SPRAY-DRIED ANIMAL PLASMA IN THE DIET OF WEANLING PIGLETS: INFLUENCE ON GROWTH PERFORMANCE AND UNDERLYING MECHANISMS

GESPROEIDROOGD BLOEDPLASMA IN VOEDERS VOOR GESPEENDE BIGGEN: INVLOED OP GROEI EN ACHTERLIGGENDE MECHANISMEN

(met een samenvatting in het Nederlands)

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Albert Jan van Dijk

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Promotor:	Prof. Dr. A.C. Beynen
Co-promotoren:	Dr. M.J.A. Nabuurs
	Dr. T.A. Niewold

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Contents

CHAPTER 1

INTRODUCTION

Introduction

Spray-dried animal plasma (SDAP) is a by-product of slaughter plants. The plasma obtained from slaughtered pigs or ruminants is spray-dried and used for the production of both human foodstuffs and animal feeds (Howell and Lawrie 1983, Gatnau 1990). It has been demonstrated in many experiments that SDAP in weaning piglets' diets can have considerable positive effects on piglets' growth performance. In a meta analysis, Van Dijk et al. (2001) calculated from 68 comparisons between SDAP containing diets and control diets that the SDAP-induced change in average daily gain (ADG) and average daily feed intake (ADFI) in the first two weeks after weaning was +26.8 % and +24.5 %, respectively. There are no other feed ingredients or additives, that have such large effects. Two experiments demonstrated that dietary SDAP can reduce post-weaning diarrhoea (Gatnau 1990, Van der Peet-Schwering and Binnendijk 1995). Moreover, there are indications that the beneficial effect of dietary SDAP is much more pronounced under conditions with a high pressure of pathogens as compared to conditions with optimal hygiene (Coffey and Cromwell 1995). In addition, the positive effect of SDAP on piglets' post weaning ADFI and ADG is more pronounced in piglets that have a low baseline value of these parameters in comparison to piglets with a higher base line growth performance (Van Dijk et al. 2001). Possibly, there are interactions between infection pressure, piglet growth performance and the effect of dietary SDAP.

There are limitations to the use of SDAP as an ingredient in piglets' diets. First, its relatively high price makes it less attractive for use than other protein sources like fishmeal or whey powder. Secondly, the use of SDAP originating from slaughter animals that have not been approved at ante and post mortem veterinary inspection can spread certain diseases like Classical and African Swine Fever and

Foot and Mouth disease (Mann and Sellers 1989, Van Oirschot and Terpstra 1989, Wilkinson 1989, Horst et al. 1997).

Given the limitations to the use of SDAP as a feed ingredient, it is important to unravel its mode of action. By getting insight in the mode of action, inclusion of SDAP in piglets diets can possibly be reduced to lower levels, e.g. by specific processing. Moreover, bioactive components responsible for the positive effects of SDAP may be identified and then isolated from other raw materials that are less expensive or that are not associated with the spread of diseases.

Another advantage of the identification of bioactive components from SDAP is that they may be used in human health-care, especially in the prevention of diarrhoea in neonates or in other categories of humans that are susceptible to diarrhoea. Research with piglets is then justified as these animals are considered to be a good model for humans as to the intestinal structure and function (Moughan et al. 1992).

To study the mode of action of SDAP, its possible intestinal flora modulating properties or its effects on the structure and function of the intestinal mucosa should be considered. Another means to get insight into the mode of action and to optimise its application is to investigate its use under conditions that have not been described before, i.e. under typical European conditions and in diets without anti microbial growth promoters (AMGP). Moreover, SDAP could be considered as an alternative for AMGP, that may be banned in the near future (Health Council 1998).

Therefore, the scope of the present thesis was as follows.

- To assess the effect of dietary SDAP in weaned piglets under typical European conditions.
- To investigate the interaction between dietary SDAP and complexity of the diet in weaned piglets.
- To investigate the interaction between SDAP and the use of AMGP in weaned piglets' diets.
- To investigate the effect of SDAP in diets for suckling piglets (creep feeds).
- To measure the effect of dietary SDAP in weaned piglets on small intestinal villus length, crypt depth, mitotic activity and brush border enzyme activity.
- To measure the effect of SDAP on the small intestinal microflora of weaned piglets.
- To investigate the effect of dietary SDAP in weaned piglets that are challenged by a pathogenic *E. coli*.

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CHAPTER 2

GROWTH PERFORMANCE OF WEANLING PIGS FED SPRAY-DRIED ANIMAL PLASMA: A REVIEW

A.J. van Dijk[a], H. Everts[b], M.J.A. Nabuurs[c], R.J.C.F. Margry[a], A.C. Beynen[b]

[a] Co-operative Central Laboratory "CCL-Nutricontrol" of Cehave Landbouwbelang, P.O. Box 107, 5460 AC Veghel, The Netherlands

[b] Department of Nutrition, Utrecht University, Faculty of Veterinary Medicine, P.O. Box 80152, 3508 TD Utrecht, The Netherlands

[c] Institute for Animal Science and Health ID-Lelystad, P.O. Box 65, 8200 AB Lelystad, The Netherlands

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Abstract

Spray-dried animal plasma (SDAP), mostly of porcine origin, is frequently used as an ingredient of weanling piglets diets in order to improve feed intake and to reduce post-weaning diarrhoea. On the basis of 15 published studies it is concluded that dietary SDAP levels up to 6 % raise both average daily gain (ADG) and feed intake (ADFI) in the first two weeks after weaning in a dose-dependent fashion. Up to 6 % SDAP also reduces feed conversion ratio (FCR). The positive effect of SDAP on ADG and ADFI is much more pronounced in the first than the second week after weaning. There is no positive carry-over effect of SDAP feeding during the period of two weeks after weaning on growth performance thereafter. SDAP is an expensive protein source and an economic evaluation should be made before including SDAP in weanling piglets diets. Multiple regression analysis indicated that, apart from SDAP dose, baseline growth rate is an important determinant of the effect of SDAP on ADG, with high baseline growth rate being associated with small effects of SDAP. It should be stressed that SDAP is a non-sterilised product that might spread certain diseases after feeding it to pigs. Porcine plasma has more beneficial effects than bovine plasma. Possible modes of action are discussed. It is suggested that, in addition to improving feed

palatability, SDAP reduces post-weaning intestinal disease by preventing attachment of pathogens.

Introduction

Weanling piglets often suffer from post-weaning diarrhoea and oedema disease. In the pathogenesis of these diseases, enteropathogenic *E. coli* strains play a major role (Van Beers-Schreurs et al. 1992, Nabuurs et al. 1993, Pluske et al. 1997, Nabuurs 1998). Low feed intake and post-weaning diarrhoea, which both occur during the first two weeks after weaning, have a negative impact on growth performance. Various measures are taken to improve feed intake and health after weaning. Amongst these measures is the addition of specified substances to the weanling piglet diet. One of these substances is spray dried animal plasma (SDAP), which usually is of porcine origin (SDPP) as a by-product of slaughter plants. An anticoagulant is added, usually sodium citrate, to the blood from 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 (Howell and Lawrie 1983, Gatnau 1990).

The type of protein in the diet of weanling piglets has consequences for feed intake, weight gain, nitrogen digestibility and pancreatic enzyme activity (Makkink et al. 1994a, Makkink et al. 1994b, Peiniau et al. 1996). The typical composition of SDAP is given in Tables 1 and 2.

Table 1

Composition (% of product) of various spray-dried animal plasma (SDAP) preparations in comparison with that of casein and soybean protein concentrate

Component	SDAP [a]	SDPP*[b]	Freeze dried porcine plasma[c]	Dried porcine plasma[c]	Dried bovine plasma[c]	Casein [a]	Soybean [a] protein concentrate
Dry matter	91	94.6	90.8	91.1	91.6	91	90
Crude protein	78	87.5	68	70	70	89	64
Crude fat	2	1	2	1.5	1.5	0.8	3
Ash	n.g.	5	11.5	11.8	10.3	n.g.	n.g.
Calcium	0.15	0.09	n.g.	n.g.	n.g.	0.61	0.35
Phosphorus	1.71	0.13	n.g.	n.g.	n.g.	0.82	0.81
Sodium	3.02	3.4	5.2	5.1	5	0.01	0.05
Chloride	1.5	n.g.	n.g.	n.g.	n.g.	0.04	n.g.
Potassium	0.2	0.13	n.g.	n.g.	n.g.	0.01	2.2
Magnesium	0.34	n.g.	n.g.	n.g.	n.g.	0.01	0.32

* Spray-dried porcine plasma [a] National Research Council (1998). [b]Delaney (1975)
[c]Howell and Lawrie (1983) n.g. = not given

Table 2

Amino acid composition and apparent ileal digestibilities of various spray-dried plasma (SDAP) preparations in comparison with casein and soybean meal

	Content (% of product)					Apparent ileal digestibility (%)		
	SDAP[a]	SDPP[b*]	SDBP[b+]	Casein[c]	Soybean meal[c]	SDAP[a]	Casein[c]	Soybean meal[c]
Alanine	n.g.	4.19	3.95	2.69	2.05	n.g.	95	85
Asparagine	n.g.	7.58	8.48	6.13	5.42	n.g.	96	87
Arginine	4.55	4.47	4.70	3.02	3.46	90	94	94
Glutamine	n.g.	11.18	11.39	18.48	8.45	n.g.	96	90
Glycine	n.g.	2.80	2.91	1.68	2.01	n.g.	94	83
Histidine	2.55	2.51	2.45	2.60	1.26	91	98	89
Isoleucine	2.71	2.79	2.53	4.37	2.15	85	95	87
Leucine	7.61	7.44	7.63	8.15	3.60	84	98	87
Lysine	6.84	6.84	7.43	6.72	2.90	87	98	89
Methionine	0.75	0.62	0.95	2.52	0.65	64	98	90
Cystine	2.63	3.03	3.16	0.34	0.70	n.g.	86	82
Phenylalanine	4.42	4.43	4.25	4.37	2.38	88	99	88
Proline	n.g.	5.90	5.71	9.41	2.38	n.g.	98	89
Serine	n.g.	4.52	5.59	4.79	2.43	n.g.	91	87
Tyrosine	3.53	3.79	3.89	4.70	1.73	n.g.	99	88
Threonine	4.72	4.54	5.54	3.61	1.82	82	94	84
Tryptophan	1.36	1.36	1.45	1.09	0.61	92	97	87
Valine	4.94	5.07	5.64	5.63	2.24	86	95	86

* Spray-dried porcine plasma
+ Spray-dried bovine plasma
[a] National Research Council (1998)
[b] Van der Peet-Schwering and Binnendijk (1997)
[c] CVB (1999)
n.g. = not given

The protein content of SDAP is lower than that of casein and has low apparent ileal digestibilities of amino acids. However, with respect to the contents of essential amino acids, SDAP is superior to soybean protein. The provision of essential amino acids from SDAP and the requirements of piglets (National Research Council 1998) are in good agreement except for methionine, which has a relatively low level in SDAP.

After weaning, the transition from sow milk to solid feed has important nutritional consequences like a switch from fat to carbohydrates as the main source of digestible energy and a shift from highly digestible animal protein to less digestible protein of plant origin (Everts et al. 1999). Addition of SDAP instead of plant protein to diets of weaned piglets as a protein source may improve post weaning performance because the amino acid composition and protein digestibility

of SDAP are more similar to those of the proteins in sow's milk (Darragh and Moughan 1998).

New-born piglets rely on colostrum as their sole source of serum antibodies and milk provides intestinal antibodies during most of the postnatal period (Bourne 1976, Blecha 1998). Coffey and Cromwell (1995) have proposed that SDPP may enhance piglet performance after weaning by improving immunocompetence due to the immunoglobulins present in SDPP.

As described below, the incorporation of SDAP into the diet can have positive effects on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) in weanling piglets. SDAP is a relatively expensive protein source and experiments have therefore been carried out to determine the optimum dose. This paper presents a review of those studies. The main objective was to establish SDAP dose-response relationships on the basis of a meta analysis and to identify additional independent variables related to the response variables ADG, ADFI and FCR. In addition, an attempt is made to describe possible mechanisms by which SDAP influences growth performance.

Multiple regression analysis.

Table 3 summarises the design and outcome of published studies on the influence of feeding SDAP on growth performance in weanling piglets. The overall, mean SDAP-induced change in ADG, ADFI and FCR in the first two weeks after weaning is +26.8 % (n=68, SEM=3), +24.5 % (n=68, SEM=2.3) and –3.2 % (n=68, SEM=1.3), respectively.

Table 3
Summary of published experiments with piglets fed spray-dried animal plasma (SDAP) during the first two weeks after weaning

Reference	Inclusion (% of diet)	n	Initial		Performance			Percentage difference vs. SDAP-free control		
			Age (days)	Weight (kg)	ADG[$]	ADFI[+]	FCR[#]	ADG	ADFI	FCR
A	0	80	17	4.9	310	497	1.60			
A	3[x]	"	"	"	313	488	1.56	1	-1.8	-2.5*
A	6[x]	"	"	"	287	512	1.79	-7.4	3	11.9*
A	9[x]	"	"	"	289	532	1.85	-6.8	7	15.6*
A	12[x]	"	"	"	268	500	1.87	-13.5	0.6	16.9*
A	0	"	18	5.3	263	402	1.53			
A	8.33[x]	"	"	"	277	470	1.70	5.3	16.9*	11.1*
A	0	"	"	"	214	251	1.18			
A	8.33[x]	"	"	"	300	399	1.34	40.2*	59.0*	13.6*
A	0	"	"	"	304	419	1.39			
A	8.33[x]	"	"	"	323	496	1.54	6.3	18.4*	10.8
A	0	"	"	"	192	269	1.43			
A	8.33[x]	"	"	"	238	352	1.48	24.0*	30.9*	3.5
A	0	120	30	7.3	155	249	1.61			
A	5[x]	"	"	"	237	391	1.65	52.9*	57.0*	2.5
A	0	"	"	"	244	363	1.49			
A	5[x]	"	"	"	343	488	1.42	40.6*	34.4*	-4.7
A	0	"	"	"	203	317	1.57			
A	5[x]	"	"	"	343	488	1.42	69.0*	53.9*	-9.6
B	0	534	21	6.4	165	206	1.27			
B	2[x]	"	"	"	206	244	1.19	24.8*	18.4*	-6.3
B	4[x]	"	"	"	217	256	1.18	31.5*	24.3*	-7.1
B	6[x]	"	"	"	240	290	1.22	45.5*	40.8*	-3.9
B	8[x]	"	"	"	247	302	1.23	49.7*	46.6*	-3.1
B	10[x]	"	"	"	255	300	1.19	54.5*	45.6*	-6.3
C	0	96	25	6.1	151	387	2.66			
C	2[x]	"	"	"	150	447	2.94	-0.7	15.5	10.5
C	4[x]	"	"	"	236	526	2.25	56.3	35.9	-15.4
C	6[x]	"	"	"	254	528	2.07	68.2	36.4	-22.2
C	8[x]	"	"	"	269	547	2.03	78.1	41.3	-23.7
C	10[x]	"	"	"	188	445	2.44	24.5	15.0	-8.3
D	0	144	24	7.2	280	330	1.12			
D	4[x]	"	"	"	360	410	1.11	28.6*	24.2*	-0.9
D	0	18	19.5	6.1	280	420	1.59			
D	14[x]	"	"	"	360	510	1.45	28.6*	21.4*	-8.8
E	0	36	n.g.	6.9	247	292	1.18			
E	10[x]	"	"	"	261	350	1.34	5.7	19.9*	13.6
E	0	"	"	"	139	213	1.46			
E	10[x]	"	"	"	261	350	1.34	87.8*	64.3*	-8.2
E	0	"	"	"	153	204	1.47			

Table 3. Continued

Reference	Inclusion (% of diet)	n	Initial Age (days)	Initial Weight (kg)	Performance ADG[$]	Performance ADFI[+]	Performance FCR[#]	Percentage difference vs. SDAP-free control ADG	Percentage difference vs. SDAP-free control ADFI	Percentage difference vs. SDAP-free control FCR
E	10[x]	"	"	"	261	350	1.34	70.6*	71.6*	-8.8
E	0	"	"	"	191	267	1.44			
E	10[x]	"	"	"	345	462	1.35	80.6*	73.0*	-6.2
E	0	"	"	"	230	300	1.74			
E	10[x]	"	"	"	345	462	1.35	50.0*	54.0*	-22.4
E	0	96	"	7.1	63	119	1.85			
E	10[x]	"	"	"	127	209	1.65	101.6*	75.6*	-10.8
F	0	236	24	7.5	262	305	1.16			
F	10[x]	"	"	"	266	302	1.12	1.5	-1.0	-3.4
F	0	204	21	5.9	315	389	1.23			
F	10.35[x]	"	"	"	444	537	1.20	41.0*	38.0*	-2.4
F	10.35[x]	"	"	"	420	487	1.16	33.3*	25.2*	-5.7
F	13.4[x]	"	"	"	413	482	1.16	31.1*	23.9*	-5.7
F	13.4[x]	"	"	"	378	437	1.15	20.0*	12.3*	-6.5
F	0	120	"	5.3	328	390	1.18			
F	10.28[x]	"	"	"	378	499	1.32	15.2	27.9	11.9
F	6.96[y]	"	"	"	327	422	1.28	-0.3	8.2	8.5
G	0	180	22	6.2	190	253	1.34			
G	3[y]	"	"	"	220	293	1.32	15.8*	15.8*	-1.5
G	6[y]	"	"	"	263	338	1.29	38.4*	33.6*	-3.7
H	0	720	28	7.9	215	270	1.30			
H	5[x]	"	"	"	250	290	1.20	16.3*	7.4*	-7.7*
H	0	"	"	"	196	260	1.33			
H	5[x]	"	"	"	249	310	1.26	27.0*	19.2*	-5.3*
I	0	960	"	7.5	192	250	1.30			
I	5[x]	"	"	"	235	290	1.23	22.4*	16.0*	-5.4*
I	5[y]	"	"	"	224	270	1.23	16.7*	8.0*	-5.4
I	3.33[x]	"	"	"	223	280	1.27	16.1*	12.0*	-2.3
I	1.66[x]	"	"	"	227	270	1.18	18.2*	8.0*	-9.2*
J	0	626	13.2	4.1	163	186	2.50			
J	2[x]	"	"	"	195	217	2.44	19.6*	16.7*	-2.4
J	4[x]	"	"	"	204	234	2.56	25.2*	25.8*	2.4
J	6[x]	"	"	"	212	236	2.44	30.1*	26.9*	-2.4
J	2[x]	"	"	"	195	222	2.50	19.6*	19.4*	0
J	4[x]	"	"	"	215	237	2.44	31.9*	27.4*	-2.4
J	6[x]	"	"	"	209	241	2.56	28.2*	29.6*	2.4
J	2[y]	"	"	"	182	204	2.50	11.7*	9.7*	0
J	4[y]	"	"	"	204	219	2.38	25.2*	17.7*	-4.8
J	6[y]	"	"	"	200	277	2.50	22.7*	48.9*	0
K	0	180	17	5	227	299	1.32			
K	2.5[z]	"	"	"	262	316	1.20	15.4*	5.7*	-9.1*
K	5[z]	"	"	"	290	341	1.18	27.8*	14.0*	-10.6*
L	0	416	15	4.3	191	248	1.30			

Table 3. Continued

			Initial		Performance			Percentage difference vs. SDAP-free control		
Reference	Inclusion (% of diet)	n	Age (days)	Weight (kg)	ADG[$]	ADFI[+]	FCR[#]	ADG	ADFI	FCR
L	5[y]	"	"	"	209	265	1.27	9.4	6.9	-2.3
L	5[x]	"	"	"	232	267	1.15	21.5*	7.7	-11.5*
L	5[x]	"	"	"	232	267	1.15	21.5*	7.7	-11.5*
M	0	360	19	5.3	311	296	0.95			
M	7.5[z]	"	"	"	333	321	0.96	7.1*	8.4*	1.0
M	0	"	"	"	329	298	0.91			
M	7.5[z]	"	"	"	337	326	0.96	2.4*	9.4*	5.8
M	0	"	"	"	295	289	0.98			
M	7.5[z]	"	"	"	313	301	0.96	6.1*	4.2*	-1.9
M	0	"	"	"	341	291	0.84			
M	7.5[z]	"	"	"	339	321	0.94	-0.6*	10.3*	12.3
M	0	"	"	"	272	259	0.95			
M	7.5[z]	"	"	"	295	293	0.99	8.5*	13.1*	4.0
M	0	"	"	"	345	304	0.88			
M	7.5[z]	"	"	"	375	342	0.91	8.7*	12.5*	3.6
N	0	45	28	7.1	138	243	2.10			
N	2[x]	"	"	"	176	276	2.34	27.5*	13.6	11.4*
N	4[x]	"	"	"	203	294	1.49	47.1*	21.0	-29.0*
N	6[x]	"	"	"	251	326	1.32	81.9*	34.2	-37.1*
N	8[x]	"	"	"	188	290	1.63	36.2*	19.3	-22.4*

A = Coffey and Cromwell (1995), B = Kats et al. (1994), C = Gatnau and Zimmerman (1992), D = de Rodas et al. (1995), E = Gatnau (1990), F = Hansen et al. (1993), G = Angulo and Cubilo (1998), H = Van der Peet-Schwering and Binnendijk (1995), I = Van der Peet-Schwering and Binnendijk (1997), J = Rantanen et al. (1994), K = Grinstead et al. (1998), L = Smith II et al. (1995), M = Nessmith et al. (1997), N = Gatnau et al. (1991)

* = statistically significant difference ($P < 0.05$)

[x] porcine plasma

[y] bovine plasma

[z] plasma of unknown origin

n.g. = not given

n = number of piglets in the experiment

[$] = average daily gain

[+] = average daily feed intake

[#] = feed conversion ratio

The data were subjected to multiple linear regression (SAS, 1988) to disclose relationships between the independent and response variables. Figure 1 shows the dose-response effects for the percentage of SDAP in the diet and percentage change in ADG, ADFI and FCR for all studies combined.

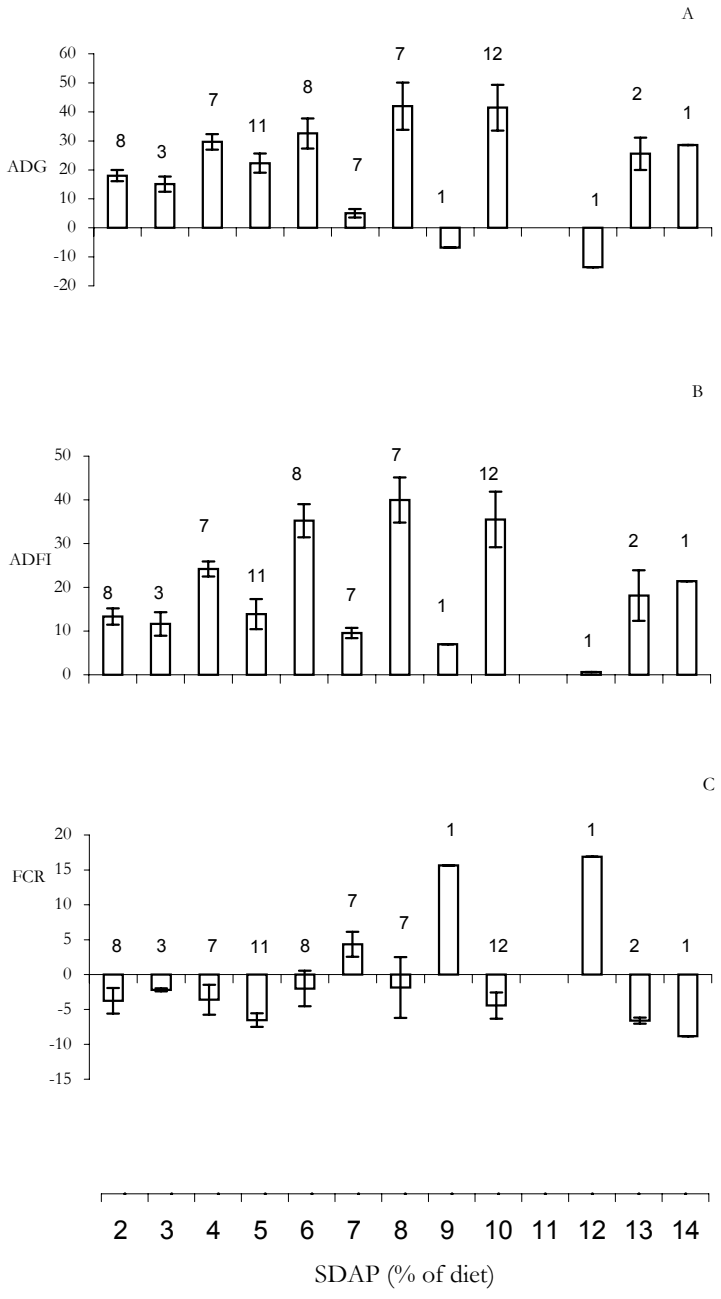

Fig. 1. Percentage change in average daily gain (ADG) (panel A), average daily feed intake (ADFI) (panel B) and feed conversion ratio (FCR) (panel C) in piglets fed spray-dried animal plasma (SDAP) during the first two weeks after weaning. SDAP dose classes were defined as the value indicated on the x axis ± 0.5 %. For the selected ranges of SDAP dose, expressed as percentage of diet, the percentage changes versus SDAP-free controls were calculated as weighted means; the SEM is given. The number of observations is indicated above the bars.

For this analysis the data were weighed according to the number of piglets studied. The response of both ADG and ADFI is more or less consistent up to 6 % SDAP in the diet, whereas the responses to higher levels of inclusion were not clear. As to FCR, the response to SDAP seems beneficial at levels below 6 %. Again, at higher levels, the response was variable.

Regression analyses for all studies combined showed that there are no significant relationships between the dose of SDAP and the response of either ADG, ADFI or FCR. When more independent variables were included in the regression model the variance in some response variables could be correlated significantly although the explained variance was not substantial (Table 4).

Table 4
Relationships between independent variables and response variables (percentage SDAP-induced change in ADG) for the first week (subscript 1) and for the period of two weeks after weaning (subscript 1+2)*

Response variable	Model[a]	R^2	P value
% Δ ADG_1	55.7 + 2.73∗%SDAP - 0.24∗$ADG_{1\ control}$	0.73	0.0001
% Δ ADG_{1+2}	65.22 + 3.77∗%SDAP - 0.29∗$ADG_{1+2\ control}$	0.54	0.0001

* SDAP = spray-dried animal plasma, ADG = average daily gain
[a] Models were selected by using the linear regression procedure of SAS (SAS, 1988).

As to ADG, there is a strong influence of the performance of the control group, with high values of ADG in the control group being associated with small effects of SDAP. This is illustrated in Figure 2 for ADG in the first week after weaning. It would appear that the feeding of SDAP improves ADG only when the piglets show suboptimal performance.

In general, the response of ADG to SDAP during the first two weeks after weaning is quite impressive. The beneficial effect of SDAP on ADG is associated with an increase in ADFI. As a consequence, the improvement of FCR is generally modest.

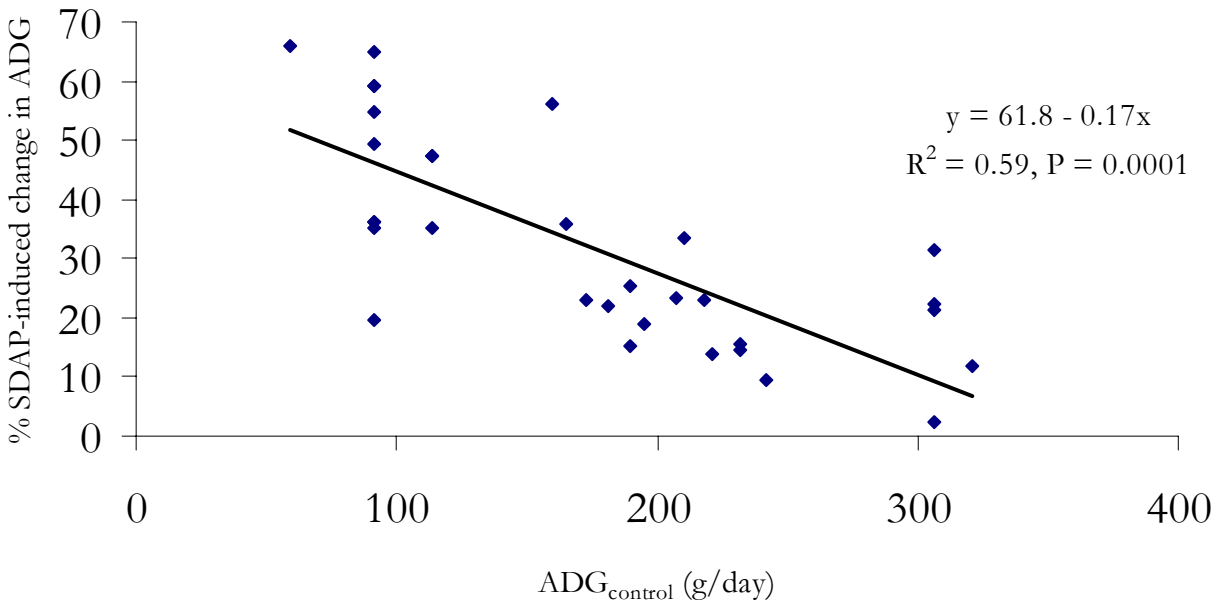

Fig. 2. Relationship between average daily gain (ADG) in control piglets and the percentage change in ADG of piglets fed spray-dried amimal plasma (SDAP) during the first week after weaning.

