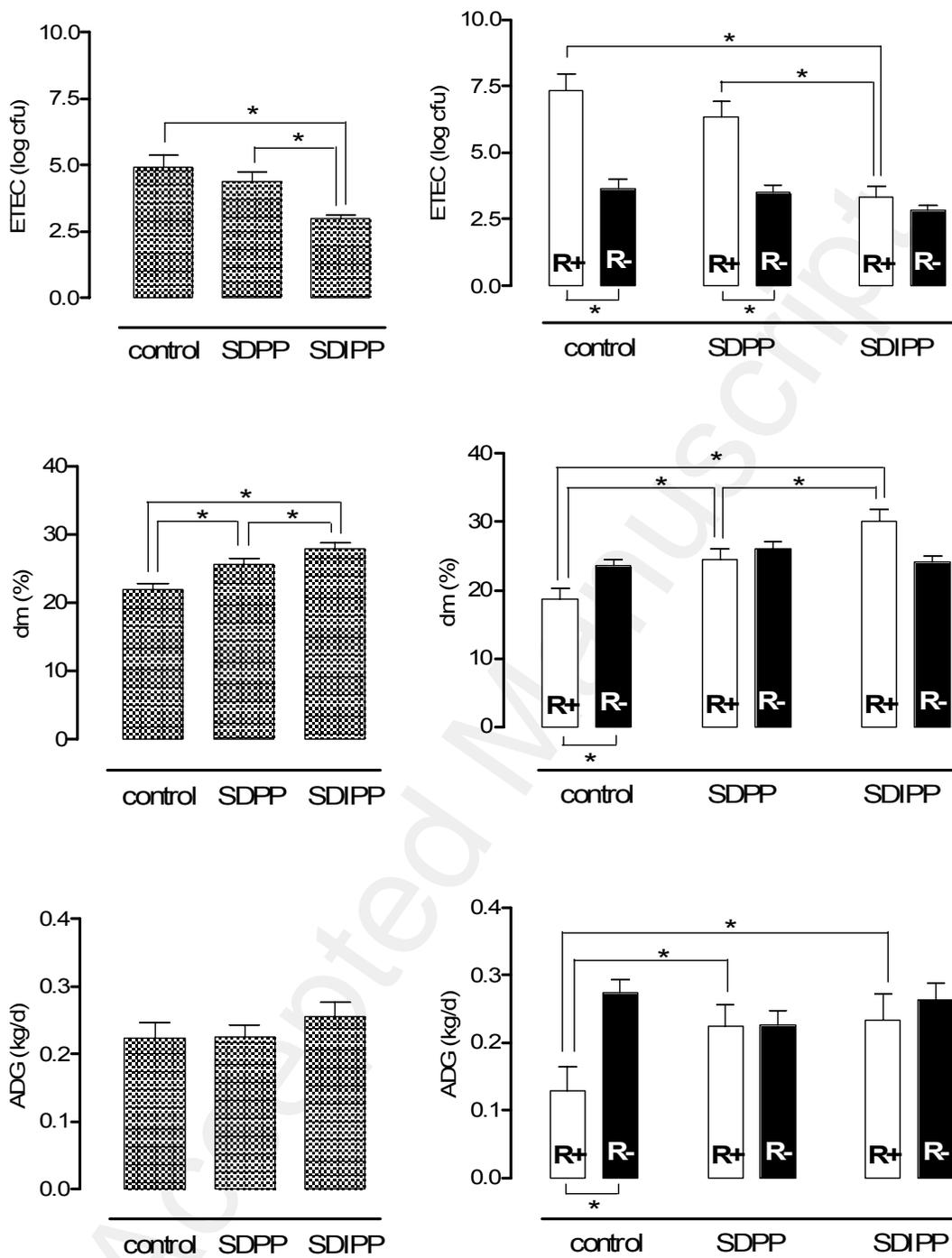


Table 1. Chemical analysis of diet, % on as fed basis

	Control	SDPP	SDPIP
Fat	5.7	4.6	4.7
Crude fibre	3.6	3.3	3.7
Moisture	10.9	9.7	9.3
Ash	6.3	4.9	4.9
Starch	34.8	40.4	41.7
Protein	17.8	17.6	17.9
IgG	n.d.	1.7	1.8

n.d.: not detectable

Table 2: Production of porcine plasma powder, and analysis of SDPP and SDPIP

A. Yield		
	SDPP	SDPIP
Blood (L)	1029	924
Plasma (L)	587	504
Plasma/blood (%)	57	55
Powder (kg)	20.4	20.3
B. Composition		
	SDPP	SDPIP
Total bacterial count (log)/g	2.5	<2.0
<i>Enterobacteriaceae</i> (log)/g	<1.0	<1.0
<i>Salmonella.spp/ 25g</i>	0	0
Protein (%)	84.3	85.1
Dry matter (%)	96.7	97.2
Solubility (%)	93.9	96.5
Antimicrobial residue	negative	negative
Immunoglobulin (g/kg)	231	253
C: Anti-F4 immunoreactivity in SDPIP (n=2) in the production process		
	Reciprocal titer $\pm$ s.d.	% Activity
Plasma (50%)	42,130 $\pm$ 3,649	100
Plasma powder (100%)	59,780 $\pm$ 2,086	71
Pellet (8%)	4,908 $\pm$ 86	73