Effective feeding period

Most papers on SDAP feeding in piglets report ADG and ADFI for the combined first two weeks after weaning, but not for the first and second week separately. The available percentage changes in ADG, ADFI and FCR for both weeks 1 and 2 are given in Table 5. It appears that the beneficial effect of SDAP on performance is much more pronounced in the first week than in the second week after weaning.

The percentage SDAP-induced changes in ADG, ADFI and FCR in the first two weeks after weaning and during the following period, when the control and SDAP-fed piglets received identical feeds, are presented in Table 5. It can be concluded that there is no positive carry-over effect of SDAP.

Table 5

Comparison of the mean percentage spray-dried animal plasma (SDAP)-induced change in average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) in the first and second week after weaning (upper part) and of the first two weeks after weaning and the following weeks when the control and SDAP-fed piglets received identical feeds (lower part). For the comparisons within each row, only experiments were used in which the data from both periods were given.

	Week after weaning						
	Week 1			Week 2			
	n	Mean	SEM	n	Mean	SEM	P value[a]
% Δ ADG	29	31.1	3.4	29	13.9	2.8	0.0002
% Δ ADFI	6	26.5	4.2	6	18.3	3.4	0.16
% Δ FCR	4	-24.5	7.4	4	1.1	1.7	0.01

	Week after weaning					
	Week 1+2			Week > 3 + 4/5		
	n	Mean	SEM	n	Mean	SEM
% Δ ADG	39	21	3	39	2.4	1.9
% Δ ADFI	39	20.9	2.6	39	0.9	1
% Δ FCR	39	0.3	1.2	39	-0.8	1.3

Data derived from experiments mentioned in Table 3.
n = number of control-SDAP comparisons
[a] Model used in t-test: y = mean + week + error

Diet composition of control group

The response to SDAP may be dependent on the kind of protein used in the control diet. In most experiments, dairy proteins were used for the control feed, but soy proteins were also used. Table 6 documents that the responses of FCR and ADG to SDAP are greater when soy protein was used in the control feed instead of dairy protein. Milk proteins are generally considered to be of high value in piglet feeding. Therefore, it is surprising that in most experiments SDAP improves piglet performance even when compared with milk proteins.

Table 6

Mean percentage spray-dried animal plasma (SDAP)-induced change in average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) during the first two weeks after weaning as determined by the kind of protein in the control feed (upper part), source of the SDAP (middle part) and form of the diet (lower part)

	Protein in the control feed						
	Milk protein			Soy protein			
	n	Mean	SEM	n	Mean	SEM	P value[a]
% Δ ADG	38	23.9	3.1	14	38.1	7.3	0.04
% Δ ADFI	38	24.5	2.8	14	28.8	5.7	0.45
% Δ FCR	38	-0.1	1.4	14	-7.9	2.3	0.006
	Source						
	Porcine SDP			Bovine SDP			
	n[d]	Mean	SEM	n[d]	Mean	SEM	P value[b]
% Δ ADG	5	24.2	2	6	17.1	3	0.07
% Δ ADFI	5	17.9	3.9	6	18.2	7.9	0.97
% Δ FCR	5	-4.7	2.4	6	-2.5	1.1	0.45
	Form						
	Meal diet			Pelleted diet			
	n	Mean	SEM	n	Mean	SEM	P value[c]
% Δ ADG	12	26.1	10.3	24	21.2	2.9	0.55
% Δ ADFI	12	29.6	7.6	24	16.3	2.5	0.05
% Δ FCR	12	4.9	2.8	24	-3.8	1.2	0.002

Data derived from experiments mentioned in Table 3.
n = number of control-SDAP comparisons
[a] Model used in t-test: y = mean + $protein_{control\ feed}$ + error
[b] Model used in t-test : y = mean + source + error
[c] Model used in t-test : y = mean + form + error
[d] Data derived from Rantanen et al. (1994), Smith et al. (1995), and Van der Peet-Schwering and Binnendijk (1997)

SDAP is lower in methionine than is casein (Table 2). In the published experiments, the diets were usually formulated on an iso-lysine basis and only occasionally on an iso-methionine basis. Hansen et al. (1993), Kats et al. (1994) and Owen et al. (1995) have suggested that insufficient absolute intake of methionine could have been the cause of the low response to SDPP in certain experiments. Low responses to dietary SDAP could also result from low intakes of other essential amino acids such as cystine, threonine and tryptophan. To exclude any effects of amino acid intake, the experimental diets should be formulated so that

the amounts of apparent, ileal-digestible amino acids are identical. Sodium citrate is frequently used as an anticoagulant. Thus, SDAP preparations may contain 2 % citrate and diets containing 10 % SDAP may contain up to 0.2 % citrate. Because citric acid might be considered as a growth promoter in swine, it could be argued that, in studies on the effects of SDAP, the control diets should be enriched with sodium citrate. However, it is unlikely that dietary citrate concentrations as low as 0.2 % would influence piglet performance.

Porcine versus bovine spray dried plasma

Hansen et al. (1993) found no response to spray dried bovine plasma (SDBP) in the first two weeks after weaning. In three other experiments, a direct comparison between SDPP and SDBP was made. The results are presented in Table 6. It can be concluded that both SDPP and SDBP improve piglet performance post weaning, but the response of ADG to SDPP is greater than that to SDBP.

Feed processing and effect of SDAP

Weanling piglets can be fed diets in either pellet or meal form. It could be suggested that the process of pelleting, during which high temperatures are reached, may damage specific bioactive components, such as immunoglobulins, in the SDAP which in turn would diminish the positive effect on performance. It appears that both meal and pelleted feed containing SDAP have a positive effect on piglet post-weaning performance (Table 6). The response of ADFI to SDAP in meal was greater than that to SDAP incorporated in pellets. FCR appears to react more favourably to SDAP in pelleted feed.

Effect on piglet health

It has been hypothesised that the feeding of SDAP reduces the incidence and/or severity of post-weaning diarrhoea. Unfortunately, health parameters were not registered in most published experiments. In two experiments, less diarrhoea was found in piglets fed SDPP during the first two weeks after weaning (Gatnau 1990, Van der Peet-Schwering and Binnendijk 1995). In one experiment, piglets given feeds with SDPP required less treatment against gastro-intestinal disorders during the first two weeks after weaning than did piglets that were fed diets without SDPP (Van der Peet-Schwering and Binnendijk 1995).

Between-experiment variation in response to SDAP

Between experiments, the response to SDAP can vary considerably (Table 3). Various factors determining the effect of SDAP, such as baseline growth and composition of the control diet, have been discussed above. It has been suggested that the response also depends on health and hygiene status (Coffey and Cromwell 1995, Bergström et al. 1997), but this suggestion is not well substantiated other than indirectly by the negative association between SDAP effect and growth of the control group (Figure 2).

Coffey and Cromwell (1995) demonstrated that the growth-enhancing properties of SDPP are unrelated to the response to antimicrobial agents, indicating that the health status of the piglets is not an important determinant of the SDAP effect.

Possible mode of action of SDAP

As to the mechanism by which SDAP enhances growth of weanling piglets, only speculative theories have been put forward. Knowledge about the mode of action could assist in identifying and/or developing feedstuffs less expensive than SDAP, but with the same properties. It is unclear whether the beneficial effects of SDAP on post-weaning ADG are caused by directly increasing feed intake or indirectly by specific bioactive components. It is likely that factors present in SDAP influence systemic and/or intestinal functions controlling growth and/or immunity.

Feed intake and digestibility

Ermer et al. (1994) performed a preference test in which weanling piglets could choose between diets containing either SDPP or dried skim milk. ADFI was higher for the feed containing SDPP and thus it has been suggested that the higher intake was associated with a greater palatability.

The higher ADFI by itself can explain most of the SDAP-induced increase in ADG. The latter increase is generally greater than the increase in ADFI, resulting in an improvement in the FCR. Hansen et al. (1993) found lower dry matter and nitrogen digestibilities for piglets consuming diets containing SDPP instead of dairy proteins. Knabe (1994) found lower apparent ileal amino acid digestibilities for SDPP than for blood meal. However, the impact of the lower digestibility of SDPP must be small because the feeding of diets with up to 6 % SDAP actually improves the ADG and FCR (Fig. 1).

Immunoglobulins (Ig) and insulin-like growth factor-I (IGF-I)

Coffey and Cromwell (1995) have proposed that SDPP may enhance piglet performance by improving immunocompetence through Ig present in SDPP. The Ig would prevent viruses and bacteria from damaging the gut wall, resulting in a more functional intestinal wall. However, there is no evidence that SDPP is more effective in environments with a greater load of pathogenic organisms. Dritz et al. (1996) have hypothesised that the Ig fraction decreases exposure of the immune system to antigens, leading to decreased production of inflammatory cytokines and, in turn, to increased feed intake. There is only indirect evidence for these ideas. Ig derived from processed blood and orally administered to colostrum-deprived new-born piglets have beneficial effects on health and performance (Drew and Owen 1988, Gatnau 1990, Gomez et al. 1998). Extrapolating these effects to non-colostrum-deprived weanling piglets is not justified, but it is clear that Ig in SDAP can have an effect on piglet health. The response to SDPP is higher than that to SDBP (Table 6) suggesting that a specific Ig effect could be involved. De Rodas et al. (1995) did not find a difference in piglet performance for two different sources of SDPP. Thus, this provides no evidence to support the hypothesis that the effect of SDPP is based on its Ig moiety.

De Rodas et al. (1995) reported that SDPP contains high levels of immunoreactive IGF-I (0.8 ng/mg), a peptide hormone in the somatotrophic axis that is involved in the regulation of growth. However, SDPP-fed piglets showed superior performance but no change in plasma IGF-I concentrations. The authors suggested that dietary IGF-I might have influenced intestinal mucosal function and gastrointestinal growth.

Glycoproteins

Andersen et al. (1980) described that binding of purified K88 antigen to porcine intestinal brush border membranes was inhibited by glycoproteins derived from porcine submaxillari mucins. In vitro studies have demonstrated that the oligosaccharide chains of glycoproteins obtained from plasma can act as binding sites for the fimbrial adhesins of *E. coli* (Sanchez et al. 1993). In this way, it may be possible that attachment of F-17 expressing *E. coli* strains to bovine mucus and brush border membranes can be prevented by glycoproteins. These authors state that this inhibition is not due to Ig present in the plasma because neither heat denaturation, nor proteolytic digestion nor removal of the antibodies from the plasma affected this inhibitory capacity. Mouricout et al. (1990) treated diarrhoea in calves due to infection by enteropathogenic *E. coli* by administration of glycoprotein glycans derived from bovine plasma. The glycan moieties of the non-Ig fraction of plasma mimicked the oligosaccharide moiety of the intestinal

receptors recognised by K99 pili. The glycoprotein glycans inhibited adhesion of bacteria to the intestine and protected colostrum-deprived calves against lethal doses of enterotoxigenic *E. coli*. There is some evidence that the feeding of large amounts of SDPP to weaned piglets offers protection against pathogenic *E. coli* (Nollet et al. 1999).

Conclusions and further research

Dietary SDAP levels up to 6 % raise both ADG and ADFI in the first two weeks after weaning in a dose-dependent fashion. Up to 6 % SDAP also reduces the FCR. The positive effect of SDAP on ADG and ADFI is much more pronounced in the first than the second week after weaning. There is no positive carry-over effect of SDAP feeding during the period of two weeks after weaning on subsequent growth. In all studies with SDAP, performance is only considered during a very short period of the piglets life. Long term effects of this expensive protein source are unknown and an economic evaluation should be made before applying SDAP in weanling diets. Baseline growth rate is an important determinant of the effect of SDAP on ADG, with high values of baseline ADG being associated with small effects of SDAP. So, it should be considered to use SDAP in weanling piglets' diets only in farms with suboptimal post-weaning performance.

There are health risks associated with the use of non-sterilised products of animal origin as feed ingredients for the same species (Horst et al. 1997). Therefore, it must be considered that the use of dietary SDAP in pigs might spread certain diseases like Classical and African Swine Fever, and Foot and Mouth disease (Mann and Sellers 1989, Van Oirschot and Terpstra 1989, Wilkinson 1989). It is recommended to only use SDPP that originates from slaughter pigs that were approved after ante- and post-mortem veterinary inspection.

The growth-promoting action of SDAP could lie in the observed increase in ADFI due to increased palatability of SDAP-containing feed. Alternatively or additionally, SDAP could have direct effects on the intestine, leading to less intestinal disease and, in turn, higher ADFI and ADG. The Ig and glycoprotein fractions of SDAP might prevent attachment of pathogens and thus support functionality of the intestine. This possible mode of action should be put to the test in vivo. Piglets fed a diet containing SDAP should show enhanced colonisation resistance against enteropathogenic bacteria. SDAP may protect against the development of mucosa damage and thus should allow less passage of inert large molecules through the intestinal wall. Experiments have to be carried out in order to either support or refute these hypotheses.

Acknowledgements

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CHAPTER 3

ZOOTECHNICAL ASPECTS

CHAPTER 3.1

GROWTH PERFORMANCE AND HEALTH STATUS IN WEANLING PIGLETS FED SPRAY-DRIED PORCINE PLASMA UNDER TYPICAL NORTHERN EUROPEAN CONDITIONS

A.J. van Dijk[a] , R.J.C.F. Margry[a], A.G. van der Lee[a], G. Hemke[a], A.C. Beynen[b]

[a] Co-operative Central Laboratory "CCL-Nutricontrol" of Cehave Landbouwbelang, p.o. box 107, 5460 AC Veghel, The Netherlands

[b] Department of Nutrition, Utrecht University, Faculty of Veterinary Medicine, p.o. box 80152, 3508 TD Utrecht, The Netherlands

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Abstract

The effect of inclusion of spray-dried porcine plasma (SDPP) in diets for weanling piglets was studied. The objectives were to determine whether SDPP would have positive effects on post weaning piglet performance and health under typical Northern European conditions. In experiment 1, 160 weanling piglets were assigned randomly to a control diet or a diet containing 3% SDPP, which was added at the expense of both fishmeal and dried skim milk. In experiment 2, 264 weanling piglets were assigned to a control diet containing whey protein, a diet without whey protein but with SDPP or a diet containing both whey protein and SDPP. In essence SDPP was added to the test diets at the expense of either whey protein or fishmeal. Piglets were fed the diets for 3 weeks. In experiment 1, the piglets fed the SDPP diet had a 7% higher average daily gain (ADG) and a 4 % lower feed conversion ratio (FCR) ($P < 0.05$) during the first 3 weeks after weaning than did those fed the control diet. There were no differences in leukocyte counts or γ -globulin. In experiment 2 there were no significant differences in ADG and

FCR among the dietary treatments. It is concluded that low amounts of SDPP in weanling diets can have positive effects on growth performance under Northern European conditions.

Introduction

A meta-analysis of published data has shown that the feeding of spray-dried porcine plasma (SDPP) improves average daily gain (ADG) and average daily feed intake (ADFI) in weanling piglets (Van Dijk et al. 2001). In two experiments, less diarrhoea was found in piglets fed SDPP during the first two weeks after weaning (Gatnau 1990, Van der Peet-Schwering and Binnendijk 1995). However, most of the experiments used in the meta-analysis were conducted under Northern American conditions, which could interfere with extrapolation to the situation in Northern Europe. European weanling diets differ from those used in the US in that the main starch source is wheat, barley or tapioca instead of corn; soybean meal is included in smaller quantities; their composition is based on the amount of apparent ileal digestible, first limiting essential amino acid instead of total amino acids and that they contain less antibiotics and zinc oxide. Moreover, the weaning age of piglets in Europe is generally higher than that in the US and there might be differences in hygiene status. The effect of SDPP on ADG and ADFI depends on various factors such as weaning age, background composition of the weanling diet and hygiene status (Van Dijk et al. 2001). Thus, the quantitative effect of SDPP may differ between management and feeding conditions.

Milk proteins are generally considered to have beneficial effects on piglets' performance (Tokach et al. 1995). It could thus be suggested that in the presence of whey protein no further influence of SDPP would be seen. The use of SDPP in diets for weanling piglets is limited by its high price in comparison with other protein sources. Dietary SDPP has clear effects at low inclusion percentages (Van Dijk et al. 2001) and thus only 3% was added to the experimental diets used in the present experiments. Blood leukocyte counts and globulin analyses can give an indication of the health status of pigs (Imlah and McTaggert 1977, Elbers et al. 1991).

Two experiments were conducted to evaluate the effect of SDPP. The objectives were to determine whether SDPP has positive effects on post-weaning piglet performance and health status under typical Northern European conditions and to study the effect of dietary SDPP in the presence or absence of a high level of dietary whey protein.

Materials and methods

Experiment 1.

Hundred sixty weanling piglets (F2 cross-bred: GY x [Finnish X Dutch Landrace]) from the closed herd of the research station 'Laverdonk' (Veghel, The Netherlands) were used. The females and castrates weighed on average 8.0 kg and were 26 days of age. The experiment had a randomised complete block design with pen as experimental unit and room as block. Each room had 6 pens and each pen contained 10 piglets. The piglets were allocated to the pens so that sex, litter origin and body weight were equally distributed. Piglets did not receive creep feed during the lactation period.

Piglets were housed in environmentally regulated rooms in pens (2.60m x 1.20m) with partially slatted floors (concrete floor: 1.10m x 1.20m) and had ad libitum access to feed and water. Each pen was equipped with a nipple waterer and a one-hole self-feeder. The room temperature was 26 °C on the first day after weaning, gradually declining to 23 °C by the end of the experiment. Day light could enter the rooms.

At weaning, piglets were assigned randomly to a diet containing either 0 or 3% SDPP (Harimex, Loenen, The Netherlands). There were eight observations (pens) per treatment. The composition of the diets is presented in Table 1. To formulate the experimental diet in relation to the control diet, SDPP was substituted for portions of both fishmeal and dried skim milk. The two diets were further formulated to contain 1.03% of apparent ileal digestible lysine and 2394 kcal NE/kg. As a consequence, there were multiple, but slight differences in the ingredient composition of the two diets. The content of apparent ileal digestible lysine was balanced by adding crystalline lysine. Piglets were fed the diets, which were in pelleted form, for 3 weeks. Subsequently, i.e. from days 22 to 33 after weaning, all piglets received the same starter diet (2270 kcal NE/kg, 16.6 % crude protein). This diet met the requirements of piglets weighing 15 to 25 kg. The experimental period lasted from weaning until 33 days after weaning. After this period, the piglets were transported to the fattening stable. Piglets and content of the feeders were weighed on d 7, 21 and 33 post-weaning so as to calculate ADG, ADFI and feed conversion ratio (FCR). Faeces scores were based on the following scale: 0= normal, solid faeces; 1 = soft, looser than normal, 2 = diarrhoea and 3=liquid faeces, severe diarrhoea. Condition scores were based on a scale of: 0 (good condition; healthy appearance, short hair, shiny skin) to 3 (poor condition; unhealthy appearance, long hair, pale and dull skin). Faeces and condition scores were recorded per pen once weekly by the same person who was unaware of treatment modality.

For leukocyte counts and globulin analyses, blood was collected from 10 randomly chosen piglets per dietary treatment 3 weeks after weaning. Blood was drawn by jugular venipuncture. The analyses were executed in the laboratory of the

Animal Health Service in the Southern Netherlands according to standard procedures. Leukocytes were counted electronically in duplicate. Leukocytes were differentiated microscopically by examining 100 leukocytes. Globulins were separated by electrophoresis on cellulose acetate and quantified densitometrically.

Experiment 2.
Two hundred and sixty-four weanling piglets, similar to those described above, were used. Piglets were housed and managed as described for experiment 1. The composition of the diets is presented in Table 2. The control diet contained 8.8 % whey-protein concentrate as a source of protein. In one of the experimental diets 3 % SDPP was added at the expense of the whey-protein concentrate ingredient, when compared with the control diet. The whey-protein level was 8.8 % to achieve that the same amount of protein was brought in the diets by the two protein sources. The second experimental diet contained both 8.8% whey-protein concentrate and 3 % SDPP, SDPP replacing a portion of the fishmeal component. The diets were pelleted and contained 1.03% of apparent ileal digestible lysine and had an energy density of 2394 kcal NE/kg, these prerequisites causing slight differences in the amounts of a limited number of ingredients. The contents of apparent ileal digestible lysine, methionine, threonine and tryptophan were balanced using crystalline amino acids. Piglets were fed the diets for 3 weeks. From days 22 to 33 after weaning, all piglets received the same starter diet as described above. There were nine observations (pens) per treatment. Piglets and content of the feeders were weighed on d 21 and 33 post-weaning. Experimental procedures, measurements and scoring methods were as described for experiment 1, except for the haematological analyses.

Analytical methods. Crude protein was determined according to Kjeldahl (EC 22-7-1993; nr. L 179/8-10). The analyses of crude fat (based on EC 3-9-1998; nr. L 257/23-25), crude fibre (based on EC 26-11-1992; nr. L334/35-37), moisture (based on EC 20-12-1971; nr. L279/ 8-11) and ash (based on ISO 936, 1992) were performed with gravimetrical methods. The starch content of the diets was determined polarimetrically according to Ewers (ISO 5554, 1993). Phosphorus was determined spectrophotometrically according to ISO 13730 (1996). Calcium, sodium, copper, iron, manganese, zinc, potassium and magnesium were analysed with atomic absorption spectrometry (ISO 6869). The amino acid content of SDPP was determined spectophotometrically.

Statistical Analysis. Analysis of variance was performed using the GLM procedures of SAS (1988). The statistical model used in experiment was Y_{ij} = mean + $diet_i$ + $room_j$ + $error_{ij}$. DF = 6 and 10 for this model in Exp. 1 and 2, respectively. In the tables, the pooled standard error of the mean (SEM) is given. The level of statistical significance was pre-set at $P < 0.05$.

Table 1

The composition and calculated nutrient content of the diets in experiment 1

Ingredient or nutrient	Control diet	SDPP diet
Ingredients, % on as fed basis		
Barley	35.00	35.00
Wheat	19.95	20.60
Corn	15.00	15.00
Fish meal	7.00	4.70
Dried whey	6.50	7.50
Toasted soy beans	6.00	6.00
Dried skim milk	5.00	2.70
Fat mixture	1.80	2.10
Organic acid mixture	1.50	1.50
L-Lysine HCl premix	0.51	0.44
Premix [a]	0.50	0.50
Limestone	0.46	0.83
Threonine premix	0.35	-
Salt	0.30	-
DL methionine premix	0.13	0.13
Spray-dried porcine plasma	-	3.00
Calculated nutrient content, on as fed basis		
NE, kcal/kg	2394	2394
Crude Protein, %	18.20	18.00
Ash, %	5.50	5.60
Fat, %	5.20	5.40
Starch, %	41.00	41.10
Crude fibre, %	2.90	2.90
Digestible[b] lysine, %	1.03	1.03
Digestible[b] methionine plus cystine, %	0.61	0.59
Digestible[b] methionine, %	0.37	0.33
Digestible[b] threonine, %	0.59	0.59
Digestible[b] tryptophan, %	0.19	0.21
Lactose, %	4.90	4.50
Calcium, %	0.80	0.83
Phosphorus, %	0.59	0.53
Digestible phosphorus, %	0.44	0.38
Potassium, %	0.96	1.00
Magnesium, %	0.13	0.13
Sodium, %	0.35	0.36

[a] Premix provided per kg of complete diet: vitamin A, 15,000 IU; vitamin D3, 1,800 IU; vitamin E, 20 mg; riboflavin, 3 mg; vitamin B12, 22.5 µg; vitamin K3, 1.1 mg, d-pantothenic acid, 8 mg; niacin, 65 mg; Fe, 80 mg; I, 0.4 mg; Co, 0.16 mg; Cu, 160 mg; Mn, 24.5 mg; Se, 0.125 mg; Zn, 150 mg; biotin, 0.04 mg; folic acid, 0.5 mg; Natuphos (phytase) 5000, 60 mg; tryptophan, 196 mg; tylosin, 40 mg.

[b] Apparent ileal digestible

Table 2

The composition and calculated nutrient content of the diets in experiment 2

Ingredient or nutrient	Control diet	SDPP diet without whey protein	SDPP diet with whey protein
Ingredients, % on as fed basis			
Barley	50.00	50.00	50.00
Corn	15.00	15.00	15.00
Whey-protein concentrate	8.80	-	8.80
Spray-dried porcine plasma	-	3.00	3.00
Fish meal	7.50	7.50	4.00
Toasted soy beans	6.00	6.00	6.00
Cassava	5.00	2.00	5.00
Fat mixture	2.30	2.45	2.50
Wheat	1.80	9.40	1.80
Organic acid mixture	1.50	1.50	1.50
Limestone	0.70	0.57	0.65
Premix [a]	0.50	0.50	0.50
Mono sodium phosphate	0.40	0.70	0.80
L-Lysine HCl premix	0.40	0.60	0.35
DL methionine premix	0.10	0.13	0.10
Threonine premix	-	0.55	-
Tryptophan premix	-	0.10	-
Calculated nutrient content, on as fed basis			
NE, kcal/kg	2394	2394	2394
Crude Protein, %	17.60	17.70	17.40
Fat, %	5.97	5.97	5.92
Ash, %	5.20	5.20	5.40
Starch, %	40.90	43.80	40.90
Crude fibre, %	3.46	3.52	3.46
Digestible[b] lysine, %	1.03	1.03	1.03
Digestible[b] methionine plus cystine, %	0.61	0.59	0.63
Digestible[b] methionine, %	0.37	0.33	0.33
Digestible[b] threonine, %	0.63	0.59	0.66
Digestible[b] tryptophan, %	0.20	0.18	0.20
Lactose, %	4.30	0.00	4.30
Calcium, %	0.92	0.84	0.86
Phosphorus, %	0.63	0.64	0.63
Digestible phosphorus, %	0.39	0.39	0.39
Potassium, %	0.66	0.58	0.66
Magnesium, %	0.14	0.13	0.13
Sodium, %	0.16	0.29	0.28

[a] Premix provided per kg of complete diet: vitamin A, 15,000 IU; vitamin D3, 1,800 IU; vitamin E, 20 mg; riboflavin, 3 mg; vitamin B12, 22.5 µg; vitamin K3, 1.1 mg, d-pantothenic acid, 8 mg; niacin, 65 mg; Fe, 80 mg; I, 0.4 mg; Co, 0.16 mg; Cu, 160 mg; Mn, 24.5 mg; Se, 0.125 mg; Zn, 150 mg; biotin, 0.04 mg; folic acid, 0.5 mg; Natuphos (phytase) 5000, 60 mg; tryptophan, 196 mg; tylosin, 40 mg. [b]Apparent ileal digestible

Results

Experiment 1

The analysed composition of the diets (Table 3) agreed well with the calculated composition, except that the fat content of the control diet and the crude protein content of the SDPP feed were somewhat lower and higher than expected, respectively (table 1). Table 3 also shows that mineral and trace element concentrations in the two experimental diets were similar, except for iron which cannot be explained.

Table 3

Analysed composition of spray-dried porcine plasma (SDPP) and the diets in experiment 1

Nutrient		SDPP	Control diet	SDPP diet
Crude protein,	%	69.1	18.4	19.1
Ash,	%	13.1	5.8	6.1
Fat,	%	1.9	4.6	5.4
Crude fibre,	%	-	2.8	2.8
Starch,	%	-	40.7	39.6
Moisture,	%	10.6	10.5	10.5
Phosphorus,	%	0.13	0.64	0.60
Calcium,	%	0.11	0.92	0.94
Sodium,	%	4.43	0.38	0.38
Potassium,	%	0.35	0.93	0.94
Magnesium,	%	0.03	0.15	0.14
Copper,	mg/kg	17	140	137
Iron,	mg/kg	101	197	287
Manganese,	mg/kg	5	54	58
Zinc,	mg/kg	9	153	152

Piglets fed the SDPP diet instead of the control diet grew 11% and 6 % faster during the first 7 days and during days 8-21, respectively (Table 4). This difference in ADG was significant only during days 8-21 ($P < 0.05$). Piglets fed the SDPP diet had a 4% and 3% lower FCR during days 1-7 and 8-21, the difference being significant only for the latter period ($P < 0.05$). During the whole three-week period of feeding the experimental diets, piglets fed the SDPP diet had a 7% higher ADG ($P < 0.05$) and a 3 % lower FCR ($P < 0.05$). ADFI was not significantly influenced by feeding SDPP. During the experiment overall health status of the piglets was good and SDPP in the diet had no significant impact on condition and faecal scores (results not shown).

During days 22-33 after weaning when all piglets received an identical diet, the feeder in one pen showed aberrations. The results of this pen are not included in the data for this period.

Table 4

The effect of SDPP in experiment 1 on growth performance of piglets weaned when weighing on average 8 kg

Criterion and days	Control diet	SDPP diet	SEM
ADG (g/day)			
1-7	176	196	9
8-21	432	457*	8
1-21	339	361*	7
22-33	522	521	29
1-33	406	416	14
ADFI (g/day)			
1-7	185	194	10
8-21	566	572	11
1-21	421	434	9
22-33	882	859	35
1-33	586	604	14
FCR			
1-7	1.06	1.02	0.05
8-21	1.29	1.25*	0.01
1-21	1.24	1.20*	0.01
22-33	1.70	1.80	0.09
1-33	1.45	1.46	0.03

* Significantly different from the corresponding value for the control diet ($P < 0.05$).

Table 5

The effect of SDPP in experiment 1 on blood variables of piglets three weeks after weaning

	Control diet	SDPP diet	SEM
Leukocytes, nr/nl	18.1	19.9	1.8
Lymphocytes, %	61.1	66.9	4.9
Monocytes, %	0.9	1.1	0.3
Eosinophils, %	1.3	1.7	0.03
Basophils, %	0.4	0.8	0.3
Stabform neutrophils, %	0.6	0.7	0.3
Segmented neutrophils, %	35.7	28.8	4.7
Total protein g/l	50.6	52.3	1.5
Albumin, %	51.9	47.9*	1.4
α -globulin, %	21.8	22.8	0.8
β -globulin, %	19.0	20.6	0.7
γ -globulin, %	7.3	8.7	0.5

* Significantly different from the corresponding value for the control diet ($P < 0.05$).

During days 22-33, there were no significant differences in the three zootechnical variables. During the whole experimental period (days 0-33) there were no significant differences in ADG, ADFI and FCR either. There were no significant diet-induced differences in faecal and condition scores after day 21 (results not shown). The blood variables are presented in Table 5. There were no significant differences between the two dietary treatments except for albumin that was significantly lower in the SDPP fed piglets than in the control piglets.

Experiment 2

The analysed composition of the diets (Table 6) was comparable to the calculated composition, with the exception of the fat content of the three diets that was higher than expected (Table 2). Mineral and trace element contents of the three diets were comparable, except for sodium.

Table 6

Analysed composition of spray-dried porcine plasma (SDPP) and the diets in experiment 2.

NUTRIENT		SDPP	Control diet	SDPP diet without whey protein	SDPP diet with whey protein
Crude protein,	%	70.1	18.0	16.9	17.7
Ash,	%	18.5	5.6	5.2	5.6
Fat,	%	2.2	6.9	7.2	7.3
Crude fibre,	%	-	4.0	4.1	4.0
Starch,	%	-	39.9	43.5	40.4
Moisture,	%	5.6	10.0	10.1	9.9
Phosphorus,	%	0.10	0.63	0.66	0.65
Calcium,	%	0.06	0.87	0.77	0.77
Sodium,	%	7.20	0.15	0.34	0.34
Potassium,	%	0.16	0.77	0.64	0.72
Magnesium,	%	0.01	0.14	0.14	0.13
Copper,	mg/kg	11	156	138	135
Iron,	mg/kg	44	256	240	242
Manganese,	mg/kg	1	39	44	45
Zinc,	mg/kg	11	198	195	171

The amino acid composition of SDPP was found to be as follows (% of product): alanine, 3.88; asparagine, 6.70; arginine, 3.96; glutamine, 9.83; glycine 2.63; histidine, 2.12; isoleucine, 2.65; leucine, 6.66; lysine, 6.17; methionine, 0.47; cystine, 2.52; phenylalanine, 4.02; serine, 4.23; threonine, 4.35; valine, 5.00.

During the first three weeks after weaning there were no significant differences in ADG, ADFI and FCR among the dietary treatments (Table 7). On a group mean level, the SDPP diet with whey protein produced a 5 % higher ADG, 3% higher ADFI and 2% lower FCR than did the control diet containing whey protein. During days 22-33, when all piglets received an identical diet, and for the whole experimental period (days 0-33) there were no significant differences either. In the third and fourth week after weaning, the piglets fed the SDPP diet with whey protein had significantly better condition scores ($P < 0.05$) than did the piglets fed the SDPP diet without whey protein.

Table 7

The effects of SDPP in the absence or presence of whey protein on growth performance and condition scores of weanling piglets in experiment 2

Criterion and days*	Control diet	SDPP diet without whey protein	SDPP diet with whey protein	SEM
ADG (g/day)				
1-21	316	314	331	7
22-33	524	511	512	10
1-33	391	385	396	7
ADFI (g/day)				
1-21	409	416	421	11
22-33	881	848	868	13
1-33	578	570	582	8
FCR				
1-21	1.30	1.32	1.27	0.03
22-33	1.70	1.68	1.71	0.03
1-33	1.48	1.48	1.47	0.02
Condition score				
7	1.11	1.22	1.11	0.21
14	1.33	1.44	1.11	0.18
21	1.22[ab]	1.56[a]	1.00[b]	0.13
28	1.44[ab]	1.67[a]	1.11[b]	0.18

[a, b] Means within the same row without a common superscript letter differ significantly ($P< 0.05$)

Discussion

When SDPP was substituted for a combination of fish and milk protein in experiment 1, a positive effect of SDPP on growth performance was seen. When the diet contained whey protein and a portion of its fish meal component was replaced by SDPP (exp. 2) there was no significant effect on growth performance but group mean values improved. Taken together the results of experiments 1 and 2, it would appear that SDPP is superior to fishmeal. Thus, SDPP may be used as an alternative for fishmeal in weanling diets. The observed improvement of ADG and FCR during the first three weeks after weaning, could justify the use of SDPP in weanling piglets diets. The magnitude of the SDPP induced improvements in growth performance is not as high as found in most other experiments conducted in the USA (Van Dijk et al. 2001). Possibly, the high hygiene status of our research station may be the cause of this, because it has been suggested that SDPP has a more pronounced effect under conditions with high infection pressure (Coffey and Cromwell 1995, Bergström et al. 1997). There were no significant differences in blood values between the two dietary treatments except for albumin that was significantly lower in the SDPP fed piglets than in the control piglets. Because there were no differences in leukocyte counts or γ-globulin, of which high values are indicative for infections (Imlah and McTaggert, 1977), it can be concluded that there was no difference in health status between the two dietary treatment groups. Overall faecal and condition scores were good, losses percentages were low (0.7% and 1.6% in exp. 1 and 2 respectively) and blood variables within the standard range from which it can be concluded that the overall health status of the piglets was good.

In experiment 2, SDPP apparently was equally effective as whey protein. The addition of SDPP to the diet in combination with the omission of whey protein had no significant effect on growth performance. However, the SDPP diet without whey protein contained extra wheat protein and no lactose. Moreover, the control diet contained less sodium than the two SDPP diets. The effect of those differences cannot be ascertained with the present experimental design. On the other hand, the lack of effect of SDPP could relate to the fact that the control diet was high in whey protein. This conclusion corroborates earlier observations in that the protein source in the control diet either masks or enhances the effect of SDPP: When the control diet contained casein instead of soy protein, the incorporation of SDPP into the diet produced a smaller stimulatory effect on growth performance in weanling piglets (Van Dijk et al. 2001). Further evidence for protein interactions comes from experiment 2. Piglets fed the diet with SDPP and whey protein showed better condition scores in weeks 3 and 4 after weaning than did the piglets fed the SDPP diet without whey protein. Possibly, the use of the combination of SDPP and whey protein is beneficial on farms with health problems or unthrifty appearance of the piglets.

In both experiments, there was no carry-over effect of SDPP feeding into the subsequent feeding period. This is in accordance with literature data (Van Dijk et al. 2001). This study corroborates earlier local reports (Van der Peet-Schwering and Binnendijk 1995 and 1997) in that a low amount of SDPP in diets for weanling piglets can have positive effects on growth performance under Northern European conditions. Possible mechanisms by which SDPP may stimulate growth performance of weanling piglets have been described elsewhere (Van Dijk et al. 2001). Probably, larger effects of SDPP on piglets' growth performance and health would be seen under less hygienic conditions and with higher inclusion levels in the diet.

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CHAPTER 3.2

THE INFLUENCE OF DIET COMPOSITION AND AN ANTI MICROBIAL GROWTH PROMOTER ON THE GROWTH RESPONSE OF WEANED PIGLETS TO SPRAY DRIED ANIMAL PLASMA

P. Bikker[a], A.J. van Dijk[b], A. Dirkzwager[a], J. Fledderus[a], M. Ubbink-Blanksma[b], A.C. Beynen[c]

[a] Institute for Animal Nutrition 'De Schothorst', P.O. Box 533, 8200 AM Lelystad, The Netherlands

[b] Co-operative Central Laboratory "CCL- Nutricontrol" of Cehave Landbouwbelang, P.O. Box 107, 5460 AC Veghel, The Netherlands.

[c] Department of Nutrition, Utrecht University, Faculty of Veterinary Medicine, P.O. Box 80152, 3508 TD Utrecht, The Netherlands

Submitted

Abstract

Two separate experiments were conducted with spray dried animal plasma (SDAP) in diets for piglets from 0-14 days post-weaning. Exp. 1 was conducted to determine a possible interaction between diet composition and SDAP in diets without anti microbial growth promoter (AMGP). Exp. 2 was conducted to determine a possible interaction between SDAP and an AMGP. Both experiments comprised four treatments in a 2 x 2 factorial arrangement. The respective factors were diet composition (simple vs. complex) and SDAP (0% vs. 4%) in exp. 1 and AMGP (0 vs. 40 ppm avilamycine) and SDAP (0% vs. 4%) in exp. 2. In exp. 1 SDAP improved average daily gain (ADG) and feed conversion ratio (FCR) during the first week post weaning by 19% and 11%, respectively ($P < 0.05$). SDAP effects were more pronounced for the complex diet. In exp. 2, SDAP improved

feed intake, ADG and FCR during the first week post-weaning by 14, 25 and 11%, respectively ($P < 0.001$). SDAP tended ($P < 0.1$) to have more effect on ADG and ADFI for the diet without avilamycine. In conclusion SDAP in weaning diets has a positive effect on growth performance of the piglets. The magnitude of the effect depends on diet complexity and is more pronounced for diets without AMGP.

Introduction

The type of protein in the weaning diet of piglets has consequences for feed intake, weight gain, nitrogen digestibility and pancreatic enzyme activity (Makkink et al. 1994a, Makkink et al. 1994b, Peiniau et al. 1996). The feeding of spray dried animal plasma (SDAP) has been shown to promote growth performance in weanling piglets (Van Dijk et al. 2001).

The use of SDAP in diets for weaned piglets in e.g. The Netherlands is limited because of the high price in comparison with other protein sources. There is an interaction between the background composition of the diet and the effect of SDAP. A meta analysis revealed that the response of average daily gain (ADG) and feed conversion ratio (FCR) to SDAP was greater when soy protein was used in the control feed instead of dairy protein (Van Dijk et al., 2001). Presumably less complex diets can be formulated if SDAP is included. Therefore it was considered necessary to determine the efficacy of SDAP added to complex Northern European diets. Moreover, because of the expected reduction in the use of anti-microbial growth promoters (AMGP) in piglet diets, the effect of SDAP in diets without AMGP is of interest. A ban on AMGP may limit production and increase health disorders like diarrhoea in newly weaned piglets (Kamphues 1999). Results of Coffey and Cromwell (1995) showed a larger effect of SDAP in piglets kept under poor hygienic conditions. There are indications that constituent immunoglobulins are in part responsible for the improved performance induced by SDAP (Godfredson-Kisic and Johnson 1997, Van Dijk et al. 2001). It was thus hypothesised that the immunoglobulins present in SDAP reduce bacterial and viral activity in the gut and have a positive effect on the integrity of the intestinal mucosa. This hypothesis would imply that SDAP has a greater effect on growth performance of weaned piglets when it is incorporated into diets without AMGP.

SDAP shows substantial effects at relatively low inclusion rates in the diet (Van Dijk et al. 2001) and it is expected that only low dosages can be economically justified. Thus, low inclusion rates of SDAP were used in the present experiments. Two experiments were conducted to evaluate the effects of SDAP as a protein source for weanling piglets. The objective of experiment 1, using weanling piglets' diets without AMGP, was to determine a possible interaction between diet ingredients and SDAP. The objective of experiment 2 was to determine a possible

interaction between SDAP and AMGP in weaned piglets fed relatively complex diets. Avilamycine was used as a prototypical AMGP.

Materials and methods

Experiment 1

Four hundred and forty weanling piglets (F_2 cross-bred: GY x [Finnish X Dutch Landrace]) from the closed herd of the research station 'Laverdonk' (Veghel, The Netherlands) were used. The females, males and castrates weighed on average 8.3 kg and were 26 days of age. The piglets were allocated to the pens so that sex, litter origin and body weight were equally distributed. Piglets were housed in environmentally regulated rooms in pens (2.60m x 1.20m) with partially slatted floors (concrete floor: 1.10m x 1.20m) and had ad libitum access to feed and water. Each room had 6 pens and each pen contained 10 piglets. Each pen was equipped with a nipple waterer and a one-hole self-feeder. The room temperature was 26 °C on the first day after weaning, gradually declining to 23 °C by the end of the experiment. Day light could enter the rooms.

The experiment had a randomised complete block design with pen as experimental unit and replicate as block. The experiment comprised four treatments from 0-14 days post-weaning in a 2 x 2 factorial arrangement. Within a room, pens were assigned randomly to one of the four dietary treatments. There were eleven replicates per treatment.

The experimental weanling diets that were used were: 1. a relatively simple diet, 2. a relatively complex diet, 3. the simple diet with SDAP (Appetein, APC, Ames, USA), 4. the complex diet with SDAP (Table 1). Simple and complex diets both met the energy and ileal digestible amino acid requirements for newly weaned piglets, but they differed in raw materials. The simple diets had barley, wheat, soybean meal, fish meal and potato protein as base components. The complex diets contained additionally maize, linseed meal and whey powder. SDAP was included at the expense of equal amounts of fishmeal and potato protein. Soya oil and pure amino acids were used to exclude any small differences in energy content and apparent ileal digestible essential amino acid levels among the experimental diets. The diets were pelleted and contained 10.3 g/kg apparent ileal digestible lysine and 9.84 MJ NE/kg. The contents of apparent ileal digestible methionine, lysine, threonine and tryptophan were balanced by adding crystalline amino acids. The four diets did not contain AMGP. After being fed the experimental diets, all piglets received the same starter diet from days 15-28 post-weaning. This diet was formulated to meet the requirements of weaned piglets in the rearing period from 8 to 25 kg.

Table 1

The ingredient composition and calculated nutrient content of the diets in experiment 1

Treatment	1	2	3	4
Diet composition	simple	complex	simple	complex
SDAP level	0%	0%	4%	4%
Ingredients, % on as fed basis				
Barley	25.00	39.94	25.00	39.93
Wheat	50.42	20.50	50.09	21.00
Maize	-	11.20	-	10.40
Linseed meal	-	2.00	-	2.00
Soybean meal	11.00	2.00	11.00	2.00
Fish meal	2.10	3.10	-	1.00
Wheypowder	-	11.59	-	11.60
Purified potato protein	3.10	3.10	1.00	1.00
Fat mixture	0.49	1.17	0.50	1.19
Soybean oil	1.52	0.50	1.95	0.96
Molasses	2.00	2.00	2.00	2.00
Monocalcium phosphate	0.91	0.29	1.14	0.51
L-Lysine HCl premix	0.71	0.67	0.67	0.64
Premix [a]	0.50	0.50	0.50	0.50
Limestone	0.88	0.84	0.91	0.87
Threonine premix	0.66	0.18	0.48	-
Salt	0.47	0.03	0.51	0.09
Tryptophan premix	0.09	0.23	-	0.05
DL methionine premix	0.15	0.16	0.25	0.26
Spray-dried animal plasma	-	-	4.00	4.00
Calculated nutrient content, on as fed basis, g/kg				
NE, MJ/kg	9.84	9.84	9.84	9.84
Crude Protein	181	172	182	173
Fat	37	37	39	40
Crude fibre	28	28	28	28
Starch	466	428	463	423
Digestible[b] lysine	10.3	10.3	10.3	10.3
Chloride	4.8	6.2	4.7	6.1
Calcium	7.0	7.0	7.0	7.0
Digestible phosphorus	3.6	3.6	3.6	3.6
Sodium	2.5	2.6	2.5	2.6

[a] Premix provided per kg of complete diet: vitamin A, 7,500 IU; vitamin D3, 1,500 IU; vitamin E, 17.5 mg; riboflavin, 3 mg; vitamin B12, 20 µg; vitamin K3, 0.75 mg, d-pantothenic acid, 6 mg; niacin, 30 mg; Fe, 80 mg; I, 0.4 mg; Co, 0.15 mg; Cu, 160 mg; Mn, 24 mg; Se, 0.125 mg; Zn, 200 mg

[b] Apparent ileal digestible

Piglets and content of the feeders were weighed on d 7, 14 and 28 post-weaning to calculate ADG, average daily feed intake (ADFI) and FCR. Faecal scores were based on the following scale: 0= normal, solid faeces; 1 = soft, looser than normal, 2 = diarrhoea and 3 = liquid faeces, severe diarrhoea. Faecal scores were recorded per pen twice weekly by the same person who was blinded to treatment modality.

Experiment 2

Two hundred and eighty-eight weanling piglets (F_2 cross-bred: GY x [Norwegian X Dutch Landrace]) from the closed herd of the research institute 'De Schothorst', (Lelystad, The Netherlands) were used. The females and castrates weighed on average 8.0 kg and were 26 days of age. The piglets were allocated to the pens so that sex, litter origin and body weight were equally distributed. Piglets were housed in environmentally regulated rooms in pens (2.00m x 1.10m) with partially slatted floors (concrete floor: 0.75m x 1.10m) and had ad libitum access to feed and water. Each room had 10 pens and each pen contained 6 piglets. Each pen was equipped with a nipple waterer and a self-feeder. The room temperature was 26 ^{o}C on the first day after weaning, gradually declining to 23 ^{o}C by the end of the experiment. The rooms were illuminated from 7.00 a.m. to 19.00 p.m.

The experiment comprised four treatments from 0-28 days post-weaning in a 2 x 2 factorial arrangement with twelve replicates. The experiment had a randomised complete block design with pen as experimental unit and replicate as block. The inclusion of SDAP into the diets fed during 14 days and that of AMGP during 28 days post-weaning were the treatments.

The experimental weanling diets that were used from day 0-14 were: 1. a diet without SDAP and without AMGP, 2. a diet with avilamycine as AMGP and without SDAP, 3. a diet with SDAP (Appetein, APC, Ames, USA) and without AMGP, 4. a diet with both SDAP and AMGP (Table 2). The four diets met the energy and ileal digestible amino acid requirements for newly weaned piglets. SDAP was included at the expense of fishmeal and potato protein. Renderer fat and pure amino acids were used to exclude any differences in energy content and apparent ileal digestible essential amino acid levels among the experimental diets. The diets were pelleted and contained 11.0 g/kg apparent ileal digestible lysine and 9.68 MJ NE/kg. The contents of apparent ileal digestible methionine, lysine, threonine and tryptophan were balanced by adding crystalline amino acids. From days 15-28 post-weaning all piglets received the same diet that was formulated to meet the requirements of weaned piglets in the rearing period from 8 to 25 kg. However, the piglets from treatments 1 and 3 received this diet without AMGP, whereas piglets from treatments 2 and 4 received this diet with AMGP. Thus, SDAP was included in treatments 3 and 4 from days 1-14 and AMGP was included in treatments 2 and 4 from days 1-28 post-weaning.

Table 2
The ingredient composition and calculated nutrient content of the diets in experiment 2

	0-14 days post-weaning				15-28 days	
Treatment	1	2	3	4	1+3	2+4
AMGP	0	40 ppm	0	40 ppm	0	40 ppm
SDAP	0 %	0 %	4 %	4 %	0 %	0 %
Ingredients, % on as fed basis						
Barley	30.0	30.0	30.0	30.0	25.0	25.0
Wheat	27.8	27.8	27.8	27.8	34.9	34.9
Maize	12.4	12.4	12.4	12.4	-	-
Manioc	0.9	0.5	0.65	0.2	11.9	11.5
Linseed meal	-	-	-	-	2.7	2.7
Soybean meal	7.05	6.65	7.05	6.65	12.5	12.1
Spray dried animal plasma	-	-	4.0	4.0	-	-
Wheypowder	11.3	11.3	11.3	11.3	-	-
Fishmeal	3.1	3.1	1.1	1.1	2.15	2.15
Renderer fat	0.8	0.8	0.94	0.99	1.0	1.0
Monocalcium phosphate	0.10	0.10	-	-	0.42	0.42
CaCO3	0.25	0.25	0.42	0.42	0.1	0.1
NaCl	0.25	0.25	-	-	0.77	0.77
Protastar, potato protein	3.0	3.0	0.8	0.8	2.1	2.1
Molasses	-	-	-	-	2.5	2.5
Soybean oil	0.5	0.5	0.5	0.5	0.24	0.24
Calciumformate	0.75	0.75	0.75	0.75	0.75	0.75
Premix[a]	0.5	0.5	0.5	0.5	0.5	0.5
Methionine premix 10%	-	-	0.51	0.51	-	-
Lysine premix 25%	0.21	0.21	0.23	0.23	0.24	0.24
Threonine premix 10%	-	-	-	-	0.73	0.73
Lys / met. premix 20/10%	0.74	0.74	0.78	0.78	1.03	1.03
Lys / try premix 18/5%	0.08	0.08	-	-	-	-
Phytase, 100,000 FTU	0.30	0.30	0.30	0.30	0.46	0.46
Avilamycine premix[b]	-	0.8	-	0.8	-	0.8
Calculated nutrient content, on as fed basis, g/kg						
NE, MJ/kg	9.68	9.68	9.68	9.68	9.42	9.42
Crude protein	182	182	184	183	170	170
Ash	55	55	54	55	53	53
Fat	38	38	37	38	37	37
Crude fibre	27	27	27	27	35	35
Starch	405	405	405	405	426	424
[c] Digestible lysine	11	11	11	11	10	10
Chloride	7.0	7.0	5.8	5.8	6.5	6.5
Calcium	6.6	6.6	6.6	6.6	5.5	5.5
Dig. phosphorus	3.6	3.6	3.6	3.6	3.2	3.2
Sodium	3.3	3.3	3.3	3.3	3.5	3.5

[a] Premix provided per kg of complete diet: vitamin A, 7,500 IU; vitamin D3, 1,500 IU; vitamin E, 15 mg; riboflavin, 4 mg; vitamin B12, 20 µg; vitamin K3, 0.75 mg, d-pantothenic acid, 6 mg; niacin, 30 mg; Fe, 80 mg; I, 0.4 mg; Co, 0.15 mg; Cu, 160 mg; Mn, 24 mg; Se, 0.125 mg; Zn, 62 mg. [b] Premix provided 40 ppm of avilamycine per kg of diet. [c]Apparent ileal digestible

Piglets and content of the feeders were weighed on d 7, 14 and 28 post-weaning to calculate ADG, ADFI and FCR. In addition faecal consistency was visually recorded twice a week by an experienced panel. Faecal scores were registered on a scale from 1-10 for which 1 = liquid stools, 10 = hard and dry stools.

Analytical methods

Crude protein was determined according to Kjeldahl (EC 22-7-1993; nr. L 179/8-10). The analyses of fat (based on EC 3-9-1998; nr. L 257/23-25), crude fibre (based on EC 26-11-1992; nr. L334/35-37), moisture (based on EC 20-12-1971; nr. L279/ 8-11) and ash (based on ISO 936, 1992) were performed with gravimetrical methods. The starch content of the diets was determined polarimetrically according to Ewers (ISO 5554, 1993) in exp. 1 and according to ISO/CD 15159 (2000) in exp. 2. Phosphorus was determined spectrophotometrically according to ISO 13730 (1996). Calcium, was analysed with atomic absorption spectrometry (ISO 6869). Pellet hardness was determined using the Kahl tester in Experiment 1 and according to Schleuniger in Experiment 2 (Thomas and Van der Poel, 1996).

Statistical Analysis

Analysis of variance was performed using the GLM procedures of SAS (1988). The statistical model used in experiment 1 was Y_{ijk} = mean + $block_i$ + feed $composition_j$ + SDAP $level_k$ + interaction + $error_{ijk}$. The statistical model used in experiment 2 was Y_{ijk} = mean + $block_i$ + AMGP $level_j$ + SDAP $level_k$ + interaction + $error_{ijk}$. Faecal scores were considered as a continuous variable. The level of statistical significance was pre-set at $P < 0.05$. In the tables, the pooled standard error of the mean (SEM) is given.

Results

Experiment 1

The analysed composition of the diets (Table 3) agreed well with the calculated composition. The level of crude protein was somewhat low for the complex diet without SDAP.

In Table 4 the main effects for SDAP and type of diet are presented. SDAP improved ADG and FCR in the first week after weaning ($P < 0.05$). There were no significant effects of SDAP in the second week post-weaning. For the entire four-week period, results were similar for diets with and without SDAP. There was a small but consistent positive effect of the inclusion of SDAP on the faecal consistency in weeks 1 and 2 post-weaning.

Table 3

Analysed composition the diets used in experiment 1

Diet composition SDAP	Simple 0%	Complex 0%	Simple 4%	Complex 4%
Component, g/kg				
Crude protein	184	169	182	181
Ash	47	50	51	56
Fat	41	39	48	42
Crude fibre	26	23	26	26
Starch	442	425	437	409
Moisture	117	112	113	108
Pellet hardness, Newton	33	41	35	46

There was no effect of the complexity of the diets on growth performance. For the entire four-week period the FCR tended ($P < 0.01$) to be less favourable for the complex diet.

For the various measures there were significant interactions between SDAP level and diet composition (Table 4). To illustrate these effects, the results of the four dietary treatments are presented in Table 5. There was a significant interaction between diet composition and SDAP for both ADG and FCR in the second week post-weaning (Table 4). For FCR there also was a tendency towards interaction during days 1-14 (Table 4). Data in Table 5 show that SDAP improved FCR when present in the complex diet but not in the simple diet for the period of two weeks post-weaning. Similar effects, although not statistically significant, were found for FCR and ADG during week 2 and for ADFI during weeks 3-4 and weeks 1-4. In the whole 4-week experimental period, piglets that were fed the simple diet without SDAP during the first two weeks after weaning grew significantly faster than did piglets fed the complex diet without SDAP.

Feed intake in weeks 3 and 4, i.e. the period after feeding the experimental diets, was higher than expected. As a consequence, for the last two replicates there was not enough diet so that their data for weeks 3 and 4 are not included in the calculations. Piglets that were fed the complex diet containing SDAP ate more of the same diet during days 15-28 than did their counterparts that had been fed the simple diet with SDAP (Table 5). In the entire 4-week period, piglets that were fed the complex diet with SDAP during the first two weeks after weaning ate significantly more than piglets fed the simple diet with SDAP.

On day 16 after weaning, the faecal score of the piglets that had previously been fed the simple diet without SDAP was significantly worse than that for the piglets that were previously fed complex diets. On day 23 after weaning, the faecal score for the piglets that had previously been fed the simple diet with SDAP was significantly less favourable than that for the piglets that were fed earlier the complex diet with SDAP.

Table 4

The main effects of inclusion of SDAP in a simple or complex diet on growth performance of weanling piglets fed the experimental diets until 14 days post-weaning followed by feeding of the same starter diet to all piglets (Exp. 1)

	Diet composition		SDAP Level		Statistical significance[a]			
	simple	complex	0%	4%	SEM	Diet composition	SDAP	Int.
Criterion and days								
ADG, g/d								
1-7	135	132	123	145	7	ns	*	ns
8-14	232	229	230	232	8	ns	ns	*
15-28	393	399	403	390	9	ns	ns	ns
1-14	184	181	176	188	6	ns	ns	ns
1-28	288	281	284	285	5	ns	ns	ns
ADFI, g/d								
1-7	159	157	153	163	6	ns	ns	ns
8-14	302	311	307	306	7	ns	ns	ns
15-28	580	601	596	584	12	ns	ns	t
1-14	231	234	230	235	6	ns	ns	ns
1-28	407	413	412	408	7	ns	ns	*
FCR								
1-7	1.20	1.25	1.29	1.16	0.04	ns	*	ns
8-14	1.32	1.38	1.36	1.34	0.03	ns	ns	*
15-28	1.50	1.53	1.49	1.53	0.03	ns	ns	ns
1-14	1.26	1.31	1.32	1.25	0.02	*	**	t
1-28	1.42	1.48	1.46	1.45	0.02	t	ns	ns
Faecal score								
2	1.36	1.36	1.45	1.27	0.09	ns	ns	ns
6	1.41	1.45	1.55	1.32	0.07	ns	*	ns
9	1.64	1.59	1.68	1.55	0.10	ns	ns	ns
13	1.17	1.17	1.22	1.11	0.09	ns	ns	ns
16	1.22	1.00	1.17	1.06	0.07	*	ns	ns
20	1.00	1.00	1.00	1.00	0.00	ns	ns	ns
23	1.33	1.08	1.17	1.25	0.10	ns	ns	t
27	1.00	1.00	1.00	1.00	0.00	ns	ns	ns

[a] Statistics: Int. = interaction diet composition x SDAP, ns = not significant, t = tendency, $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Table 5

The individual treatment effects of inclusion of SDAP in a simple or complex diet on growth performance of weanling piglets fed the experimental diets until 14 days post-weaning followed by feeding of the same starter diet to all piglets (Exp. 1)

Diet	simple	complex	simple	complex	SEM
SDAP	0%	0%	4%	4%	
Criterion and days					
ADG, g/d					
1-7	121	124	149	141	10
8-14	242	218	223	241	9
15-28	408	398	378	401	12
1-14	182	171	186	191	8
1-28	296[a]	273[b]	281[ab]	288[ab]	7
ADFI, g/d					
1-7	152	155	167	160	8
8-14	309	305	296	316	9
15-28	602[ab]	591[ab]	557[a]	610[b]	16
1-14	230	230	231	238	8
1-28	419[ab]	405[ab]	395[a]	421[b]	9
FCR					
1-7	1.27[ab]	1.32[a]	1.14[b]	1.18[ab]	0.05
8-14	1.29[b]	1.44[a]	1.35[ab]	1.33[ab]	0.04
15-28	1.50	1.49	1.50	1.56	0.04
1-14	1.27[a]	1.37[b]	1.25[a]	1.26[a]	0.02
1-28	1.43	1.49	1.42	1.47	0.03
Faecal score					
2	1.45	1.45	1.27	1.27	0.14
6	1.55	1.55	1.27	1.36	0.10
9	1.73	1.64	1.55	1.55	0.14
13	1.22	1.22	1.11	1.11	0.13
16	1.33[a]	1.00[b]	1.11[ab]	1.00[b]	0.09
20	1.00	1.00	1.00	1.00	0.00
23	1.17[ab]	1.17[ab]	1.50[a]	1.00[b]	0.13
27	1.00	1.00	1.00	1.00	0.00

[a,b] Means within the same row without a common superscript letter differ significantly ($P < 0.05$).

Experiment 2

The analysed composition of the diets (Table 6) agreed well with the calculated composition although the analysed fat content was slightly higher than that calculated (Table 2). The main effects of SDAP and avilamycine are presented in Table 7. From 1-14 days AMGP did not significantly affect the variables whereas inclusion of SDAP into the diet significantly improved ADG, ADFI, FCR and

faecal scores. The effects of SDAP were most prominent from 1-7 days and diminished from 8-14 days post-weaning.

Table 6

Analysed composition of the diets used in experiment 2

	0-14 days post-weaning				15-28 days	
Treatment	1	2	3	4	1+3	2+4
Component, g/kg						
Crude protein	183	186	184	184	169	170
Ash	52	53	51	51	50	50
Fat	42	42	41	42	49	49
Crude fibre	25	25	25	23	32	31
Starch	383	399	396	397	398	400
Moisture	141	135	139	135	140	139
Pellet hardness, Newton	24	28	33	29	11	12

The faecal consistency was significantly better on the SDAP diets during the first week post-weaning without significant effects thereafter. In week 3-4 post-weaning AMGP significantly improved ADFI and ADG. In week 3-4 post-weaning the piglets that previously had received the SDAP diet showed a lower ADFI and ADG than their counterparts that had been previously fed a diet without SDAP. Furthermore, the piglets receiving a diet with AMGP showed a higher ADG during days 15-28 than the piglets fed a diet without AMGP. The FCR for days 15-28 was similar for all treatments. Over the four-week period, inclusion of the growth promoter into the diet tended to improve ADG without significant effects on ADFI or FCR. For the period of days 1-28 SDAP did not have a significant effect on ADG or ADFI, but significantly improved the FCR.

There were tendencies ($P < 0.1$) for an interaction between the effects of AMGP and SDAP. To illustrate the interactions Table 8 presents results for the individual treatments. The inclusion of SDAP into the diet had more significant effects in the absence than in the presence of AMGP. Results for days 1-7 and 8-14 demonstrate a considerable effect of SDAP on ADFI and ADG when the diet did not contain AMGP, whereas the effects were less pronounced for the diet with AMGP. Similarly, AMGP tended to have greater effects when added to diets without SDAP. Furthermore, for the period of 1-14 days SDAP more effectively influenced performance variables than did AMGP. In general, performance variables during weeks 1-2 were significantly better when the diet contained both SDAP and AMGP instead of none of the additions. In week 3-4 AMGP increased both ADFI and ADG and tended to have a greater effect in piglets that had not previously been fed diets with SDAP. For the entire four-week period AMGP increased ADFI and ADG in piglets fed diets without SDAP, but not in their counterparts that had been fed diets with SDAP during days 1-14 post-weaning.

Table 7

The main effects of inclusion of avilamycine and SDAP on growth performance of weanling piglets (Exp. 2)

	avilamycine		SDAP			Statistical significance[a]		
	0	40 ppm	0	4 %	SEM	avilamycine	SDAP	Int.
Criterion and days								
ADG, g/d								
1-7	204	204	181	227	5	NS	***	ns
8-14	349	360	344	365	8	ns	t	ns
15-28	518	536	536	518	6	*	*	ns
1-14	272	277	258	292	5	ns	***	t
1-28	391	403	392	401	4	t	ns	ns
ADFI, g/d								
1-7	211	206	195	222	4	ns	***	ns
8-14	446	454	440	459	8	ns	t	ns
15-28	769	793	794	768	8	*	*	ns
1-14	321	322	310	333	5	ns	**	t
1-28	538	550	544	544	5	ns	ns	t
FCR								
1-7	1.06	1.02	1.10	0.98	0.02	ns	***	ns
8-14	1.28	1.27	1.29	1.26	0.02	ns	ns	ns
15-28	1.49	1.48	1.48	1.49	0.01	ns	ns	ns
1-14	1.19	1.17	1.21	1.15	0.01	ns	**	ns
1-28	1.38	1.37	1.39	1.36	0.01	ns	**	ns
Faecal score								
week 1	6.0	5.9	5.5	6.4	0.2	ns	**	ns
week 2	5.5	5.5	5.4	5.6	0.1	ns	ns	ns
week 3-4	5.7	5.7	5.7	5.7	0.1	ns	ns	ns

[a] Statistics: Int. = interaction avilamycine x SDAP, ns = not significant, t = tendency, $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Table 8

The individual treatment effects of inclusion of avilamycine and SDAP on growth performance of weanling piglets (Exp. 2)

Avilamycine, d 0-28	0	40 ppm	0	40 ppm	SEM
SDAP, d 0-14	0	0	4 %	4 %	
Criterion and days					
ADG, g/d					
1-7	176[a]	187[a]	231[b]	222[b]	7
8-14	331[a]	357[ab]	367[b]	362[ab]	12
15-28	525[ab]	546[b]	510[a]	526[ab]	8
1-14	249[a]	266[a]	295[b]	288[b]	7
1-28	383[a]	402[b]	399[ab]	404[b]	6
ADFI, g/d					
1-7	194[a]	196[a]	228[b]	215[b]	6
8-14	427[a]	453[ab]	464[b]	455[ab]	11
15-28	774[a]	812[b]	762[a]	773[a]	11
1-14	303[a]	317[ab]	339[c]	328[bc]	7
1-28	531[a]	557[b]	544[ab]	543[ab]	8
FCR					
1-7	1.13[a]	1.06[b]	0.99[c]	0.97[c]	0.02
8-14	1.28	1.28	1.27	1.25	0.03
15-28	1.47	1.49	1.49	1.47	0.01
1-14	1.22[a]	1.20[a]	1.15[b]	1.14[b]	0.02
1-28	1.39[a]	1.39[a]	1.36[ab]	1.35[b]	0.01
Faecal score					
week 1	5.5[a]	5.5[a]	6.6[b]	6.2[ab]	0.3
week 2	5.3	5.5	5.8	5.5	0.2
week 3-4	5.6	5.8	5.7	5.7	0.1

[a,b] Means within the same row without a common superscript letter differ significantly ($P < 0.05$).

Discussion

Although SDAP was exchanged for high value protein sources in exp. 1, positive effects of relatively low inclusion levels of SDAP were apparent. This is in accordance with the outcome of a meta analysis, conducted by Van Dijk et al. (2001). So, SDAP can be used as an alternative for the combination of milk proteins, fish meal and potato protein, thereby even improving ADG and FCR during the first week after weaning, which may economically justify its use. In experiment 1 there was no positive carry over effect of SDAP. This also agrees with the meta analysis (Van Dijk et al. 2001). For the whole experimental period, there were no treatment effects on growth performance. It seems that piglets in the control groups compensated for the lower weight gain during the initial weeks of the experiment. In exp.1, in which no AMGP's were present in the diets, there was

a small but systematic, positive effect of SDAP on faecal consistency. Likewise, in the experiments of Gatnau (1990) and Van der Peet-Schwering and Binnendijk (1995) less diarrhoea was found in piglets fed SDAP. It might be anticipated that SDAP addition to weaning piglets diets may help to prevent post-weaning diarrhoea. This would be especially relevant when AMGP are banned and, as a consequence, more gastro-intestinal disorders can be expected (Kamphues 1999).

In experiment 1, the use of a complex diet with similar nutrient content, but more high quality ingredients, did not result in an improved performance. It may be speculated that the higher pellet hardness of the complex diets, probably caused by the higher inclusion level of whey powder, caused the lack of the expected better performance. A similar suggestion was made earlier by Makkink (1993). The overall daily gain in experiment 1 was relatively low, which is presumably caused by the use of diets without AMGP's. Inclusion of SDAP into the diet considerably improved ADG and FCR during the first week post-weaning but not in the second week after weaning. This observation corroborates the literature review by Van Dijk et al. (2001). There were significant interactions between diet composition and the effect of SDAP in that SDAP generally had beneficial effects on performance when added to complex diets in stead of simple diets. We had hypothesised that SDAP would improve performance more when added to a simple diet when compared to a complex diet. Possibly, our hypothesis is not supported by the results of this experiment because of the complex diet without SDAP produced poor growth performance so that SDAP could act beneficially. The positive effect of SDAP was seen for complex diets without AMGP. Coffey and Cromwell (1995) also demonstrated that the growth-enhancing properties of SDAP are unrelated to the response to antimicrobial agents.

The positive effects of SDAP on growth performance were not accompanied by a higher feed intake. This would indicate that the positive effect of SDAP on weight gain was not caused by a better palatability of the SDAP containing diets, but rather by an improvement of digestibility or absorptive capacity. Literature data generally point at an improved palatability of diets containing SDAP (Van Dijk et al. 2001).

During the four-week period of experiment 2 the piglets showed on average an ADFI of 540 g/d, an ADG of 400 g/d and a FCR of 1.38. Even for the negative control treatment 1, the performance results were quite good. Inclusion of avilamycine as growth promoter into the experimental diets improved feed intake and growth rate, but not for the first two weeks post-weaning. These results are in agreement with earlier work at De Schothorst (unpublished data) in which avilamycine improved ADFI and ADG by approximately 5% during week 3-4 post-weaning, without having an effect during week 1-2. In contrast, SDAP significantly improved ADFI (by 14%), ADG (by 25%) and FCR (by 11%) during week 1 post-weaning and did also, but to a lesser extent in week 2 post-weaning, the effects being enhancements by 4%, 6% and 2%, respectively. These results are

in good agreement with those of Van der Peet-Schwering and Binnendijk (1995, 1997). The better palatability of the diets with SDAP, and a protective effect of immunoglobulins present in SDAP might explain the positive effects. The favourable characteristics of SDAP make it a valuable feed ingredient in diets for newly weaned piglets.

During the entire four-week period, FCR was significantly improved by the feeding of SDAP during the first two weeks, but feed intake and daily gain were not significantly affected. After the withdrawal of SDAP the piglets showed a lower group mean ADG and ADFI than those that had not received SDAP during weeks 1-2. It can be speculated that after the withdrawal of SDAP the piglets have to adapt to diets with a lower palatability and without the protective effect of the immunoglobulins. In several experiments Coffey and Cromwell (1995) did not find a negative carry-over effect after withdrawal of SDAP. It is therefore of interest to study how reduced performance after the changeover in diets can be avoided. The information obtained could increase the benefit of the use of SDAP under practical conditions.

Coffey and Cromwell (1995) showed larger effects of SDAP in piglets kept under poor hygienic conditions. This may indicate a protective effect of SDAP against colonisation of pathogenic bacteria. Therefore it was hypothesised that SDAP would have a bigger effect when added to diets without growth promoter, because when using diets without AMGP (pathogenic) bacteria are more likely to colonise the digestive tract. Our hypothesis is supported by the results of experiment 2. During week 1 and 2, SDAP improved ADFI, ADG and FCR by 12, 18 and 6%, respectively, when SDAP was added to the diet without growth promoter, whereas the improvements were only 3, 8 and 5% when the diet contained a growth promoter. The mechanism of the interaction between SDAP and avilamycine is not clear. Immunoglobulins from SDAP might bind bacteria and prevent bacteria and viruses from damaging the gut wall. In addition, recent data point at a reduction in intestinal inflammation after weaning piglets onto diets with porcine plasma (Jiang et al. 2000). There also was a plasma-induced reduction in weight of the small intestine (Jiang et al. 2000) which is characteristic for the response of animals ingesting antibiotics with the diet (Visek 1978). Our results are contradictory to those of Coffey and Cromwell (1995) who reported similar effects of SDAP in diets with or without growth promoter. The reason for the discrepancy is not clear, but Coffey and Cromwell (1995) used much higher levels of a combination of various antibiotics and copper sulphate than we did. In any case, the observed larger effect of SDAP in the diet without growth promoter suggests that SDAP has an extra benefit in antibiotic free diets.

Conclusions

It can be concluded from experiment 1. that a relatively low inclusion level of SDAP in weaning piglets diets without AMGP has positive effects on growth performance and possibly on health and that these effects are dependent of diet complexity. The results of experiment 2. show that SDAP in the diet can significantly improve ADFI, ADG, FCR and faecal consistency during the first two weeks post-weaning. The effect of SDAP is bigger when added to a diet without avilamycine. This indicates an increased value of plasma in diets without AMGP.

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CHAPTER 3.3

PRE- AND POSTWEANING PERFORMANCE OF PIGLETS FED PRE-WEANING DIETS CONTAINING EITHER SPRAY-DRIED PORCINE PLASMA, WHEY PROTEIN CONCENTRATE OR WHEY POWDER

A.J. Van Dijk[a] , M. Ubbink-Blanksma[a], J.G.P. Van der Palen[a], A.C. Beynen[b]

[a] Co-operative Central Laboratory Nutricontrol Cehave-Landbouwbelang, Veghel, The Netherlands

[b] Department of Nutrition, Utrecht University, Faculty of Veterinary Medicine, Utrecht, The Netherlands

Submitted

Abstract

The effect of the addition of either whey powder, spray dried porcine plasma (SDPP) or whey protein concentrate (WPC) to creep feed on pre- and post weaning performance and health of piglets was studied. The experiment had a randomised complete block design with litter as the experimental unit. Piglets remained with their littermates in the same pen after weaning. During the period from thirteen days before weaning until weaning, the piglets were offered one of three experimental creep feeds followed by the same diet after weaning. During the pre-weaning period there were no significant differences in feed intake and daily gain between the treatment groups. During the first week after weaning, the piglets that had been fed the SDPP diet before weaning, had a significantly higher average daily feed intake (ADFI) than did the piglets that were fed the WPC diet before weaning. During the fourth week after weaning, the piglets given the creep feed with SDPP before weaning had a significantly higher average daily gain (ADG) and lower feed conversion ratio (FCR) than the piglets that were fed the creep feed

with whey powder. It is concluded that the type of protein in creep feed can have positive carry-over effects on post weaning growth performance.

Introduction

Modern sow genotypes give birth to large litters of piglets that have a high growth capacity (Mackenzie and Revell 1998). Milk production of those sows is too low to accommodate the piglets' growth potential (Harrell et al. 1993). Given the tendency towards larger litters and the limitation in milk production, it seems desirable to stimulate the intake of supplementary feed by suckling piglets. Extra feed intake would attest to the piglets' growth capacity and would prevent a pronounced negative energy balance the lactating sow.

The feeding of diets to piglets during suckling, so called creep feeds, could be expected to have beneficial effects on piglets' performance and health before and after weaning. However, the experiments on feeding piglets during lactation have not yielded conclusive results (Pluske 1993). Feed intake during lactation generally is small and variable and publications report either beneficial effects or no effects on piglet performance before and/or after weaning. Piglets that were fed creep feed before weaning had longer small intestinal villi after weaning than did their non-fed littermates (Nabuurs et al. 1993, Nabuurs et al. 1996), but this effect was not seen in an other experiment (Hampson 1986). Mean net absorption of fluid and sodium after weaning by the small intestine of piglets that had been fed creep feed was greater than in their non-fed littermates (Nabuurs et al. 1996). Possibly, piglets that receive creep feed during lactation have an improved functioning of the small intestine after weaning.

The inclusion of spray-dried animal plasma (SDAP) in a diet for weaned piglets generally has a positive effect on average daily gain (ADG) and average daily feed intake (ADFI) in comparison with other protein sources (Hansen et al. 1993, Kats et al. 1994, Coffey and Cromwell 1995, Van Dijk et al. 2001a). The growth promoting action of SDAP could lie in the observed increase in ADFI due to increased palatability of SDAP-containing feed (Ermer et al. 1994). Alternatively, or additionally, SDAP may have direct effects on the intestine, leading to less intestinal disease which in turn has a beneficial effect on ADFI and ADG (Van Dijk et al. 2001a). Whey protein concentrate (WPC) can be used to replace SDAP in diets for weanling piglets without reducing performance (Grinstead et al. 2000, Van Dijk et al. 2001b). The effect of SDAP in diets supplied before weaning has only been described by Cheng and Yen (1995), who found no significant differences in pre and postweaning growth performance between a spray dried porcine plasma (SDPP) containing creep feed (inclusion level 7.5%) compared to a fish meal and skim milk containing creep feed. However, the growth performance data in the latter experiment are relatively low and not comparable to that of high

production sow units. It could be suggested that the addition of either SDAP or WPC to creep feed may increase piglets' feed intake before weaning. The aim of the present study was to investigate whether the addition of SDPP or WPC in creep feed influences pre- and post weaning growth performance and health.

Materials and Methods

Fifty-one litters, comprising five hundred and forty-two suckling and subsequently weaned piglets from the closed herd of the research station 'Laverdonk', Veghel were used. On the first day after parturition, some piglets were re-allocated to the sows so as to achieve equal litter sizes of 10 or 11 piglets per sow. The piglets (F2 cross-bred: GY x [Finnish X Dutch Landrace]) consisted of females and castrates. The male piglets were castrated at the age of 3 days. Piglets were weaned on average at the age of 25 days.

During the period from thirteen days before weaning, when the piglets weighed on average 4.5 kg, until weaning, the piglets were offered either a control diet containing 16.7 % dried whey or one of two experimental diets containing either 5.0 % SDPP (Harimex, Loenen, The Netherlands) or 13.1 % WPC (Nutrifeed, Veghel, The Netherlands). SDPP or WPC were exchanged for whey powder in the control diet on an isonitrogenous basis. The three diets were further formulated to contain 1.25 % of apparent ileal digestible lysine, 10.8 MJ NE/kg and 7.5 % lactose. The contents of apparent ileal digestible lysine, methionine, tryptophan and threonine were balanced by adding crystalline amino acids. The composition of the diets is presented in Table 1. Within parity classes of the sows, litters were assigned randomly to one of the three dietary treatments. Piglets were offered the experimental diets ad libitum in bowls to which the sows had no access.

The sows and their piglets were housed in conventional farrowing crates (2.4 m x 1.8 m). There were 6 crates per room. In the crates, the sow had a restricted area, but the piglets could roam freely. One portion of the floor of the crates consisted of concrete and the remaining area of triangular metal bars. Part of the concrete floor was heated with warm water pipes and a lamp to create a lying place for the piglets with a temperature of 27 °C. Room temperature was kept at 22 °C. Sows and piglets had free access to a water nipple. During the first eleven days of lactation, the sows were fed a commercial lactation diet (dietcode 359, Cehave, Veghel, The Netherlands), the amount supplied gradually increasing from 0.5 to 7.0 kg per day. During the subsequent days of lactation, the sows were fed this diet on ad libitum basis.

Table 1

Ingredient composition and calculated nutrient content of the creep feeds.

Item	WP	SDPP	WPC
Ingredients (% on as fed basis)			
Corn	41.18	43.05	43.31
Barley	10.00	10.00	10.00
Toasted soy beans	9.00	9.00	9.00
Fish meal	6.00	6.00	6.00
Soybean meal	5.00	5.00	5.00
Wheat	5.00	5.00	5.00
Whey permeate	-	9.50	0.96
Spray-dried porcine plasma	-	5.00	-
Dried whey	16.70	-	-
Whey protein concentrate	-	-	13.10
Soybean oil	2.90	2.46	2.36
Premix [a]	1.00	1.00	1.00
Soycomil	1.51	0.95	1.40
Monocalcium phosphate	0.51	0.79	0.77
Ca-formiate	0.60	0.60	0.60
Potassium chloride	-	0.50	0.39
Limestone	0.09	0.31	0.22
Salt	-	0.28	0.51
Flavour	0.05	0.05	0.05
Sweetener	0.03	0.03	0.03
DL-Methionine premix	0.11	0.12	0.05
L-Lysine HCl premix	0.31	0.30	0.21
Tryptophan premix	0.01	0.03	0.04
Threonine premix	-	0.03	-
Calculated nutrient content (on as fed basis)			
NE. MJ/kg	10.80	10.80	10.80
Crude Protein. %	21.00	21.00	21.00
Fat. %	7.38	7.00	7.15
Digestible[b] lysine. %	1.25	1.25	1.25
Digestible[b] methionine plus cystine. %	0.70	0.70	0.70
Digestible[b] methionine. %	0.42	0.40	0.41
Digestible[b] threonine. %	0.70	0.67	0.74
Digestible[b] tryptophan. %	0.21	0.21	0.21
Lactose. %	7.50	7.50	7.50
Calcium. %	0.85	0.85	0.85
Digestible P. %	0.50	0.50	0.50
Sodium. %	0.42	0.42	0.42

[a] Main micro-nutrients provided by this premix per kilogram of complete diet: vitamin A, 12,000 IU; vitamin D3, 2,000 IU; vitamin E, 50 mg; Cu, 160 mg; avilamycine, 40 mg.
[b] Apparent ileal digestible

After weaning, the litters were moved to environmentally regulated rooms, each containing 6 pens. The pens were 2.60 x 1.20 m with partially slatted floors (concrete floor: 1.10 m x 1.20 m). Piglets remained with their littermates in the same pen after weaning. The piglets had ad libitum access to feed and water. All piglets received the same weaner diet from weaning until 14 days after weaning (dietcode 302, Cehave, Veghel, The Netherlands) and were subsequently changed to the same starter diet (dietcode 315, Cehave, Veghel, The Netherlands) which was given until 28 days after weaning (end of the experiment). The weaner and starter diets met the requirements of piglets weighing 8 to 25 kg. Each pen was equipped with a nipple waterer and a one-hole self-feeder. The room temperature was 26 °C on the first day after weaning, gradually declining to 23 °C by the end of the experiment.

Before weaning, the piglets were weighed after birth, 13 days before weaning and at weaning. Contents of the bowls were weighed on days 13 and 7 before weaning and at weaning. Postweaning, the piglets and contents of the feeders were weighed after 7, 14, 21 and 28 days so as to calculate ADG, ADFI and feed conversion ratio (FCR).

Samples of the experimental diets and of SDPP and WPC were analysed. Crude protein was determined according to Kjeldahl (EC 22-7-1993; nr. L 179/8-10). The analyses of crude fat (based on EC 3-9-1998; nr. L 257/23-25), crude fibre (based on EC 26-11-1992; nr. L334/35-37), moisture (based on EC 20-12-1971; nr. L279/ 8-11) and ash (based on ISO 936, 1992) were performed with gravimetrical methods. The starch content of the diets was determined polarimetrically according to Ewers (ISO 5554, 1993). The total immunoglobulin content was determined with protein-G affinity chromatography (Pharmacia/ LKB), followed by UV detection.

Data were analysed as based on a randomised complete block design with litter as the experimental unit and parity class or birth date as block factors. There were 17 replicates (litters) per treatment. Treatments were compared using pair-wise t-tests using the general linear models procedure of SAS (1988). Kolmogorov's and Levene's tests were applied to check the assumptions of normality and homogeneity of variances. The statistical model used for the data before weaning was: y = mean + diet effect + parity class effect + error. The statistical model used for the data after weaning was: y = mean + diet effect + birth date effect + error. The level of statistical significance was pre-set at $P < 0.05$. In the tables, the pooled standard error of the mean (SEM) is given.

Results

The analysed composition of the creep feeds (Table 2) agreed well with the calculated composition (Table 1).

Table 2

Analysed composition of the protein sources and experimental creep feeds

	SDPP	WPC	WP diet	SDPP diet	WPC diet
Chemical analysis , % on as fed basis					
Crude protein	82.0	34.1	20.1	20.5	20.2
Fat	0.1	0.5	6.6	6.7	6.6
Crude fibre	-	-	1.9	1.9	2.0
Moisture	7.6	3.8	11.1	11.1	10.9
Ash	5.5	6.8	6.4	6.3	6.2
Starch	-	-	35.4	35.8	36.2
Immunoglobulins, g/kg	199	12	ND	11	ND

SDPP = Spray dried porcine plasma
WPC = Whey protein concentrate
WP = Whey powder
ND = Not detected

Growth performance before weaning is presented in Table 3. There were no significant treatment effects on feed intake and daily gain. On average, piglets fed the SDPP diet ate more during the period of 7 days before weaning until weaning than did the other two groups, but piglets fed the WPC diet grew somewhat faster than those fed the diet containing whey powder. Before weaning, the piglets were healthy and showed no signs of diarrhoea.

Table 3

The effects of protein source in creep feed on growth performance of piglets before weaning.

Diet	WP	SDPP	W P C	SEM
Number of litters	17	17	17	
ADG (g/day)				
13 days before weaning until weaning	295	310	314	9
ADFI (g/day)				
13 days before weaning until 7 days before weaning	8	9	9	2
7 days before weaning until weaning	17	22	18	3
13 days before weaning until weaning	12	15	13	2

See footnote to Table 1.

The results for the period after weaning are presented in Table 4. During the first week after weaning, piglets that had been fed the SDPP diet before weaning, had a higher ADFI than did the piglets that were fed the WPC diet before weaning ($P < 0.05$). Piglets that had been fed the diet with whey powder showed intermediate values for ADFI during the first week after weaning. As from week one after weaning, there were no significant treatment effects on ADFI. During the first 3 weeks after weaning, there were no significant treatment effects on ADG. However during the fourth week after weaning, piglets fed the SDPP creep feed had a higher ADG than the piglets that were fed the diet with whey powder before weaning ($P < 0.05$). The experimental creep feeds with SDPP and WPC induced a non-significant higher weight at the end of experiment (Fig 1). During the fourth week after weaning, piglets fed SDPP creep feed had a significantly better FCR than did their counterparts fed WPC creep feed. During the post-weaning period all piglets were healthy and showed no signs of diarrhoea.

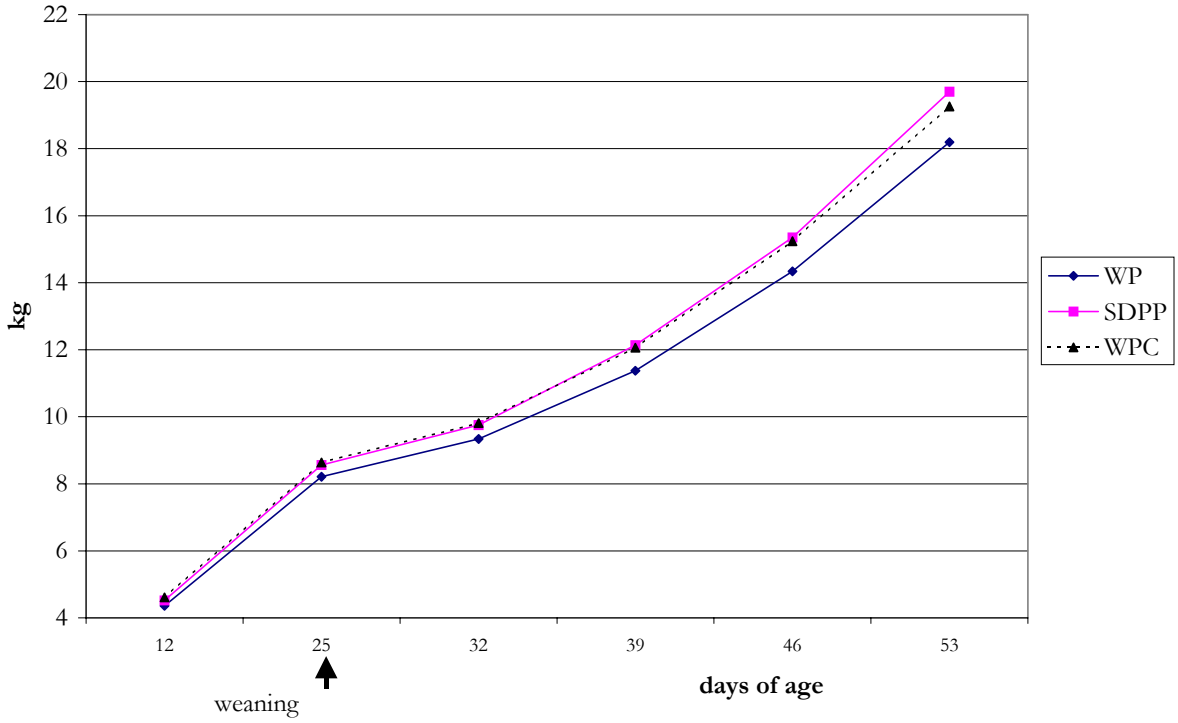

Fig 1: Mean body weight of the piglets during the experimental period. Before weaning creep feeds with different protein sources were given; after weaning all piglets received the same diet. There were 17 litters per treatment.
SDPP = Spray dried porcine plasma
WPC = Whey protein concentrate
WP = Whey powder

Table 4

The effects of protein source of creep feed on growth performance of piglets after weaning when they were al fed the same diet

Diet	WP	SDPP	WPC	SEM
Number of litters	17	17	17	
ADG (g/day)				
Days after weaning				
1-7	162	170	167	16
8-14	290	333	321	21
15-21	428	464	454	24
22-28	550[a]	621[b]	586[ab]	24
1-14	226	251	244	17
15-28	489	542	519	20
1-28	358	397	381	16
ADFI (g/day)				
Days after weaning				
1-7	171[ab]	192[a]	156[b]	11
8-14	361	399	383	21
15-21	616	692	633	36
22-28	837	909	912	33
1-14	266	295	270	14
15-28	726	801	771	32
1-28	496	548	519	22
FCR				
Days after weaning				
1-7	1.12	1.18	1.06	0.06
8-14	1.19	1.23	1.23	0.05
15-21	1.50	1.51	1.40	0.07
22-28	1.54[ab]	1.48[a]	1.58[b]	0.03
1-14	1.20	1.19	1.15	0.04
15-28	1.49	1.48	1.48	0.03
1-28	1.39	1.38	1.36	0.02

SDPP = Spray dried porcine plasma
WPC = Whey protein concentrate
WP = Whey powder
[a,b] Means within the same row without a common superscript letter differ significantly ($P < 0.05$).

Discussion

We speculated that higher intakes of creep feed would be associated with higher feed intakes immediately after weaning. We also expected that the type of protein source in creep feed would affect feed intake before weaning. In the present experiment the protein-induced differences in creep feed intake were not statistically significant. However, during the week before weaning the creep feed with SDPP produced a more than 20% higher feed intake than did the creep feeds with either WPC or whey powder. There was a carry-over effect in that during the first week after weaning ADFI for the piglets fed the creep feed with SDPP was 23 % higher than for those fed the diet with whey powder. Possibly, the analysed high level of immunoglobulins in SDPP had protective and/or stimulatory effects on the intestine, leading to a better functioning of the intestine after weaning as reviewed earlier by Van Dijk et al. (2001a). Alternatively, the palatability of SDPP (Ermer et al. 1994) could be involved. During the period of lactation palatability would enhance the intake of creep feed which in turn would facilitate the uptake of dry feed as sole source of nutrition after weaning. It should be stressed that the positive effect of SDPP on post-weaning feed intake was seen during the first week only. After the first week post weaning there was no significant influence of the type of protein in the creep feed and thus SDPP, WPC and whey powder can be considered equally effective.

During the post-weaning period there were effects of the type of protein in creep feed on ADG and FCR but the effects were limited to days 22-28. It is unknown why the carry-over effects became apparent during one specific post weaning period only. In any event, the piglets fed creep feed with SDPP had a significantly higher ADG during days 22-28 after weaning than did the piglets fed the creep feed with whey powder. Cheng and Yen (1995), who conducted a comparable experiment using two experimental creep feeds (control and SDPP), found a similar, although non-significant post weaning weight gain effect for SDPP creep feed fed piglets. It is difficult to see that the higher ADG is a result of the higher ADFI during the first week. The higher ADG during days 22-28 after weaning for the piglets fed the creep feed with SDPP was associated with an unchanged ADFI and thus significantly lower FCR, at least when compared with the piglets fed creep feed with WPC. Again it should be stressed that for the entire post-weaning period there were no major differential effects on ADG, ADFI and FCR as mediated by either SDPP, WPC or whey powder in the creep feed.

It can be concluded that even within high quality protein sources in creep feed there still may be a difference in growth performance and that the protein source in creep feed can have a positive carry over effect on post weaning growth performance, but the observed effects were not systematic. Nevertheless, it would appear that the use of SDPP and/or WPC in creep feed, of which only minute amounts are consumed, might be justified for their inclusion in pre-weaning diets.

Acknowledgements

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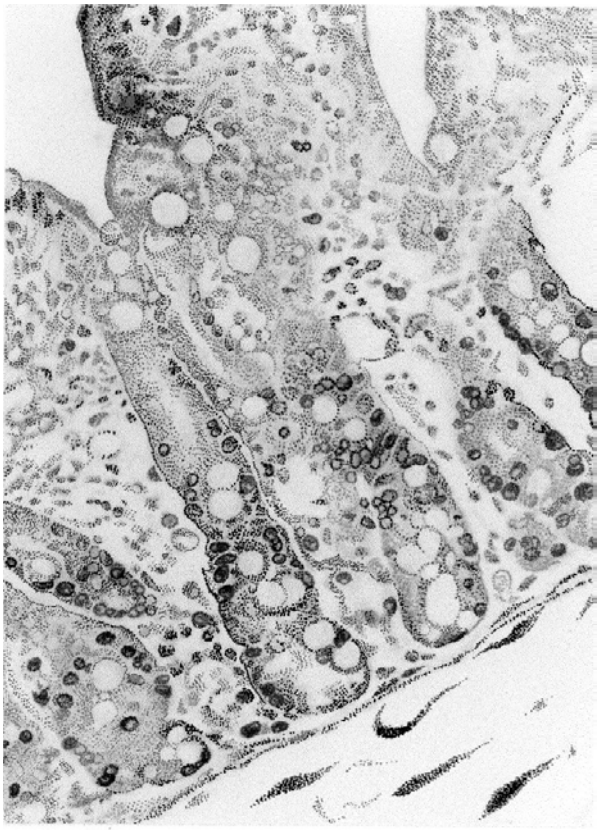
CHAPTER 4

SMALL INTESTINAL MORPHOLOGY AND FUNCTION



Jejunal cypts of piglets. BrdU positive cells, an indicator of cell mitotic activity, are visible as relatively dark-stained cells. Magnification 400 X.

CHAPTER 4.1

SMALL INTESTINAL MORPHOLOGY IN WEANED PIGLETS FED A DIET CONTAINING SPRAY-DRIED PORCINE PLASMA

A.J. Van Dijk[a], T.A. Niewold[b], R.J.C.F. Margry[a], S.G.C. Van Den Hoven[a], M.J.A. Nabuurs[b], N. Stockhofe-Zurwieden[b], A.C. Beynen[c]

[a]Co-operative Central Laboratory "CCL- Nutricontrol" of Cehave Landbouwbelang, P.O. Box 107, 5460 AC Veghel, The Netherlands,

[b]Department of Immunology, Pathobiology and Epidemiology, Institute for Animal Science and Health, ID-Lelystad, P.O. Box 65, 8200 AB Lelystad, The Netherlands,

[c]Department of Nutrition, Utrecht University, Faculty of Veterinary Medicine, P.O. Box 80152, 3508 TD Utrecht, The Netherlands

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Abstract

The hypothesis tested in this study was that the reported beneficial effects of spray-dried porcine plasma (SDPP) on piglet post-weaning performance and health are associated with a trophic effect on small intestinal mucosa. At 24 days of age, the piglets of 7 sows were assigned to one of three treatment groups. One group remained to be suckled. The other two groups were weaned and offered a diet containing either 15% SDPP or casein. From each treatment group, one piglet was anaesthetised and samples were taken from the small intestinal wall at 26, 28 and 31 days of age. There were no significant effects of SDPP versus casein on villus length. On average, there was less mitotic activity in the SDPP-fed piglets than in those fed casein on days 4 and 7 after weaning. Because less mitotic activity leads to less immature enterocytes, this may provide a mechanism for the reported beneficial effects of SDPP on performance and health.

Introduction

In weaned piglets, diarrhoea occurs frequently during the first two weeks after weaning at the age of 21-28 days, which causes impaired growth performance and high mortality. The diarrhoea is associated with the proliferation and activity of enterotoxigenic *E. coli* and low feed intake in the first week after weaning (Van Beers-Schreurs et al. 1992). Histological examination of the small intestine has shown that weaning is associated with a marked hyper-regeneration following villus atrophy (Hall and Byrne 1989). The villus atrophy impairs digestive and absorptive function of the gut, contributing to poor performance after weaning (Hampson and Kidder 1986, Nabuurs et al. 1994, Pluske et al. 1997). Kelly et al. (1991), Pluske et al. (1996) and Van Beers et al. (1998) demonstrated that higher feed intake after weaning results in less villus atrophy.

Pluske et al. (1997) suggested that cell-kinetic changes after weaning are similar to those described in starvation followed by re-feeding, which caused a decreased cell production rate in the crypts followed by an increase (reviewed by Pluske et al. 1997). An increased mitotic activity results in more immature enterocytes (Wild and Murray 1992), leading to an impaired digestive and absorptive function (Dauncey et al. 1983, Smith 1984, Smith 1985, Wild and Murray 1992) and increased sensitivity to bacterial toxins and increased toxin migration through the enterocyte membrane (Mezoff et al. 1991, Chu and Walker 1993, Nabuurs et al. 1994). As suggested by Hampson (1986) and Nabuurs et al. (1994), these effects explain the increased susceptibility of the piglet to diarrhoea and growth depression in the post-weaning period.

Various measures are taken to improve feed intake and health of piglets after weaning. Amongst these is the addition of specific substances to the weaner pig diet. One of these substances is spray-dried animal plasma (SDAP) which usually is of porcine origin.

The addition of SDAP to diets of weaned piglets generally has a positive effect on average daily feed intake (ADFI) and average daily gain (ADG) (Van Dijk et al. 2001). In two experiments, less diarrhoea was found in piglets fed spray-dried porcine plasma (SDPP) during the first two weeks after weaning (Gatnau 1990, Van der Peet-Schwering and Binnendijk 1995).

Several studies have been conducted to unravel the mode of action of SDAP. The growth promoting action of SDAP could lie in the observed increase in ADFI due to increased palatability of SDAP-containing feed (Ermer et al. 1994). Alternatively, or additionally, SDAP may have direct effects on the intestine, leading to less intestinal disease which in turn has a beneficial effect on ADFI and ADG (Van Dijk et al. 2001). It could be hypothesised that SDPP prevents post weaning villus atrophy by a protective or trophic effect on the small intestinal epithelium. It

may be possible that SDAP contains active components like Epidermal growth factor or Insulin like growth factor-1, that are trophic to the small intestinal villi, as described for milk products by Odle et al. (1996). There are four reports in abstract form on the effect of feeding SDAP on small intestinal morphology in weaned piglets (Cain et al. 1992, Gatnau et al. 1995, Touchette et al. 1997, Spencer et al. 1997). However, the experimental designs and methods used are not described in detail and the outcomes of the experiments are contradictory. Therefore we examined the effects of dietary SDPP not only on small intestinal morphology, but also on enterocyte mitotic activity in piglets during the first week after weaning. Two groups of weaned piglets were fed a diet containing either 15% SDPP or casein. A third group of piglets remained with the sow. Thus, it was expected that our experiment would provide information about the extent to which the feeding of SDPP may normalise intestinal morphology and enterocyte mitotic activity. High concentrations of SDPP in the diet have considerable positive effects on piglet post weaning performance (Van Dijk et al. 2001) and by using a high level in the experimental diet, we anticipated that maximum trophic or protective effects on the small intestinal mucosa would be seen. Casein is generally considered to be a high value protein source for piglets. As a control protein for SDPP, casein may not enhance the contrast, but it does exclude possible confounding effects on small intestinal morphology because, unlike plant protein sources, casein would be expected not to damage the intestinal epithelium (Hall and Byrne 1989).

Materials and methods

Animals and design

Animal care and use. The experimental design was approved by the Animal Experiments Committee of ID-Lelystad.

Seventy piglets from the closed herd of the research station 'Laverdonk', Veghel were used. The piglets (F2 cross-bred: GY x [Finnish X Dutch Landrace]) were females and castrates aged 24 d with average weight of 7.9 kg. The experiment had a complete randomised block design with each block consisting of 10 piglets from the same litter. At the age of 24 days, the piglets of 7 sows were assigned randomly to one of three treatments. One group (3 piglets per litter) remained on the sow. Two groups were weaned and offered a diet containing either SDPP (3 piglets per litter) or casein (3 piglets per litter). To obtain baseline values, at the age of 24 days, one randomly chosen piglet from each litter was anaesthetised to provide samples of the small intestinal wall and was killed immediately afterwards. From each treatment group, one randomly chosen piglet from each sow was sampled and killed at 26, 28 and 31 days of age. As a result, there were 21 piglets for each feeding treatment.

Housing Environments. The piglets that had been weaned were kept in groups of 3 animals (per dietary treatment) from the same litter. The groups were housed in pens (2.60m x 1.20m) with rubber-coated, expanded metal floors. The pens were placed in an environmentally regulated nursery. The piglets had free access to feed and water. Each pen was equipped with a water nipple and a feeding bowl. The room temperature was 26 °C. The unweaned piglets were housed with their dam in conventional farrowing crates (2.4 m x 1.8 m). In the crates, the sow was restricted to one area and the piglets could roam freely. One part of the floor of the crates consisted of concrete and the other part of triangular metal bars. Room temperature was kept at 22 °C. Piglets had free access to a water nipple. No creep feed was provided.

Feeding. The composition of the experimental diets is shown in Table 1. The diets, which were in meal form, contained either 15% (w/w) casein or SDPP. The diets were formulated to contain 1.32 % apparent ileal digestible lysine and 2394 kcal NE/kg.

Measurements.

Individual body weight was measured at 24, 26, 28 and 31 days of age. For the piglets that were weaned, feed intake per group was measured daily from 24 to 31 days of age.

The crude protein content of the feed was determined according to Kjeldahl (EC 22-7-1993; nr. L 179/8-10). The analyses of fat (based on EC 3-9-1998; nr. L 257/23-25), crude fibre (based on EC 26-11-1992; nr. L334/35-37), moisture (based on EC 20-12-1971; nr. L279/ 8-11) and ash content (based on ISO 936, 1992) were performed with gravimetrical methods. The starch content of the feed was determined polarimetrically according to Ewers (ISO 5554, 1993). The total immunoglobulin content of SDPP and the feed was determined with protein-G affinity chromatography (Pharmacia/LKB), followed by UV detection.

To assess cell mitotic activity, the incorporation rate of 5-bromo-2'-deoxyuridine (BrdU) into the small intestinal enterocytes was determined by an immunocytochemical technique (Wynford-Thomas and Williams 1986, Dolbeare 1995). The piglets received BrdU (Sigma-Aldrich Fine Chemicals, B-5002) by intraperitoneal injection. Each animal received a volume of 5 ml, which contained 40 mg BrdU/ml saline, two hours before sampling the small intestine.

Before samples from the small intestine were taken, the piglets were anaesthetised with a mixture of nitrous oxide, oxygen and halothane administered through a facemask. Five intestinal tissue samples of 2 cm length each were removed along the small intestine. One sample was taken adjacent to the stomach at 10 per cent of the length of the small intestine. The other four samples were taken further distally at 25, 50, 75, and 95 per cent of the length of the small intestine. The tissue was cut at the contra-mesenteric side and pinned with the serosal side on a piece of cork. After the samples had been taken, the piglets were

euthanised by an intracardial injection of T61 (Hoechst Roussel Vet, Brussels, Belgium).

Table 1.

Composition of the experimental diets

	Control	SDPP
Ingredient composition	% on as fed basis	
Spray-dried porcine plasma	-	15.00
Casein	15.00	-
Dried whey	10.00	10.00
Monocalcium phosphate	0.88	1.60
Limestone	1.02	0.60
Soybean oil	1.30	1.70
Premix[a]	0.50	0.50
Barley	40.00	20.30
DL-Methionine	-	0.30
Corn	30.80	50.00
Salt	0.50	-
Calculated composition		
NE kcal/kg	2394	2394
Crude protein, %	23.40	22.50
Fat, %	3.50	4.20
Crude fibre, %	2.50	2.00
Digestible[b] lysine, %	1.37	1.32
Digestible[b] methionine plus cystine, %	0.73	0.73
Digestible[b] methionine, %	0.54	0.34
Digestible[b] threonine, %	0.84	0.83
Lactose, %	5.00	5.00
Calcium, %	0.73	0.74
Digestible[b] phosphorus, %	0.40	0.41
Sodium, %	0.50	0.50

[a] Premix provided per kilogram of complete diet: vitamin A, 7,500 IU; vitamin D_3, 1,500 IU; vitamin E, 17.5 mg; riboflavin, 2 mg; vitamin B_{12}, 20 µg; vitamin K_3, 0.75 mg, d-pantothenic acid, 6 mg; niacin, 30 mg; iron, 80 mg; iodine, .45 mg; cobalt, .15 mg; copper, 160 mg; manganese, 24 mg; selenium, .175 mg; zinc, 150 mg; zincbacitracin, 50 mg.

[b] Apparent ileal digestible

The tissue samples were fixed in 10 per cent neutral buffered formalin with the mucosal side downwards so that the villi were fixed vertically. After fixation, a portion of the sample was embedded in paraffin wax by standard techniques. From each sample, two transverse sections were selected, stained with

haematoxylin and eosin and examined with a binocular microscope using an ocular micrometer. Length and depth of at least 10 villi and crypts were measured in each sample (Nabuurs et al. 1993). An immunohistochemical technique was used to stain cells labelled with BrdU. Tissue sections of about 4 μm thickness were deparaffinized and rehydrated, endogenous peroxidase was blocked and sections were treated with 0.1 % proteinase K (Sigma P-6556). Sections were first incubated with a monoclonal antibody against bromodeoxyuridine (Dako X0902, 1:15 dilution, room temperature) followed by an incubation with a biotinylated secondary antibody (rabbit-anti-mouse, Dako E0413, 1:200 dilution, room temperature). An incubation with a streptavidin/biotin complex (Dako P 0397) and diaminobenzidine (DAB, Sigma D 5637) as substrate were used to visualise binding of the first antibody. Stained cells showed a brown to darkbrown colour and were counted in ten randomly selected crypt-areas of 0.062 mm^2 per specimen by light microscope at a magnification of 400 fold. This immuno-stain was applied on the samples taken at 10, 25, 50, and 75 per cent of the length of the small intestine. Histological examination was done by one person while he was blinded to treatment modality.

Statistical Analyses

Pen was the experimental unit for growth performance data. The individual pig was considered as experimental unit for the data from small intestinal morphology and mitotic activity. Growth performance and villus-crypt data were analysed using the model: y = mean + sow effect + treatment effect + error. Mitotic activity data were analysed using the model: y = mean + treatment effect + error. Sow as block factor was left out of the latter model because, due to absence of staining of some BrdU samples, the number of piglets originating from one sow was not equal for each treatment. Crypt depth and villus length data are all based on 7 replicates (individual piglets) per treatment-day combination. Statistical analysis was performed using the general linear models procedure of SAS (1988). The level of statistical significance was pre-set at $P < 0.05$. Kolmogorov's and Levene's tests were applied to check the assumptions of normality and homogeneity of variances.

Results

The data presented in Table 2 show that the analysed values for feed composition were similar to those calculated (Table 1).

For ADG data some in-equalities of variance were found. After log transformation of the ADG data the variances were found to approach equality so that further statistical analyses were performed with the transformed data. During

the first 4 days, the unweaned piglets grew much faster than the weaned piglets. (Table 3).

Table 2.

Chemical analysis of the experimental diets

	control	SDPP
Chemical analysis	% on as fed basis	
Crude protein	23.4	21.0
Fat	3.1	3.9
Crude fibre	3.3	2.6
Moisture	10.3	10.8
Ash	5.3	5.3
Starch	39.8	43.1
Immunoglobulins (g/kg)	4.0	37.0

Table 3.

Daily weight gain in the three treatment groups and feed intakes in the weaned piglets

	Unweaned piglets	Weaned piglets		
Measure		Control diet	SDPP diet	SEM
Average Daily Gain, g				
Days[1] 24-25	597[a]	36[b]	16[b]	40
DAYS 26-27	411[a]	228[b]	239[b]	46
Days 28-30	212	240	158	55
Average Daily Feed Intake, g				
Day 24		4	13	5
Day 25		16	33	9
Day 26		118	124	19
Day 27		170	202	24
Day 28		217	173	37
Day 29		301	217	66
Day 30		260	278	43

Data presented as means for 6 or 7 animals and pooled SEMs.
[1]Days refer to age of the piglets. Piglets were weaned at the age of 24 days.
[a,b] Means within the same row without a common superscript letter differ significantly ($P < 0.05$).

There were no significant differences in ADG between unweaned and weaned piglets during the last 3 days of the experiment. For the weaned piglets, there was no significant diet effect on ADG. ADFI was similar for the two dietary treatments.

Villi were significantly shorter in the weaned than unweaned piglets at the age of 26 and 28 days (Fig. 1). No such difference in villus length was seen at the age of 31 days. Diet composition did not significantly influence the villus length reduction seen immediately after weaning.

At the ages of 26 and 28 days there were no differences in crypt depth among treatments (Fig. 2). At the age of 31 days however, the unweaned piglets generally showed shorter crypts than did their weaned counterparts groups. The difference was statistically significant for the site at 75% of the length of the small intestine.

The villus-height/crypt-depth ratio is presented in Figure 3. The ratio was systematically higher in the unweaned piglets than in the weaned piglets. There was no significant diet effect in the weaned piglets. At days 4 and 7 after weaning, the overall mean villus-height/crypt depth ratio was higher for the weaned piglets fed the SDPP diet instead of the casein diet, but the difference was not significant.

BrdU data are based on 3-7 replicates per treatment. In 66 out of the total of 280 samples there was absence of staining and those samples were excluded from the calculations. The excluded samples consisted of 9, 30 and 27 samples of the unweaned, casein and SDPP fed groups, respectively. It was concluded that the major cause of the absence of staining was erroneous injection of BrdU into the bladder or the intestinal lumen because there was a total absence of BrdU incorporation in either the mucosa or submucosa. At the age of 26 days, the overall mean numbers of BrdU-labelled epithelial cells in the small intestine were significantly lower in the weaned piglets than in their unweaned counterparts (Fig. 4). However, at the ages of 28 and 31 days, the overall mean number of BrdU-labelled cells was lower in the unweaned group than in the weaned groups. This difference was significant at the age of 31 days. At day 7 after weaning, piglets fed SDPP generally showed less mitotic activity than did the piglets receiving the casein diet; the difference only reached statistical significance for the site at 25% of the length of the small intestine. For the piglets aged 26, 28 and 31 days, linear regression was done for the number of BrdU-labelled epithelial cells and either villus length or crypt depth. The analysis was carried out with the mean values of all five sites for the entire small intestine. Only at 26 days of age these relationships were found to be statistically significant (Fig. 5).

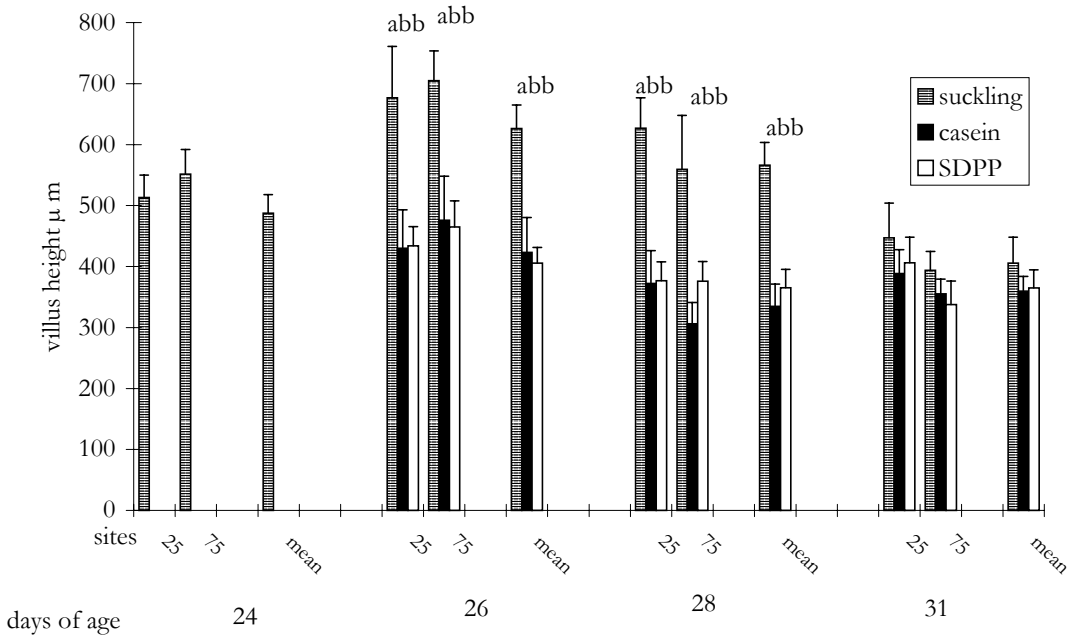

Fig 1: Villus height (+SEM) at the 25% and 75% sites along the entire length of the small intestine and the mean villus height of all sites of unweaned piglets and weaned piglets given either a casein or SDPP containing diet. Days refer to age of the piglets. Weaning was done at the age of 24 days. [a,b] Bars, within each set of comparable data without a common superscript letter differ significantly ($P < 0.05$).

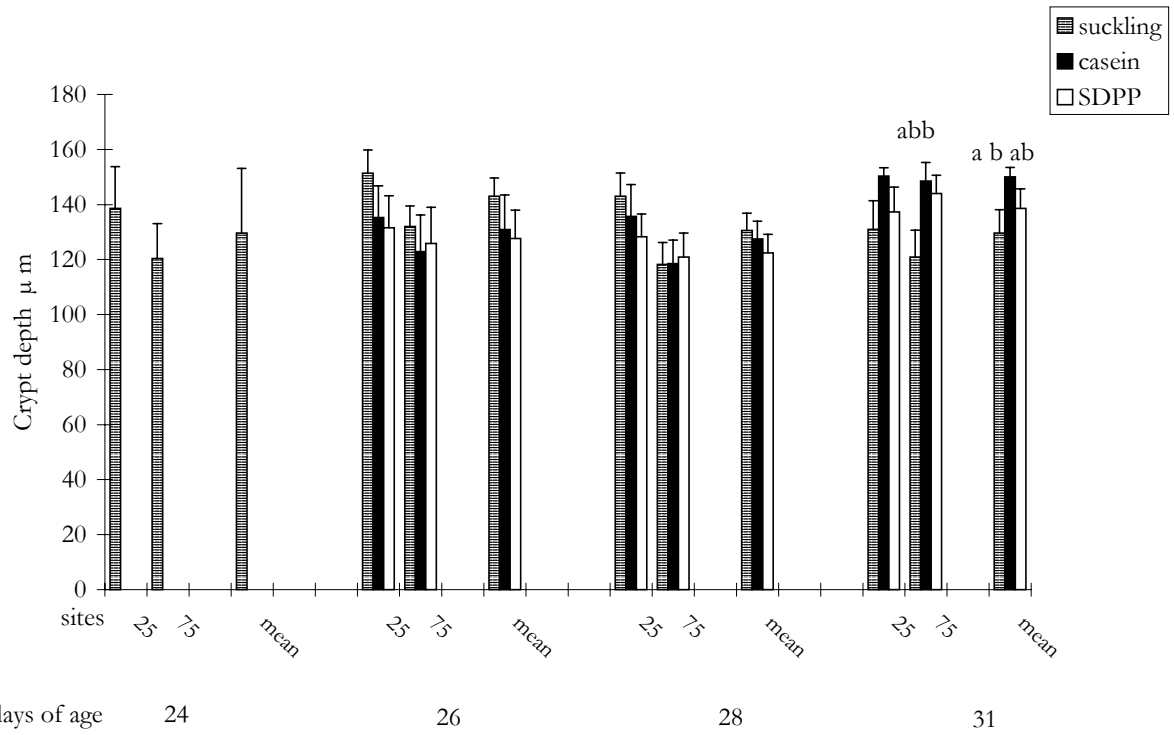

Fig 2: Crypt depth (+SEM) at the 25% and 75% sites along the entire length of the small intestine and the mean crypt depth of all sites of unweaned piglets and weaned piglets given either a casein or SDPP diet. Days refer to age of the piglets. See also legend to Fig. 1.

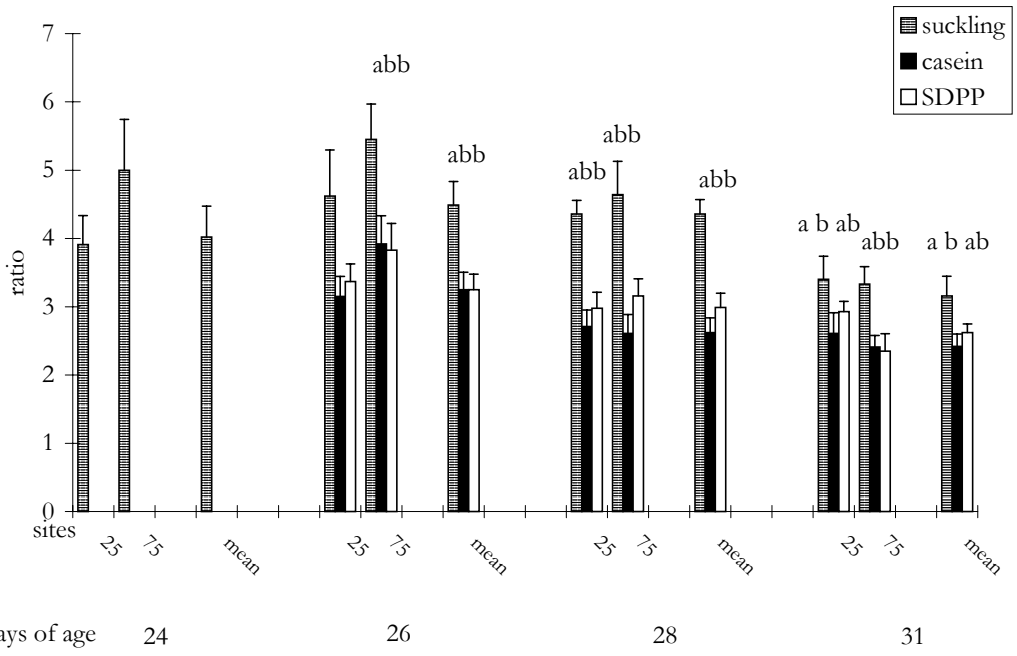

Fig 3: Villus height-Crypt depth ratio (+SEM) at the 25% and 75% sites along the entire length of the small intestine and the mean ratio of all sites of unweaned piglets and weaned piglets given either a casein or SDPP diet. See also legend to Fig. 1.

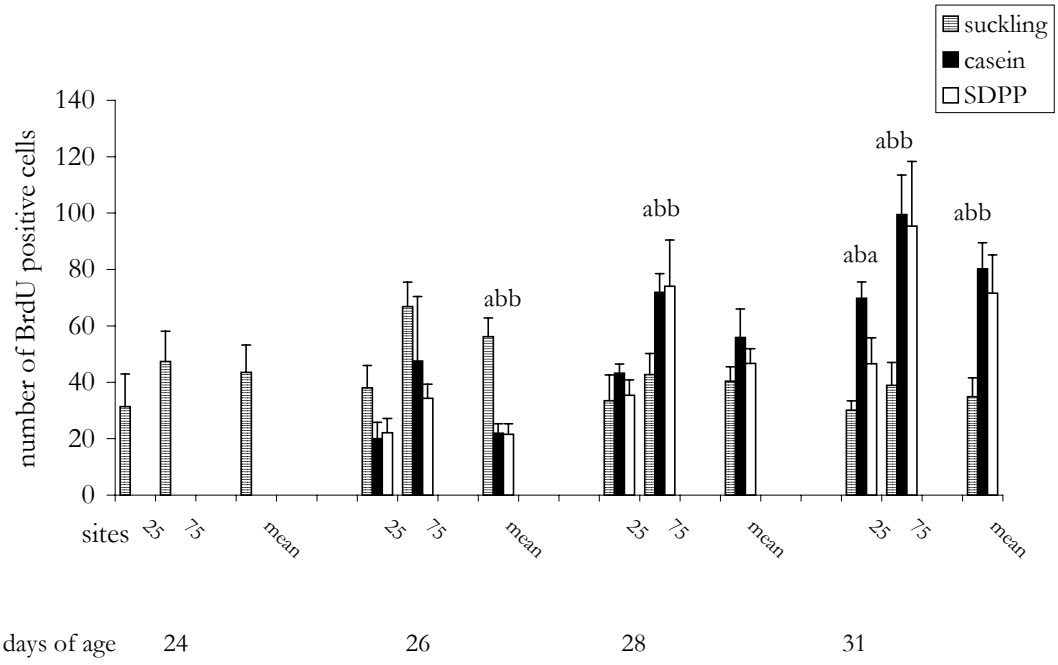

Fig 4: Number of BrdU positive epithelium cells (+SEM) at the 25% and 75% sites along the entire length of the small intestine and the mean number of all sites of unweaned pigs and weaned piglets given either a casein or SDPP diet. See also legend to Fig. 1.

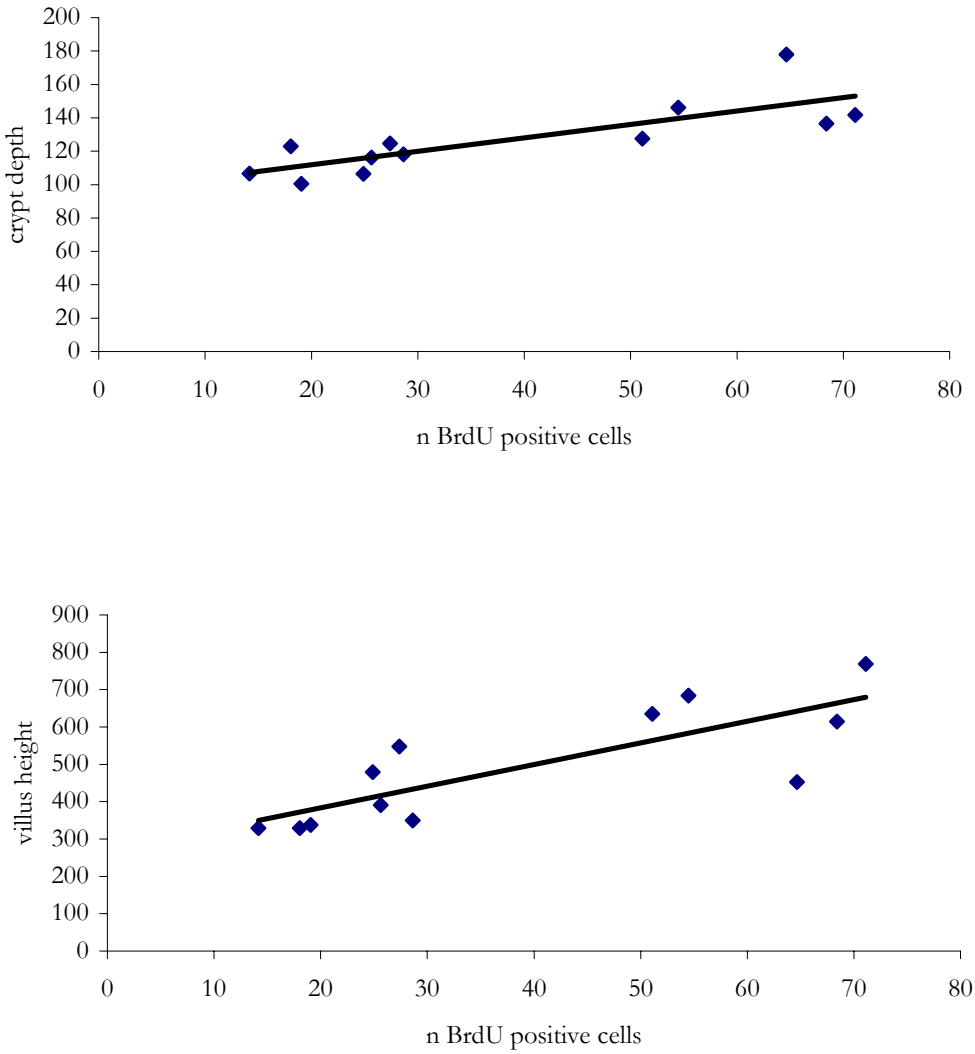

Fig 5: The relationship between the average number of BrdU positive epithelial cells and either crypt depth or villus height (µm) at day 26 of age. Model equations were: Crypt depth = 95.7 + 0.8 x number of BrdU positive cells (R^2=0.65, P=0.002) and Villus height= 267 + 5.8 x number of BrdU positive cells (R^2=0.65, P=0.002).

Discussion

This report describes the effect of dietary SDPP on small intestinal morphology and enterocyte mitotic activity in weaned piglets. Unweaned piglets served as reference. In our study there was no positive effect of SDPP on ADFI and ADG in the first week after weaning. This may be explained by the adequate ADFI and ADG in the control group for the age interval of 24 – 30 days. Van Dijk et al. (2001) demonstrated in a meta analysis that high baseline ADG and ADFI values

are associated with a lack of effect of SDAP. Because SDPP did not affect ADFI, this experiment is suitable to identify specific trophic effects of SDPP, if any. A change in feed intake would by itself alter villus height (Kelly et al. 1991, Pluske et al. 1996, Van Beers et al. 1998).

The finding that villi were significantly shorter in the weaned piglets versus unweaned piglets is in accordance with the experiments of Nabuurs et al. (1993) and Van Beers et al. (1998). However, contrary to the experiments of Nabuurs et al. (1993) and Van Beers et al. (1998), the shorter villi in weaned piglets were not seen at 7 days after weaning in our experiment. Possibly in this experiment the adequate consumption of good quality feed had caused an increase in mitotic activity, resulting in increased villus height. In the weaned piglets, there was no significant effect of dietary SDPP on villus length, indicating that that SDPP has no specific trophic effects to small intestinal villi. This is in accordance with the outcome of an experiment of Jiang et al. (2000).

At the age of 26 days, the mean numbers of BrdU-labelled epithelial cells were significantly lower in the weaned piglets than in their unweaned counterparts (Fig. 4). However, at the ages of 28 and 31 days, the difference was opposite. The increased mitotic activity at days 4 and 7 after weaning, fits into the concept of hyper-regeneration following villus atrophy. It also supports the suggestion of Pluske et al. (1997) that cell-kinetic changes after weaning are similar to those described in starvation followed by re-feeding, which caused a decreased cell production rate in the crypts followed by an increase (reviewed by Pluske et al. 1997). As stated in the introduction, the increased mitotic activity at days 4 and 7 after weaning can explain the increased susceptibility of the piglet to diarrhoea and growth depression in the post-weaning period.

Hall and Byrne (1989), using the vincristine technique that arrests dividing cells in metaphase, found that there was less mitotic activity in crypts at 3 days after weaning and suggested that this induced shorter villi rather than villus damage. This suggestion agrees with our findings in that there was a positive relationship between mitotic activity and villus length at day 2 after weaning (Fig 5).

At day 7 after weaning, the SDPP-fed piglets showed less mitotic activity than did the piglets receiving the casein diet, but the difference only reached statistical significance for the site at 25% of the length of the small intestine. When the BrdU data of days 4 and 7 after weaning were combined and were adjusted for sow and age, the comparison of SDPP-fed with casein-fed piglets showed for the former significantly less mitotic activity in epithelial cells at the site at 25% of the length of the small intestine. A similar, but non-significant effect at day eight after weaning in the proximal jejunum was reported by Jiang et al. (2000). The mechanism by which SDPP may affect mitotic activity is not known. There could have been secondary effects on mitotic activity, such as effects mediated by changes in the intestinal microflora. As explained above, higher mitotic activity predisposes to disease (Nabuurs et al. 1994). The lower mitotic activity in piglets

fed SDPP might explain the better health and performance found in most investigations on SDPP feeding in weaned piglets (Van Dijk et al. 2001).

Acknowledgements

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CHAPTER 4.2

SMALL INTESTINAL MORPHOLOGY AND DISACCHARIDASE ACTIVITIES IN EARLY-WEANED PIGLETS FED A DIET CONTAINING SPRAY-DRIED PORCINE PLASMA

A.J. Van Dijk[1], T.A. Niewold[2] M.J.A. Nabuurs[2], J. Van Hees[1], P. De Bot[1], N. Stockhofe-Zurwieden[2], M. Ubbink-Blanksma[1] and A.C. Beynen[3]

[1] Co-operative Central Laboratory "CCL-Nutricontrol" of Cehave Landbouwbelang, P.O. Box 107, 5460 AC Veghel, The Netherlands,

[2]Department of Immunology, Pathobiology and Epidemiology, Institute for Animal Science and Health, ID-Lelystad, P.O. Box 65, 8200 AB Lelystad, The Netherlands,

[3]Department of Nutrition, Utrecht University, Faculty of Veterinary Medicine, P.O. Box 80152, 3508 TD Utrecht, The Netherlands

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Abstract

The goal of this study was to test whether dietary spray-dried porcine plasma (SDPP) in early-weaned piglets prevents small intestinal villus atrophy by trophic or protective activity. Fifty-four weaned, 18-days old piglets were used to determine the effect of dietary SDPP on small intestinal villus length, crypt depth, enterocyt mitotic activity and brush border enzyme activities during the first week after weaning. The piglets were offered a diet containing either 8% SDPP or 8% casein.

At 2 and 7 days after weaning, piglets were anaesthetised to provide samples of the small intestinal wall and killed immediately afterwards. There were no differences in daily gain and daily feed intake between the two dietary treatments. At day 2 after weaning, all piglets showed a marked reduction in villus height when compared with baseline values. In all piglets, small intestinal enterocyte mitotic activity had decreased by day two and was increased again on day 7. There were no significant effects of dietary SDPP on small intestinal villus length, crypt depth and enterocyt mitotic activity. This indicates that SDPP has no trophic effect on the small intestinal mucosa and that it does not protect against the damaging effect on the small intestinal villi that is associated with the process of weaning. There was no effect of SDPP on lactase, sucrase or maltase specific activities that are a measure of the digestive function of the small intestine. It can be concluded that SDPP versus casein has no effect on small intestinal morphology and disaccharidase activities in early-weaned piglets kept under low infection pressure.

Introduction

In piglets, the process of weaning is associated with small intestinal villus atrophy (Hall and Byrne, 1989), which impairs digestive and absorptive function of the gut (Hampson and Kidder 1986, Nabuurs et al. 1994, Pluske et al. 1997). The addition of spray-dried animal plasma (SDAP) to diets of weaned piglets generally has a positive effect on average daily feed intake (ADFI) and average daily gain (ADG) (Van Dijk et al. 2001a) and may diminish the incidence of diarrhoea (Gatnau 1990, Van der Peet-Schwering and Binnendijk 1995).

So far, the mode of action of SDAP remains obscure. Van Dijk et al. (2001b) investigated the effects of dietary spray-dried porcine plasma (SDPP) versus casein on small intestinal morphology and enterocyt mitotic activity in piglets that were weaned at 24 days of age. There was no significant effect of dietary SDPP on villus length or crypt depth, but the SDPP-fed piglets tended to have less mitotic activity. Similar results have been reported by Jiang et al. (2000). Van Dijk et al. (2001b) found an increased mitotic activity at days 4 and 7 after weaning in weaned versus unweaned piglets. An increased mitotic activity results in more immature enterocytes (Wild and Murray 1992), leading to an impaired digestive and absorptive function (Dauncey et al. 1983, Smith 1984, Smith 1985, Wild and Murray 1992) and increased sensitivity to bacterial toxins and increased toxin migration through the enterocyte membrane (Mezoff et al. 1991, Chu and Walker 1993, Nabuurs et al. 1994). As suggested by Hampson (1986) and Nabuurs et al. (1994), these effects explain the increased susceptibility of the piglet to diarrhoea and growth depression in the post-weaning period. Less mitotic activity is associated with less immature enterocytes, which might explain the beneficial effects of SDPP (Van Dijk et al. 2001b). It could be suggested that weaning at an

earlier age than in our previous experiment (Van Dijk et al. 2001b) would result in more pronounced effects of SDPP because weaning-induced morphological changes in the small intestine are more conspicuous when weaning occurs at an earlier age (Pluske et al. 1997). The objective of the present study was to determine the effect of dietary SDPP on villus length, crypt depth and enterocyt mitotic activity of piglets that were weaned at the age of 18 days. The specific activity of brush-border (BB) enzymes is a measure of the digestive function of the small intestine (Smith 1985, Pluske et al. 1997). Immature enterocytes have a lower BB enzyme activity than older enterocytes (Smith 1985). The effect of SDPP, if any, on the activity of the BB enzymes lactase, sucrase and maltase was unknown, which prompted us to determine these activities also.

Materials and methods

Animals and design

Animal care and use. The experimental design was approved by the Animal Experiments Committee of ID-Lelystad.

Fifty-four piglets from the closed herd of the research station 'Laverdonk', Veghel were used. The piglets (F2 cross-bred: GY x [Finnish X Dutch Landrace]) were females and castrates aged 18 d with an average weight of 6.3 kg. The experiment had a complete randomised block design with each block consisting of 8 piglets from the same litter. At the age of 18 days, the piglets of 6 sows were weaned and assigned randomly to one of two treatments. The piglets were offered a diet containing either SDPP (4 piglets per litter) or casein (4 piglets per litter). To obtain baseline values, at the age of 18 days, one randomly chosen piglet from each litter was anaesthetised to provide samples of the small intestinal wall and was killed immediately afterwards. From each treatment group, two randomly chosen piglets from each sow were sampled and killed at 2 and 7 days after weaning. As a result, there were 24 piglets for each feeding treatment.

Housing Environments. The piglets that had been weaned were penned in individual cages (1.2 m x 0.42 m) with slatted metal floors. The cages were placed in an environmentally regulated room. The piglets had free access to feed and water. Each pen was equipped with a water nipple and a one-hole self-feeder. The room temperature was 26 °C. No creep feed was provided during the lactation period.

Feeding. The composition of the experimental diets is shown in Table 1. The diets, which were in pellet form, contained either 8% (w/w) casein or SDPP. The diets were formulated to contain 1.3 % apparent ileal digestible lysine and 2352 kcal NE/kg. Pelleting temperatures were kept low (65 °C and 67 °C for the control and

SDPP diet, respectively) to minimise damage of any bioactive components in the SDPP.

Table 1.

Composition of the experimental diets

	Control	SDPP
Ingredient composition	% on as fed basis	
Wheat	49	49
Barley	25	25
Soybean-meal	10	10
Spray-dried porcine plasma	-	8
Casein	8	-
Molasses	2	2
Fat mixture	2.15	2.6
Limestone	1.1	0.99
Monocalcium phosphate	0.9	1.19
Premix[a]	0.5	0.5
Salt	0.56	0.33
DL-Methionine premix	-	0.31
L-Lysine HCl premix	0.09	0.08
Threonine premix	0.7	-
Calculated composition		
NE kcal/kg	2352	2352
Crude protein, %	20.50	19.83
Fat, %	3.60	4.11
Crude fibre, %	2.69	2.70
Starch, %	45.55	45.16
Digestible[b] lysine, %	1.30	1.30
Digestible[b] methionine plus cystine, %	0.61	0.59
Digestible[b] methionine, %	0.38	0.38
Digestible[b] threonine, %	0.67	0.67
Calcium, %	0.72	0.77
Digestible[b] phosphorus, %	0.36	0.36
Sodium, %	0.38	0.39

[a] Premix provided per kilogram of complete diet: vitamin A, 7,500 IU; vitamin D_3, 1,500 IU; vitamin E, 17.5 mg; riboflavin, 2 mg; vitamin B_{12}, 20 μg; vitamin K_3, 0.75 mg, d-pantothenic acid, 6 mg; niacin, 30 mg; iron, 80 mg; iodine, 0.45 mg; cobalt, 0.15 mg; copper, 160 mg; manganese, 24 mg; selenium, 0.175 mg; zinc, 150 mg

[b] Apparent ileal digestible

Measurements

Chemical analysis of dietary constituents and assessment of cell mitotic activity were performed as described in our previous article (Van Dijk et al., 2001b). The incorporation rate of 5-bromo-2'-deoxyuridine (BrdU) into the small intestinal enterocytes was used as an index of mitotic activity.

Individual body weight and feed intake were measured daily. Faeces and condition scores were recorded per piglet daily by the same person who was blinded to treatment modality. Faeces scores were based on the following scale: 0 = normal, solid faeces; 1 = soft, looser than normal, 2 = diarrhoea and 3 = liquid faeces, severe diarrhoea. Condition scores were based on a scale of: 0 (good condition; healthy appearance, short hair, shiny skin) to 3 (poor condition; unhealthy appearance, long hair, pale and dull skin).

Samples from the small intestine were taken as described in our previous article (Van Dijk et al. 2001b). Five intestinal tissue samples of 2 cm length each and two of 10 cm length (for biochemical analyses, see further) were removed along the small intestine. One 2 cm sample was taken adjacent to the stomach at 10 per cent of the length of the small intestine. The other four 2 cm samples were taken further distally at 25, 50, 75, and 95 per cent of the length of the small intestine. The two 10 cm samples were taken at 25 and 50 per cent of the length of the small intestine. The histological processing and examination was also done exactly as described in our previous article (Van Dijk et al. 2001b).

The 10 cm small intestinal samples were cut open, rinsed in 4 ºC sodium chloride solution (9 g/l saline), gently blotted, wrapped in aluminium foil, frozen in liquid nitrogen and then stored at -85°C prior to enzyme and protein determinations. The samples were allowed to thaw, mucosa was scraped off with a blunt spatula and weighed accurately. The scrapings were sonicated in 4 parts chilled sodium chloride solution (9 g/l saline) and were stored over-night at 4°C prior to the biochemical determination of protein, lactase, sucrase and maltase II and III. Lactase was determined using 56 mM lactose (4-chloromercuribenzoate was added to inhibit lactases other than the brush-border enzyme) and sucrase using 56 mM sucrose as substrate. Maltase II and III activities were determined using 28 mM maltose as substrate after first heating homogenates at 55°C for 30 min to inactivate sucrase-isomaltase by the method of Kidder and Manners (1980). Glucose released by hydrolysis of disaccharide was then determined using the glucose-6-phosphate dehydrogenase (EC 1.1.1.49)-hexokinase (EC 2.7.1.1) assay (Boehringer, Mannheim, Germany). Enzyme activities were calculated as μmol substrate split/min and expressed for specific activity (per gram protein). Protein was measured by the method of Markwell et al. (1978).

Statistical analyses

The individual piglet was the experimental unit. Treatments were compared with a t-test using the general linear models procedure of SAS (1988). Kolmogorov's and

Levene's tests were applied to check the assumptions of normality and homogeneity of variances. Faecal and condition scores were compared using a proportional odds model using the logistic procedure of SAS (1988). The statistical model used was: Y = mean + diet effect + sow effect + error. Mitotic activity data were analysed using the model: y = mean + diet effect + error. Sow as block factor was left out of the latter model because, due to absence of staining of some BrdU samples, the number of piglets originating from one sow was not equal for each treatment. Crypt depth and villus length data are all based on 12 replicates (individual piglets) per treatment-day combination. The level of statistical significance was pre-set at $P < 0.05$. In the tables, the pooled standard error of the mean (SEM) is given.

Results

The total immunoglobulin content of SDPP was 171.4 g/kg. The data presented in Table 2 show that the analysed values for feed composition were in line with those calculated.

There was no systematic diet effect on ADG and ADFI. On day 4 after weaning the control piglets grew significantly faster than those given the SDPP-diet (Table 3). SDPP had no significant impact on condition and faecal scores (results not shown).

Table 2. Chemical analysis of the experimental diets

	control	SDPP
Chemical analysis	% on as fed basis	
Crude protein	20.1	20.3
Fat	3.7	4.1
Crude fibre	2.7	2.7
Moisture	10.8	10.6
Ash	5.1	5.4
Starch	43.4	42.5
Immunoglobulins (g/kg)	2.0	12.9

After weaning, villus length had dropped significantly (Fig 1), but diet composition did not significantly influence the villus length. Likewise, there were no differences in crypt depth between the two treatments (Fig. 2). BrdU data are based on 8-12 replicates per treatment (Fig. 3). In 35 out of the total of 270 samples there was absence of staining and those samples were excluded from the calculations. The excluded samples consisted of 5, 20 and 10 samples of the

unweaned, casein and SDPP group, respectively. It was concluded that the major cause of the absence of staining was erroneous injection of BrdU into the bladder or the intestinal lumen because there was a total absence of BrdU incorporation in either the mucosa or submucosa. On day 7 after weaning, piglets fed SDPP generally showed more mitotic activity than did the piglets receiving the casein diet, but the differences were not statistically significant.

Brush-border enzyme activities are presented in Figures 4, 5 and 6. On day 2 after weaning, lactase, sucrase and maltase activities for the site at 50% of the length of the small intestine had dropped. The fall in lactase activity was most pronounced. On day 7 after weaning, lactase activity was still low, but maltase activities had reached values above those seen at weaning. There were no statistically significant differences in lactase, sucrase or maltase specific activities between the two dietary treatments

Table 3. Daily weight gain and feed intakes in the two treatment groups

Measure	Control diet	SDPP diet	SEM
Average Daily Gain, g			
Day[1] 1	-244	-258	14
Day 2	-68	-100	21
Day 3	57	47	75
Day 4	206[a]	35[b]	56
Day 5	218	238	64
Day 6	155	270	60
Day 7	195	255	74
Average Daily Feed Intake, g			
Day 1	4	3	1
Day 2	18	15	6
Day 3	72	142	58
Day 4	303	120	98
Day 5	147	219	81
Day 6	236	263	39
Day 7	304	395	57

Data presented as means for 12 (days 1-2) or 6 animals per experimental diet (days 3-7) and pooled SEMs.

[1]Days refer to the day after weaning. Piglets were weaned at the age of 18 days.

[a,b] Means within the same row without a common superscript letter differ significantly ($P < 0.05$).

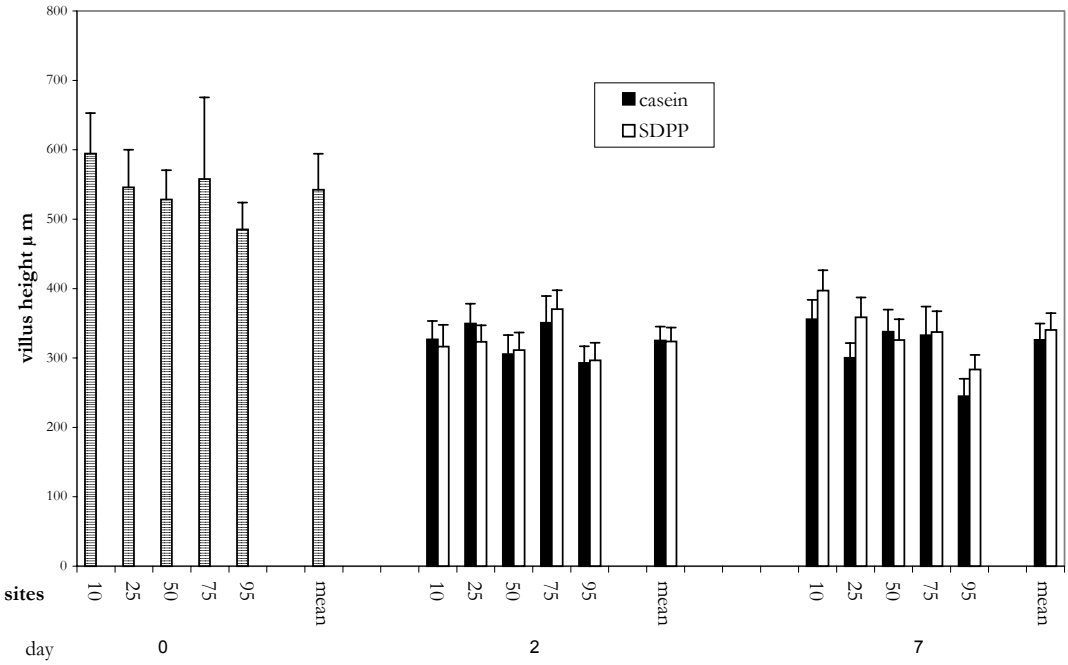

Fig 1: Villus height (+SEM) at the 10%, 25%, 50%, 75% and 95% sites along the entire length of the small intestine and the mean villus height of all sites of weaned piglets given either a casein or SDPP containing diet. Days refer to the days after weaning. □ = control.

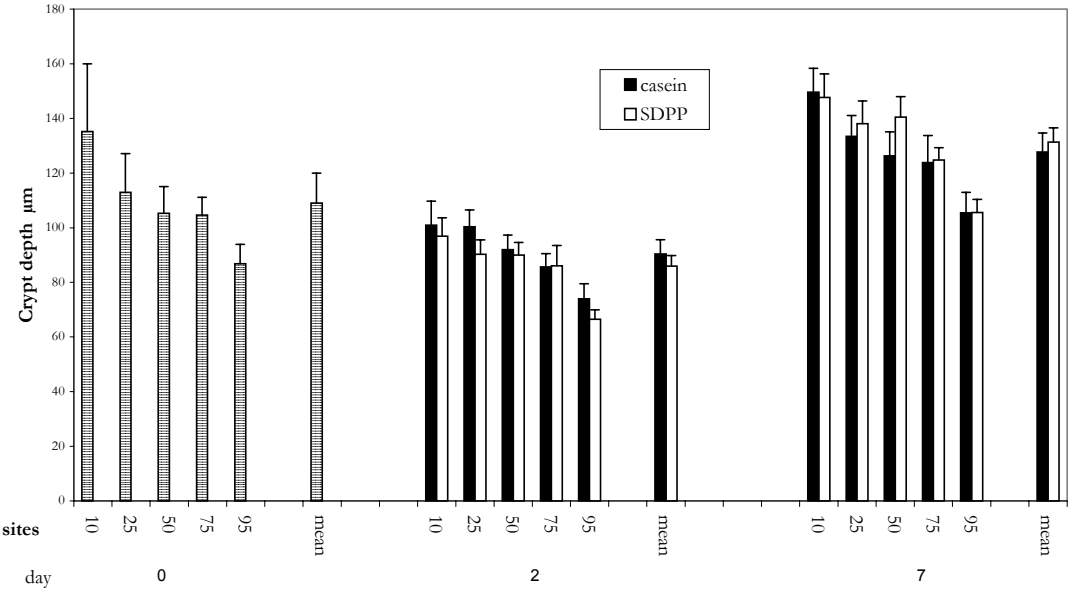

Fig 2: Crypt depth (+SEM) at the 10%, 25%, 50%, 75% and 95% sites along the entire length of the small intestine and the mean crypt depth of all sites of weaned piglets given either a casein or SDPP containing diet. Days refer to the days after weaning. □ = control.

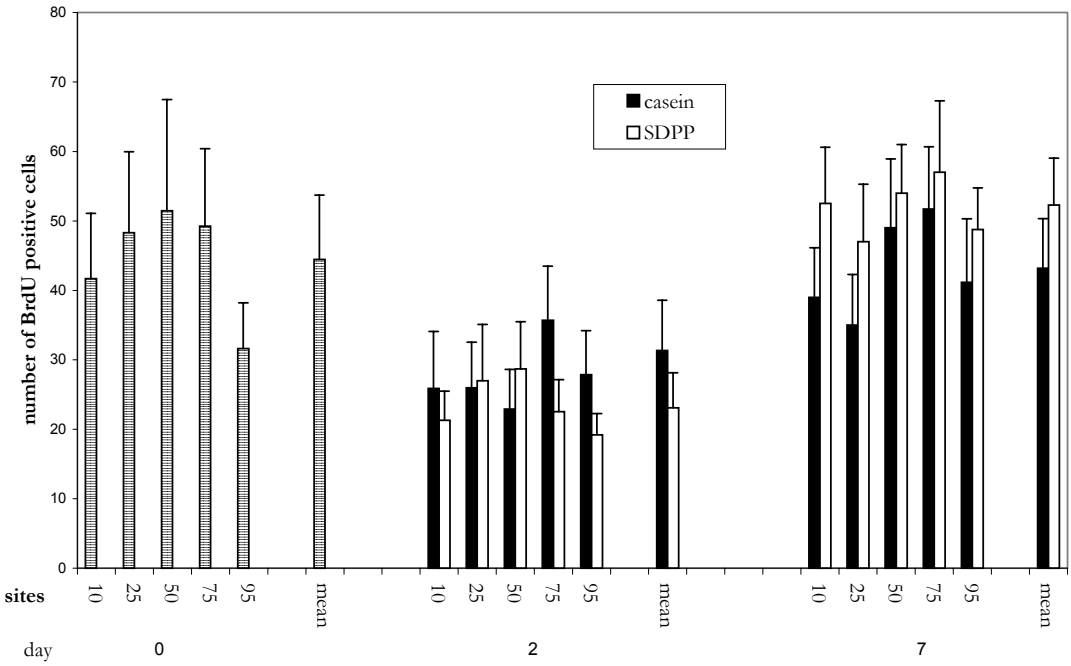

Fig 3: Number of BrdU positive epithelium cells (+SEM) at the 10%, 25%, 50%, 75% and 95% sites along the entire length of the small intestine and the mean number of BrdU positive epithelium cells of all sites of weaned piglets given either a casein or SDPP containing diet.

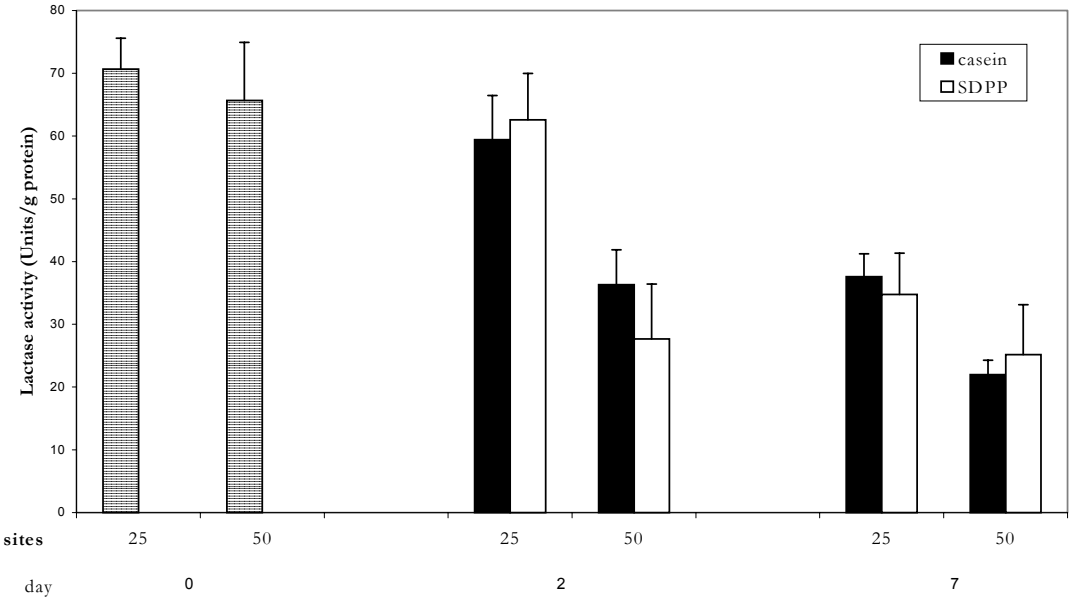

Fig 4: The activity of lactase (+SEM) at the 25% and 50% sites along the entire length of the small intestine of weaned piglets given either a casein or SDPP containing diet. Days refer to the days after weaning. □ = control.

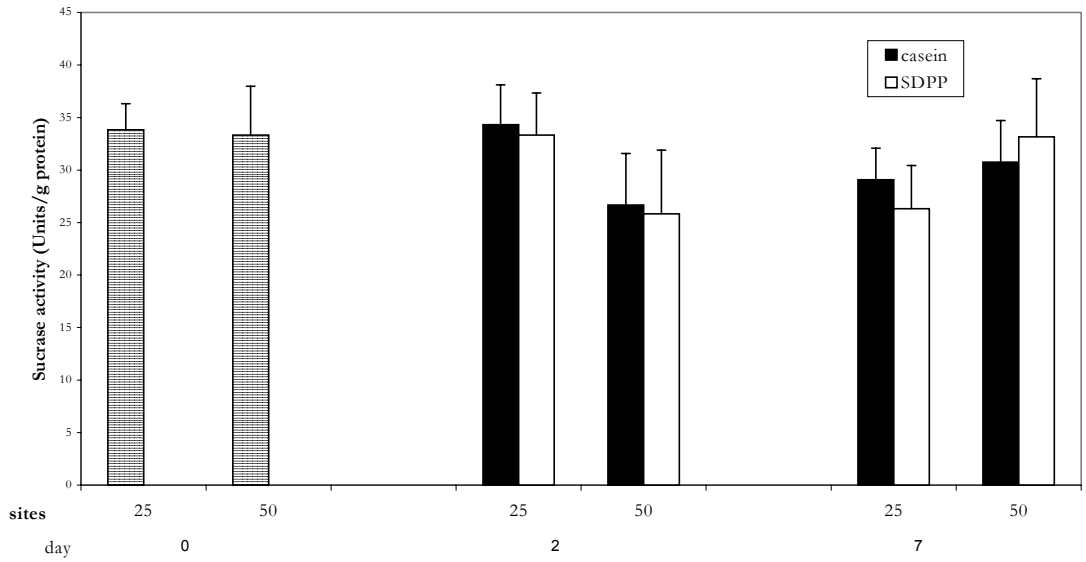

Fig 5: The activity of sucrase (+SEM) at the 25% and 50% sites along the entire length of the small intestine of weaned piglets given either a casein or SDPP containing diet. Days refer to the days after weaning. □ = control.

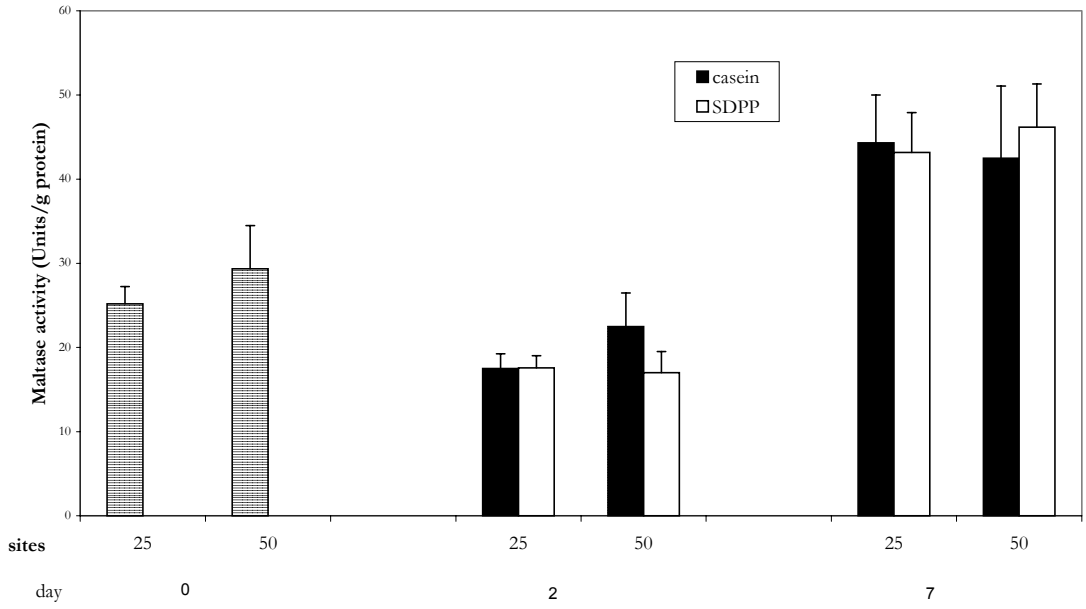

Fig 6: The activity of maltase (+SEM) at the 25% and 50% sites along the entire length of the small intestine of weaned piglets given either a casein or SDPP containing diet. Days refer to the days after weaning. □ = control.

Discussion

In an experiment with piglets that were weaned at 24 days of age, dietary SDPP versus casein had no effect on villus length nor on crypt depth but tended to decrease enterocyt mitotic activity on days 4 and 7 after weaning (Van Dijk et al. 2001b). In the present experiment weaning was done at the age of 18 days. In this study as in our previous experiment, there was no positive effect of SDPP on ADFI and ADG in the first week after weaning. The lack of effect of SDPP may be explained by the high hygienic conditions under which the experiments were carried out. It has been suggested that the positive effect of SDPP on growth performance is seen only under conditions of infection pressure (Van Dijk et al. 2001a). However, it could be suggested that because SDPP did not affect ADFI, this experiment is suitable to identify specific trophic effects of SDPP as a change in feed intake would by itself alter villus height (Kelly et al. 1991, Pluske et al. 1997, Van Beers et al. 1998). The villus atrophy on day two after weaning was, as would be expected (Pluske et al. 1997), more pronounced in this than in our previous experiment, the reductions in villus length being 42% and 17%, respectively. Thus, even under conditions of severe villus atrophy, SDPP did not prevent it, not even partly. It can be concluded from this experiment and from the experiments of Van Dijk et al. (2001b) and Jiang et al. (2000) that dietary SDPP does not prevent villus atrophy that is associated with the process of weaning.

Upon weaning, the number of BrdU positive cells in the small intestinal mucosa had dropped after two days and then rose by day 7. This is in accordance with our earlier experiment (Van Dijk et al. 2001b) and is explained by the concept of hyper-regeneration following villus atrophy (Hall and Byrne 1989, Pluske et al. 1997). On day 7 after weaning, the piglets fed SDPP showed higher group mean mitotic activity than did the piglets receiving the casein diet, but the differences were not statistically significant. The present result is in contrast with our earlier experiment (Van Dijk et al. 2001b), in which the effect of SDPP tended to be opposite. There is no obvious explanation for the discrepancy, but it may relate to the lower SDPP inclusion level in the diet. In our previous experiment the diminishing effect of SDPP on mitotic activity was restricted to the first part of the small intestine (SI), suggesting poor survival of the active component in the GI tract. This could explain why a lower percentage (8%) of SDPP fails to produce an effect. A similar effect has been found of orally administered epidermal growth factor in piglets by Zijlstra et al. (1994). Clearly, in this experiment there was no stimulatory effect of SDPP on villus length, illustrating that SDPP had no trophic effect on the small intestinal villi. In our experiment we used casein as a control protein source. Casein is generally considered to be a high value protein source for piglets. As a control protein for SDPP, casein may not enhance the contrast, but it does exclude possible confounding effects on small intestinal morphology because, unlike plant protein sources, casein would be expected not to damage the intestinal

epithelium (Hall and Byrne 1989). This reasoning implies that SDPP could have a trophic effect on small intestinal villi when it is incorporated in standard rations.

This is the first paper describing small intestinal BB-disaccharidase activity in piglets fed SDPP. The analysed values of the BB-enzyme activities are in line with those found by Kidder and Manners (1980). There were no statistically significant effects of SDPP on lactase, sucrase and maltase specific activities indicating that there was no SDPP effect on digestive functionality of the small intestine as to disaccharides. Immature enterocytes have a lower BB enzyme activity than do older enterocytes (Smith 1985). So, the lack of effect of SDPP on mitotic activity is in accordance with that on BB enzyme activities.

In conclusion, villus length, mitotic activity and BB-disaccharidases were not influenced by SDPP in piglets kept under low infection pressure. Thus, the reported (van Dijk et al. 2001a) beneficial effects of SDPP cannot be explained by changes in the small intestinal morphology. Recently, evidence has been put forward in that the beneficial effect of SDPP may be caused by diminishing the sensitivity to pathogens in the small intestinal lumen (Nollet et al. 1999, Jiang et al. 2000, van Dijk et al. 2001c).

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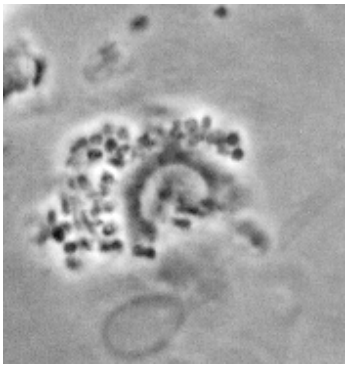
CHAPTER 5

INFLUENCE ON INTESTINAL MICROFLORA



Brush borders of piglet jejunal enterocytes, with numerous adherent pathogenic *E. coli* bacteria. Magnification 1000X.

CHAPTER 5.1

EFFECT OF SPRAY-DRIED PORCINE PLASMA ON INTESTINAL MICROFLORA AS MEASURED IN VITRO AND IN WEANLING PIGLETS

A.J. Van Dijk[a*], S.G.C. Van den Hoven[a], P.M.M. Enthoven[a], and A.C. Beynen[b]

[a]Co-operative Central Laboratory "CCL- Nutricontrol" of Cehave Landbouwbelang, P.O. Box 107, 5460 AC Veghel, The Netherlands.

[b]Department of Nutrition, Utrecht University, Faculty of Veterinary Medicine, P.O. Box 80152, 3508 TD Utrecht, The Netherlands

Submitted

Abstract

The hypothesis tested was that the reported positive effects of dietary spray-dried porcine plasma (SDPP) on growth performance and health of weanling piglets relates to an effect on the gastrointestinal microflora. Weanling piglets were offered a diet containing either 8% SDPP or 8% casein and 7 days later they were anaesthetised to take samples of gastrointestinal contents. In the stomach, the number of Enterobacteriaceae was significantly lowered by the SDPP diet, whereas the number of sulphite reducing Clostridia was significantly raised. There was a non-significant, but systematic SDPP-induced increase in Lactobacilli in different gastrointestinal compartments. SDPP stimulated the growth of *L. plantarum* in pure cultures, this effect becoming smaller with higher SDPP concentrations. There was also a growth stimulating effect of SDPP on pure cultures of *E. faecalis*. The growth of pure *C. perfringens* was inhibited by SDPP in a dose-dependent fashion.

In isolated caecal contents, the growth of Enterococci was inhibited with increasing SDPP concentrations, whereas there was a growth-stimulating effect on sulphite reducing Clostridia. The observed bacterial effects of SDPP do not readily explain the beneficial effects of dietary SDPP on post-weaning growth performance of piglets.

Introduction

In piglets, the process of weaning is associated with impaired feed intake and growth performance, diarrhoea and high mortality. The diarrhoea is associated with the proliferation and activity of enterotoxigenic *E. coli* (Van Beers-Schreurs et al. 1992). The addition of spray-dried animal plasma (SDAP) to diets of weaned piglets generally has a positive effect on average daily feed intake (ADFI) and average daily gain (ADG) (Van Dijk et al. 2001a) and reduces diarrhoea (Gatnau 1990, Van der Peet-Schwering and Binnendijk 1995).

There is suggesting evidence that SDAP has antibacterial activity. Coffey and Cromwell (1995) showed a greater effect of SDAP on growth performance of piglets kept under poor hygienic conditions than in their counterparts kept under optimal hygienic conditions. Recent data point at a reduction in intestinal inflammation in weanling piglets fed diets with porcine plasma (Jiang et al., 2000). Wijngaards et al. (1995) demonstrated that bovine plasma antagonised the proliferation of *Pseudomonas fluorescens*. It could be hypothesised that the feeding of SDAP reduces microbial growth or activity in the small intestine so that nutrient utilisation by the host is enhanced, a mechanism explaining increased growth in animals given anti-microbial growth promoters (AMGP) (Visek 1978, Vervaeke et al. 1979, Dierick et al. 1986ab). In addition, SDAP may be antagonistic towards pathogenic bacteria, including those causing diarrhoea. Thus, it was the goal of the present study to investigate the effect of spray dried porcine plasma (SDPP) on gastrointestinal microbial activity. A feeding trial was performed with weanling piglets given a diet without or with SDAP and selected intestinal bacteria were quantified. Further, the effect of SDAP on selected bacteria under *in vitro* conditions was studied.

Materials and methods

Experiment 1.

Eighteen piglets were used in this feeding trial. Details on the experimental design are described elsewhere (van Dijk et al. 2001b). Briefly, the experiment had a complete randomised block design with litter as block and nine piglets per treatment. At the age of 18 days, the piglets were weaned and assigned to one of

two dietary treatments and penned in individual cages. The piglets were offered a pelleted diet containing either 8% spray-dried porcine plasma (SDPP) or 8% casein.

The piglets were sampled at 7 days after weaning. They were anaesthetised with a mixture of nitrous oxide, oxygen and halothane administered through a facemask. The stomach, caecum and a segment of the distal part (at 95 per cent of the length) of the jejunum were aseptically ligated and removed. Then, the piglets were euthanised by an intracardial injection of T61 (Hoechst Roussel Vet, Brussels, Belgium). The samples were placed on ice in sterile plastic containers. Within two hours the samples were transported to the laboratory. Contents of the segments were frozen at –85 °C until determination of bacterial counts. After thawing at 43 °C, 10-fold serial dilutions were made from stomach or enteral contents using a physiological salt solution as a diluent. Fifty microliters of each dilution were spread by spiral plating on Petri dishes containing growth media specific for each bacterial group. Total Lactobacilli were determined by growth on Rogosa agar (Merck 1.05413), total Enterococci using Slanetz and Bartley agar (S & B Oxoid CM377), total Enterobacteriaceae using Violet Red Bile Glucose agar (VRBG, Merck 1.10275) and total sulphite reducing Clostridia using Sulphite Polymyxine Yeast extract agar (SPY). The SPY extract agar consisted of Tryptone (Oxoid L42, 15 g/L), Yeast Extract (Oxoid L21, 10 g/L), ammonium iron(III) citrate (Merck 3761, 1 g/L) and bacteriological agar (Oxoid L11, 15 g/L); after sterilisation (15 min at 121 °C) the following filter (0,2 μm) sterilised components were added aseptically: sodium sulphite (Merck 6657, 2.5%, 1 ml/100 ml) and polymixine-B-sulfate (Serva 47976, 0.1%, 1 ml/100 ml). An agar overlay method to reduce oxygen was used. For the overlay method, dilutions were spread on SPY extract agar, and plates were overlaid with 10 ml of liquid SPY agar at 45°C. Plates were incubated at 37 °C for 18-24 h (Enterobacteriaceae) or 42-48 h (Clostridia and Enterococci) or 72-76 h (Lactobacilli). The culture media for Clostridia were incubated anaerobically (BBL GasPak, Becton Dickinson 270304) and Lactobacilli were grown under micro-aerophylic conditions (BBL GasPak, Becton Dickinson 270304).

Experiment 2

The following bacteria were used for this *in vitro* experiment: *Escherichia coli* (strain ATCC 25922), *Enterococcus faecalis* (strain ATCC 29212), *Clostridium perfringens* (strain ATCC 13124) and *Lactobacillus plantarum* (strain Bd 99-00553). All cultures were grown for 18 h at 37 °C in brain heart infusion (BHI) broth (Oxoid CM225). *E. coli* and *E. faecalis* were grown aerobically, *C. perfringens* was grown anaerobically and *L. plantarum* was grown under micro-aerophylic conditions.

A culture medium was prepared that would mimic the intestinal contents of piglets and allow growth of the test strains. The composition of the medium (pH 6.75) was as follows: A phosphate buffer at pH 6.75 was made by mixing di-

potassium hydrogen phosphate (Merck 1.05104, 19.16 g/L) and potassium dihydrogen phosphate (Merck 1.04873, 14.97 g/L). Subsequently, the following components were added: sodium chloride (Merck 1.06404, 8.50 g/L), manganese(II)sulphate monohydrate (Merck 5963, 0.05 g/L), magnesium sulphate heptahydrate (Merck 5886, 0.20 g/L), peptone (Merck 1.07214, 1.00 g/L), yeast extract (Oxoid L21, 2.00 g/L), ammonium ferro(III)citrate (Merck 3761, 0.02 g/L), sodium acetate trihydrate (Merck 1.06267, 3.00 g/L) and D(+)glucose monohydrate (Merck 1.08342, 1.00 g/L). After preparation, the medium was sterilised during 15 minutes at 121 °C. SDPP (Harimex, Loenen, the Netherlands) was added to the culture medium at concentrations of 100.0, 50.0, 12.5 and 6.25 mg/ml. Tubes were filled with 12 ml of culture medium, 1.5 ml of bacterial culture (100 times diluted) and 1.5 ml SDPP containing medium. Tubes with 13.5 ml culture medium and 1.5 ml bacterial culture served as reference. Tubes were incubated anaerobically at 37 °C for 4 h. At the start and end of the incubation period, 10-fold serial dilutions were made of the contents of the tubes using a physiological salt solution as a diluent. Fifty microliters of each dilution were spread by spiral plating on Petri plates containing growth media specific for each bacterial type. *E. coli* was determined by growth on VRBG agar, *E. faecalis* using S&B agar, *L. plantarum* using Rogosa agar and *C. perfringens* using SPY agar as described above.

Bacterial growth was expressed as the log change in cfu/ml after incubation for 4 h. Bacterial inactivation or activation was determined by subtracting the bacterial growth in reference culture from that in the test culture.

Experiment 3.

For this *in vitro* experiment materials and methods were identical to those in Exp. 2, except that instead of a culture of a specific bacterium 1.5 ml of piglets' caecal contents was added to the tubes. The caecal contents had been diluted 100 times with physiological salt solution. Caecal contents were stored at –85 °C and thawed at 43 °C 45 minutes before use. Caecal contents had been collected earlier from anaesthetised, healthy piglets under aseptical conditions. Total Lactobacilli, Enterococci, Enterobacteriaceae and sulphite reducing Clostridia were determined as described above for Exp. 1. Bacterial inactivation or activation was determined as described for Exp. 2.

Statistical analyses

In Exp. 1, the individual piglet was the experimental unit. Treatment effects were evaluated with a t-test using the general linear models procedure of SAS (1988). The statistical model used was: Y = mean + diet effect + sow effect + error. The level of statistical significance was pre-set at $P < 0.05$. The results of Exp. 2 and 3 were statistically analysed by linear regression analysis (SAS 1988).

Results

Experiment 1

The bacterial counts are presented in Table 1. In the stomach, the number of Enterobacteriaceae was significantly lowered by the SDPP diet, whereas the number of sulphite reducing Clostridia was slightly but significantly increased. There was a non-significant, systematic SDPP-induced increase in Lactobacilli in the three intestinal compartments. Further, no effects of SDPP on bacterial counts were observed.

Table 1.

Effect of dietary SDPP on bacteria (log_{10} cfu/g intestinal contents) in the gastro-intestinal tract of piglets.

	Control diet	SDPP diet	SEM
Stomach			
Enterococci	3.92	3.71	0.43
Enterobacteriaceae	1.79	0.89 *	0.30
Sulphite reducing Clostridia	1.68	2.09 *	0.12
Lactobacilli	7.19	7.89	0.49
Jejunum			
Enterococci	4.72	4.59	0.44
Enterobacteriaceae	4.93	5.75	0.66
Sulphite reducing Clostridia	0.90	1.19	0.23
Lactobacilli	7.06	8.25	0.69
Caecum			
Enterococci	5.62	5.30	0.33
Enterobacteriaceae	6.62	6.16	0.40
Sulphite reducing Clostridia	3.39	3.36	0.23
Lactobacilli	7.78	8.48	0.41

* Significant difference versus control group ($P < 0.05$).

Experiment. 2.
SDPP stimulated the growth of *L. plantarum* but this effect diminished with higher concentrations (Table 2). There also was a growth-stimulating effect of SDPP on *E. faecalis* that was concentration independent. The growth of *C. perfringens* was inhibited by SDPP in a dose-dependent fashion. There was no consistent effect of SDPP on *E. coli.*

Table 2.

Effect of varying concentrations of SDPP on growth of pure cultures of gastrointestinal bacteria.

SDPP concentration, mg/ml	0.625	1.250	5.000	10.000
Enterococcus faecalis	0.66	0.68	0.54	0.55
Escherichia coli	-0.32	0.06	0.10	-0.10
Clostridium perfringens	-0.48	-0.74	-0.80	-0.94
Lactobacillus plantarum	0.80	0.79	0.43	0.16

Bacterial growth in reference cultures was substracted from that in SDPP-treated cultures and the difference expressed as the $\log_{10}$ change in cfu/ml after incubation for 4 h.

Experiment. 3.
In isolated caecal contents, the growth of Enterococci was inhibited with increasing SDPP concentrations, whereas the growth of sulphite reducing Clostridia was stimulated (Table 3). There was no effect of SDPP on Lactobacilli or Enterobacteriaceae.

Table 3.

Effect of varying concentrations of SDPP on growth of bacteria in isolated caecal contents.

SDPP concentration, mg/ml	0.625	1.250	5.000	10.000
Enterococci	-0.09	-0.19	-0.81	-0.70
Enterobacteriaceae	-0.01	-0.01	-0.14	-0.24
Sulphite reducing Clostridia	0.09	0.09	0.24	0.57
Lactobacilli	0.03	-0.07	0.15	0.02

Bacterial growth in reference cultures was substracted from that in SDPP-treated cultures and the difference expressed as the $\log_{10}$ change in cfu/ml after incubation for 4 h.

Discussion

In the feeding trial SDPP had significant effects on bacteria only in the stomach. Possibly, any antibacterial component of SDPP was digested or diluted more distally in the intestine. It is unfortunate that samples from the proximal jejunum were not taken. The significant SDPP-induced reduction of Enterobacteriaceae in the stomach might explain the earlier observed reduction in diarrhoea (Gatnau 1990, Van der Peet-Schwering and Binnendijk 1995). However, there was no effect of SDPP on Enterobacteriaceae in the distal jejunum. Moreover, under *in vitro* conditions no diminishing effect of SDPP on *E. coli* could be demonstrated. The systematic SDPP-induced increase in the number of Lactobacilli the gastrointestinal tract and the SDPP-induced increase in *L. plantarum* counts in pure cultures could contribute to the anti-diarrhoeal properties of SDPP. Lactobacilli may increase colonisation resistance of the intestine against pathogenic bacteria (Mackie et al. 1999).

The SDPP-induced increase in sulphite reducing Clostridia both *in vivo* in the stomach and *in vitro* on caecal contents seems to be at variance with the SDPP-induced decrease in Clostridium perfringens counts in pure cultures. We have no explanation for this discrepancy and a relationship, if any, with the health and growth promoting properties of SDPP is not clear. There were also contradictory effects of SDPP on Enterococci in the stomach and in vitro in caecal contents. It is feasible that the *in vivo* and *in vitro* experiments yield different results in relation to the experimental conditions. As mentioned above, SDPP and its components may undergo changes during the digestive processes. In the feeding trial, casein addition to the diet was used as a control treatment. The in vitro experiments were carried out with control treatments without added protein source so that the specificity of the SDPP cannot be assessed. In addition, there are less interactions of constituents within the *in vitro* systems than in the *in vivo* situation.

The present experiments do not support the hypothesis that the beneficial effects of dietary SDPP on post-weaning growth performance and health of piglets can be explained by its antimicrobial properties. However, it cannot be excluded that SDPP has an effect on bacterial species other than those investigated in the present experiments.

Van Dijk et al. (2001c) and Nollet et al. (1999) investigated the effect of SDPP in diets of piglets that were challenged with a pathogenic *E. coli*. As opposed to Van Dijk et al. (2001c), Nollet et al. (1999) demonstrated a SDPP-induced reduction in faecal *E. coli* counts, but the latter authors used extremely high, non realistic SDPP levels in the diet. The response to dietary SDAP in germ-free piglets is of interest; a positive effect on growth performance would suggest that the mode of action of SDAP is not based on an interaction with the microbial flora.

Acknowledgements

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CHAPTER 5.2

THE EFFECT OF DIETARY SPRAY-DRIED PORCINE PLASMA ON CLINICAL RESPONSE IN WEANED PIGLETS CHALLENGED WITH A PATHOGENIC ESCHERICHIA COLI

A.J. Van Dijk[1], P.M.M. Enthoven[1], S.G.C. Van den Hoven[1], M.M.M.H. Van Laarhoven[2], T.A. Niewold[3], M.J.A. Nabuurs[3] and A.C. Beynen[4]

[1] Co-operative Central Laboratory "CCL- Nutricontrol" of Cehave Landbouwbelang, P.O. Box 107, 5460 AC Veghel, The Netherlands.

[2]Animal Nutrition Group, Wageningen Institute of Animal Sciences, P.O. Box 338, 6700 AH Wageningen, The Netherlands.

[3]Department of Immunology, Pathobiology and Epidemiology, Institute for Animal Science and Health, ID-Lelystad, P.O. Box 65, 8200 AB Lelystad, The Netherlands.

[4]Department of Nutrition, Utrecht University, Faculty of Veterinary Medicine, P.O. Box 80152, 3508 TD Utrecht, The Netherlands.

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Abstract

Weaned piglets were used to determine the effect of dietary spray-dried porcine plasma (SDPP) on the clinical response to an infection with a pathogenic *E. coli* O139:K82 LT $^{-}$. The piglets were divided into two groups of 10 animals each. One group was fed the control diet containing soybean(meal) plus whey powder. The test piglets were fed a diet with 8 % SDPP. Piglets were orally infected with the challenge strain on days 6 and 7 after weaning. The experimental period lasted 14

days after which the piglets were euthanised and necropsied. Faecal samples were collected daily for bacteriological analysis. Segments of jejunum, caecum and rectum were removed for bacteriological analysis post mortem. Feed intake and weight gain, faecal and condition scores and body temperature were measured daily. In the control and SDPP group 6 and 7 piglets died from diarrhoea. The average daily feed intake (ADFI) and average daily gain (ADG) were substantially higher in the SDPP group than in the control group. SDPP-fed piglets generally had a more favourable faecal score and a healthier appearance than did the control piglets. The faecal excretion of *E. coli* O139:K82 was similar for control and test piglets. There were no diet effects on the *E. coli* O139:K82 counts at different sites of the intestine. In this experiment the inclusion of SDPP at an economically acceptable percentage in the diet could not prevent piglet losses due to challenge with a pathogenic *E. coli*, but improvements of ADG, ADFI and faecal and condition scores were achieved.

Introduction

Weaned piglets often suffer from post-weaning diarrhoea (PWD) or oedema disease (OD), which causes impaired growth performance and high mortality. PWD occurs mainly during the first week after weaning and is associated with the proliferation of enterotoxigenic *E. coli* (ETEC) and toxins produced by these bacteria, like heat labile enterotoxin (LT). OD is associated with the proliferation of enterotoxemic *E. coli* (ETEEC) and release of their toxins (Shiga-like toxins) (Van Beers-Schreurs et al. 1992, Nabuurs et al. 1993, Nagy and Feteke 1999, Bertschinger 1999). Other factors, such as changes in the flora, function and morphology of the intestine also are involved in the development of PWD and OD (Nabuurs 1998).

Various measures are taken to improve feed intake and health of piglets after weaning. Amongst these is the addition of specific substances to the weaner pig diet. One of these substances is spray-dried animal plasma (SDAP) which usually is of porcine origin. The addition of SDAP to diets of weaned piglets generally has a positive effect on average daily feed intake (ADFI) and average daily gain (ADG) in comparison to the feeding of other commonly used protein sources (Van Dijk et al. 2001a). In two experiments, less diarrhoea was found in piglets fed spray-dried porcine plasma (SDPP) during the first two weeks after weaning when compared to piglets fed milk and soy proteins (Gatnau 1990, Van der Peet-Schwering and Binnendijk 1995). Thus, SDAP addition to weaning piglets' diets may help to prevent PWD and OD. The possible underlying mechanisms, protection of the intestine by immunoglobulins or inhibition of binding of ETEC/ETEEC to the brush borders by glycoproteins, are described in detail by Van Dijk et al. (2001a).

The oral challenge of piglets with pathogenic *E. coli* is used as a model of PWD or OD (Sarmiento et al. 1988, Nagy et al. 1992, Deprez et al. 1996, Meijer et al. 1997, Jeyasingham et al. 1999, McDonald et al. 1999, Nollet et al. 1999). In many cases clinical signs cannot be provoked by an oral challenge alone and a stressor such as cold stress is introduced in the model (Sarmiento et al. 1988, McDonald et al. 1999). In four experiments the number of bacteria used for challenge was counted in the faeces after the infection in order to quantify the faecal excretion of the challenge strain. The CFU of a specific pathogenic *E. coli* strain in faeces of diarrhoeic weaned piglets was positively related with the proliferation of that specific strain in the small intestine (Nabuurs et al. 1993). Resistance to colonisation of pathogenic bacteria is reflected by low bacterial counts in faeces (Bovee-Oudenhoven et al. 1997). Moreover, large numbers of pathogenic bacteria in faeces increase the shedding of these pathogens to the environment, causing a higher risk for the disease.

Nollet et al. (1999) demonstrated that the feeding of SDPP derived from non-vaccinated pigs (non-immune plasma) reduced the excretion of pathogenic *E. coli* with faeces. However, Nollet et al. (1999) studied the effects of daily intakes of SDPP as high as 45 and 90 g per piglet. These intakes are extremely high, may have caused non-specific effects, and are certainly not economical. The aim of the present experiment was to determine if a relatively low dosage of SDPP (8%) in the diet of piglets challenged with pathogenic *E. coli* could reduce the faecal excretion of the bacterium challenged with and could also reduce morbidity and mortality. We used a challenge model more severe than that was used by Nollet et al. (1999). Our piglets were pre-treated with colistin (Meijer et al. 1997), received a double instead of single oral challenge, and were exposed to moderate cold stress.

Materials and methods

Animals

The experimental design was approved by the Animal Experiments Committee of ID-Lelystad. Twenty piglets from the closed herd of the research station 'Laverdonk', Veghel were used. The piglets (F2 cross-bred: GY x [Finnish X Dutch Landrace]) were females and castrates aged 19 d. The piglets were divided into two groups of 10 animals each that were group housed. The piglets in each group were randomly selected from the litters of 10 different sows so that litter origin distributions were identical. Each group was randomly assigned to one of two dietary treatments: the control or SDPP containing diet. The average weight of the piglets at the beginning of the experiment was 6.62 kg for the control group and 7.19 kg for the SDPP group. The piglets did not receive creep feed during the lactation period.

Housing

The groups were housed in pens (2.33 x 3.65 m) with concrete floors covered with sawdust bedding. The 2 pens were located in one environmentally regulated room in an isolated stable. The two pens were separated from each other by an empty space of 100 cm so that physical contact between piglets of the two groups was excluded. Separate boots for each pen were used by the person that entered the pens. The piglets had free access to feed and water. Each pen was equipped with a water nipple and a one-hole self-feeder. Room temperature was kept at 24 °C to induce moderate cold stress.

Bacteria

The challenge strain used in this experiment was an *E. coli* O139:K82 LT $^-$ isolated from a clinical case with PWD. Strains of O serogroup 139 have a well-established association with both OD and PWD (Van Beers-Schreurs et al. 1992, Bertschinger 1999). The strain was haemolytic and was resistant to amoxicillin and spectinomycin dihydrochloride. The bacteria were grown in brain heart infusion broth (Oxoid CM225) at 37 °C during 24 h. Bacteria were harvested by centrifugation, washed with 0.20 M sodium phosphate buffered saline (PBS), pH 7.0, and resuspended in PBS at a concentration of 1 x 10^9 bacteria.ml^{-1}

Diets

The composition of the two experimental diets is shown in Table 1. The diets, which were pelleted at a temperature below 60 °C, contained either soybean(meal) plus whey powder or SDPP (Harimex, Loenen, The Netherlands) as main protein source. The diets were formulated to contain 1.03 % apparent ileal digestible lysine, 2352 kcal NE/kg and 4.7 % lactose. The crude protein content of the feed was determined according to Kjeldahl (EC 22-7-1993; nr. L 179/8-10). The analyses of fat (based on EC 3-9-1998; nr. L 257/23-25), crude fibre (based on EC 26-11-1992; nr. L334/35-37), moisture (based on EC 20-12-1971; nr. L279/ 8-11) and ash (based on ISO 936, 1992) were performed with gravimetrical methods. The starch content of the feed was determined polarimetrically according to Ewers (ISO 5554, 1993). The total immunoglobulin content of SDPP, whey powder and the diets was determined with protein-G affinity chromatography (Pharmacia/LKB) followed by UV detection.

Table 1.

Ingredient composition and calculated nutrient concentrations in the experimental diets

	Control	SDPP
Ingredient composition, % on as fed basis		
Spray-dried porcine plasma	-	8.00
Soybean meal	15.00	6.20
Dried whey	12.15	-
Monocalcium phosphate	0.29	0.87
Limestone	0.02	1.35
Premix[a]	0.50	0.50
Barley	35.95	45.00
Wheat	23.70	26.20
Toasted soybeans	3.79	-
DL methionine premix	0.26	0.29
L-Lysine HCl premix	0.74	0.44
Threonine premix	0.84	-
Lactose	-	4.70
Salt	0.28	0.10
Molasses	3.00	3.00
Fat mixture	3.48	3.35
Calculated composition		
NE kcal/kg	2352	2352
Crude protein, %	17.60	16.90
Fat, %	5.30	4.80
Crude fibre, %	2.80	2.70
Digestible[b] lysine, %	1.03	1.03
Digestible[b] methionine plus cystine, %	0.59	0.68
Digestible[b] methionine, %	0.33	0.33
Digestible[b] threonine, %	0.58	0.58
Lactose, %	4.70	4.70
Calcium, %	0.90	0.90
Digestible[b] phosphorus, %	0.36	0.36
Sodium, %	0.30	0.35

[a] Premix provided per kilogram of complete diet: vitamin A, 7,500 IU; vitamin D_3, 1,500 IU; vitamin E, 17.5 mg; riboflavin, 2 mg; vitamin B_{12}, 20 μg; vitamin K_3, 0.75 mg; d-pantothenic acid, 6 mg; niacin, 30 mg; iron, 80 mg; iodine, 0.45 mg; cobalt, 0.15 mg; copper, 160 mg; manganese, 24 mg; selenium, 0.175 mg; zinc, 150 mg

[b] Apparent ileal digestible

E. coli challenge trial

On day 1 and 2 after weaning, the piglets did not receive feed to induce maximum villus atrophy (Pluske et al. 1997), but drinking water was freely available. From day 3, the piglets were offered either the experimental or control diet ad libitum. From days 1 to 5 after weaning, the piglets received colistin (Dopharma, Raamsdonksveer, The Netherlands) in their drinking water at a dosage of about 5 mg/kg live-weight. Colistin pre-treatment increases the sensitivity of the piglets towards pathogenic *E. coli* (Meijer et al. 1997). On the sixth and seventh day after weaning all piglets were perorally infected with 1 x 10^{10} CFU of the *E. coli*, suspended in 10 ml PBS.

Methods used to assess the clinical response to E. coli challenge

For a period of 2 weeks after weaning, each piglet was monitored daily. Faecal and condition scores were assigned by the same person who was blinded to treatment modality. Faecal scores were based on the following scale: 0 = normal, solid faeces; 1 = soft, looser than normal faeces, 2 = diarrhoeal faeces and 3 = liquid, severe diarrhoeal faeces. Condition scores were based on a scale of which the extreme values are described as: 0 = good condition (healthy appearance, short hair, shiny skin) and 3 = poor condition (unhealthy appearance, long hair, pale and dull skin). Individual rectal temperature and body weight and feed intake per group were measured daily. Faecal samples were collected daily upon rectal stimulation by either the thermometer or a swab-induced defaecation. The faecal samples were immediately put into sterile plastic containers, placed on ice and transported within two hours to the laboratory where they were frozen at –80 °C until being processed for determination of bacterial counts. Examination of the piglets that died during the experiment was limited to gross observation at necropsy to determine the cause of death. Segments of jejunum, caecum and rectum were removed for bacteriological analysis.

On day 15 after weaning, the piglets were killed by intracardiac injection of 3 ml of T61 (Hoechst Roussel Vet, Belgium) and necropsied. Segments of jejunum, caecum and rectum were removed for bacteriological analysis. All samples were placed on ice in sterile plastic containers and transported to the laboratory within two hours. Contents of the segments were frozen at –80 °C until determination of bacterial counts.

For enumeration of *E. coli* O139:K82 in faeces and intestinal contents, the material was diluted ten times with peptone physiological salt solution (PFS). Serial dilutions were made in PFS and numbers of bacteria per gram of wet faeces were determined by surface plating techniques on blood agar (Oxoid CM271) with 7% defibrinated sheep blood (Biotrading) to which 80 mg amoxicillin (Sigma A-8523) and 40 mg spectinomycin dihydrochloride (Sigma S-4014) per litre had been added. After 20-24 h of incubation at 37°C, the colonies were counted. Randomly picked

colonies were identified by slide agglutination with specific antiserum (ID-Lelystad 7432110).

Statistical analyses

The individual piglet was considered to be the experimental unit. Treatments were compared with a t-test using the general linear models procedure of SAS (1988). Faecal and condition scores were compared with a proportional odds model using the logistic procedure of SAS (1988). The statistical model used was: Y = mean + diet effect + error. The level of statistical significance was pre-set at $P < 0.05$. In the tables, the pooled standard error of the mean (SEM) is given.

Results

The data presented in Table 2 show that the analysed values for feed composition agreed with those calculated (Table 1), except for the protein content in the control feed which was lower than calculated. This may be explained by a lower actual than calculated protein content of the whey powder and/or soybean(meal) used. The immunoglobulin contents of SDPP and whey powder were 19.5 % and 0.6 %, respectively.

Table 2.

Analysed composition of the experimental diets

	Control	SDPP
Chemical analysis % on as fed basis		
Fat	6.6	5.7
Crude fibre	2.5	2.6
Moisture	11.9	11.5
Ash	6.5	5.6
Starch	36.5	40.4
Crude protein	16.1	17.4
Immunoglobulins	ND	1.5

ND = not detected

During the experiment, the piglets huddled closely together when they were at rest. In the control and SDPP group 6 and 7 piglets died during the experiment. The number of the control piglets that died on the various days after weaning were as follows: d8: 1, d11: 1, d12: 3 and d13: 1. In the SDPP group the days and

numbers were: d11: 1, d12: 4, d13: 1 and d14: 1. The difference in total losses between treatment groups was not statistically significant. Only one piglet (in the control group) showed nervous signs before it died, but no signs of OD were seen. Post mortem findings of the piglets that died during the experiment were dilated and hyperaemic small intestines with watery contents and swollen mesenteric lymph nodes. In both groups, the majority of the piglets showed greyish, watery diarrhoea some days before they died. Based on the clinical signs and post mortem findings it was concluded that pneumonia was the cause of death of the piglet in the control group that died on d 8.

Daily feed intake per treatment group is presented in Figure 1. Feed intake in the SDPP group was higher than in the control group and there was no feed intake depression after infection unlike in the control group. Daily feed intake could only be determined per pen so that statistical analysis could not be performed.

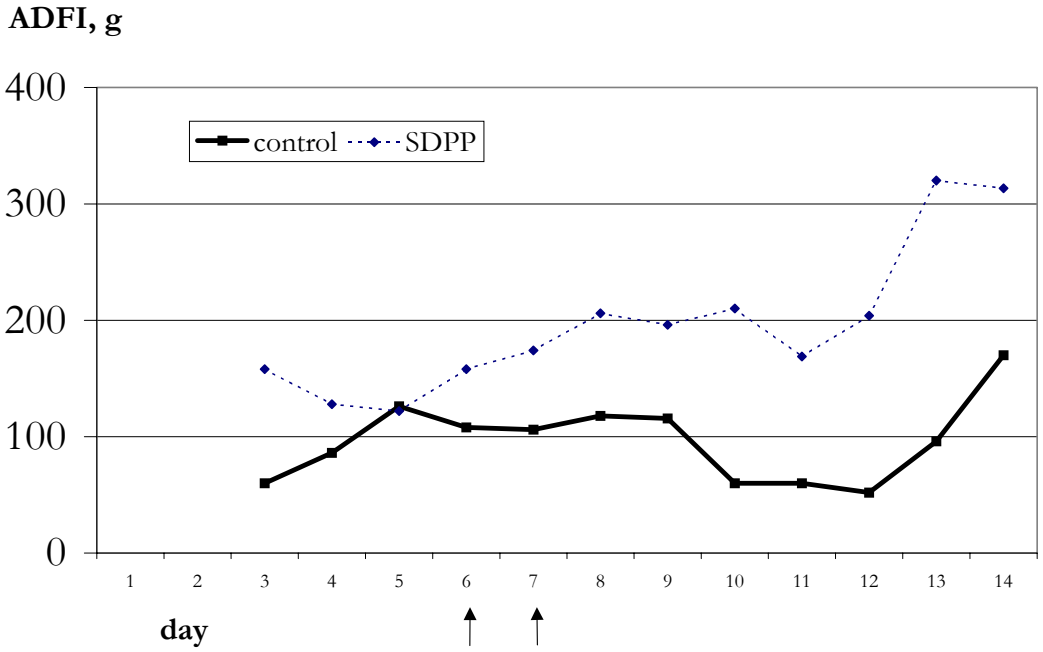

Fig 1: Average daily feed intake per dietary treatment. The piglets were weaned on d 0, with-held from feed during d 1 and 2 and challenged on both d 6 and 7 (indicated by arrows).

The ADG was significantly higher in the SDPP group, both before and after infection (Table 3). From days 7-11, the post-infection period during which most piglets still were alive, SDPP-fed piglets gained weight whereas the control piglets continued to lose weight.

Table 3.

Daily weight gain (g) in piglets fed either a control diet or a diet containing SDPP and challenged with *E. coli*.

	Control diet		SDPP diet		
		n		n	SEM
Days[1] 1-6	-46[a]	10	-1[b]	10	13.3
Days 6-11	-24[a]	9	74[b]	10	22.1
Days 6-14	-30	4	63	4	31.2
Days 1-14	-47[a]	4	42[b]	4	22.2

The piglets were weaned on d 0, withheld from feed during d 1 and 2 and challenged on both d 6 and 7.

Faecal scores during days 8-11 were more favourable in the SDPP-diet fed piglets than in the controls (Fig 2), but the difference for each separate day was not significant. Based on repeated measurement analysis, the SDPP-diet fed piglets tended to have better faecal scores than the control piglets (P = 0.09).

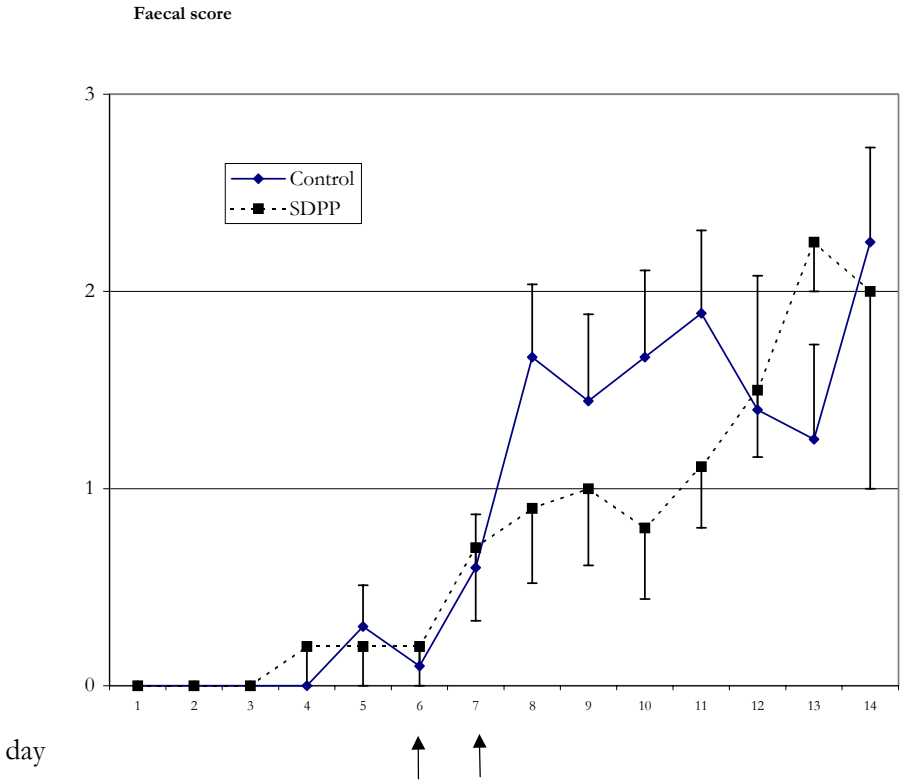

Fig 2: Faecal scores (means ± SEM) during the experimental period. Faecal scores were based on the following scale: 0 = normal, solid faeces; 1 = soft, looser than normal faeces, 2 = diarrhoeal faeces and 3 = liquid, severe diarrhoeal faeces. See also legend to Fig. 1.

Condition scores were more favourable for SDPP-diet fed piglets than the control piglets on days 11-14, but the difference for each separate day was not significant (Fig 3). When a repeated measurement analysis was applied on the data for days 11-14, the SDPP-diet fed piglets were found to display a significantly better condition score than the piglets fed the control diet ($P = 0.03$).

Rectal temperature measurements are presented in Figure 4. On days 6, 7, 8 and 9 SDPP-fed piglets had significantly higher rectal temperatures than the control piglets. The faecal excretion of *E. coli* O139:K82 only showed a significant diet effect on day 14. On the other days there were no diet-induced differences in the numbers excreted (Fig 5). The *E. coli* O139:K82 counts for different sites of the intestine collected post mortem either after spontaneous death or after euthanasia showed no significant differences between the treatment groups (Table 4). Bacterial counts, especially for jejunum contents, were higher in the piglets that died spontaneously than in those euthanised on day 15.

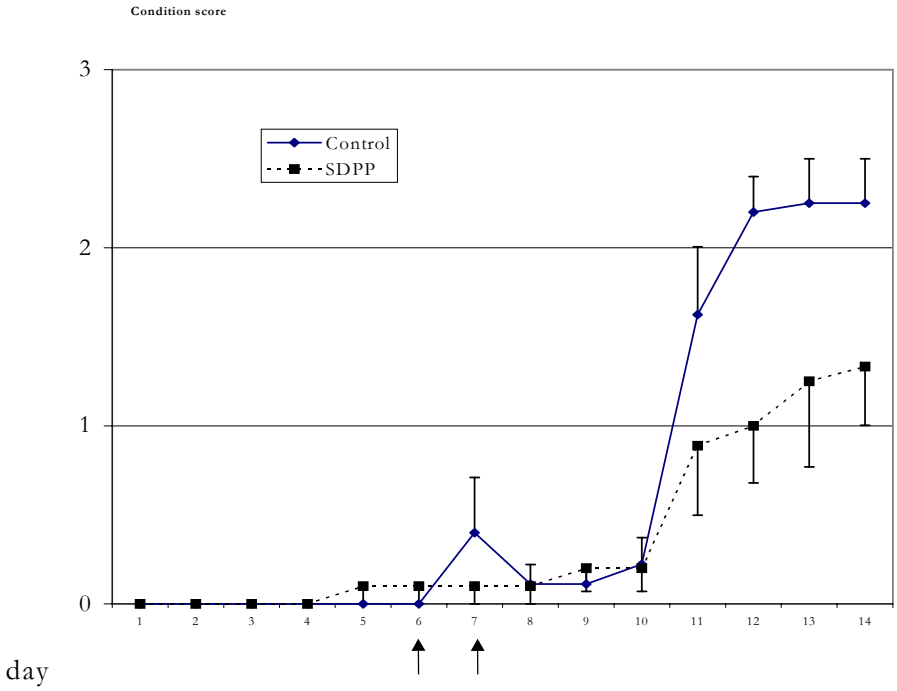

Fig 3: Condition scores (means ± SEM) during the experimental period. Condition scores were based on a scale of which the extreme values are described as: 0 = good condition (healthy appearance, short hair, shiny skin), 3 = poor condition (unhealthy appearance, long hair, pale and dull skin). See also legend to Fig. 1.

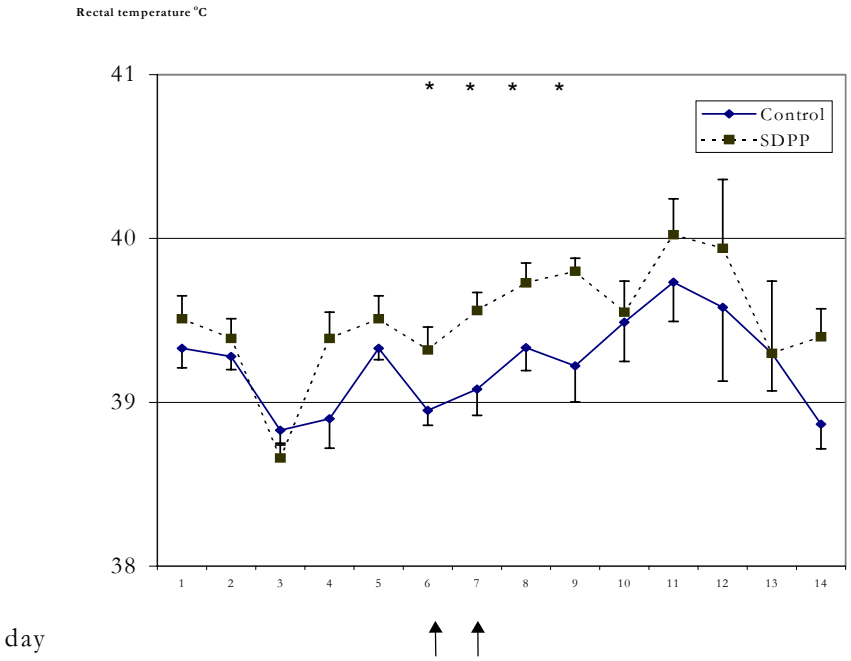

Fig 4: Rectal temperatures (means ± SEM) of the piglets during the experimental period. ★ = significant difference ($P < 0.05$). See also legend to Fig. 1.

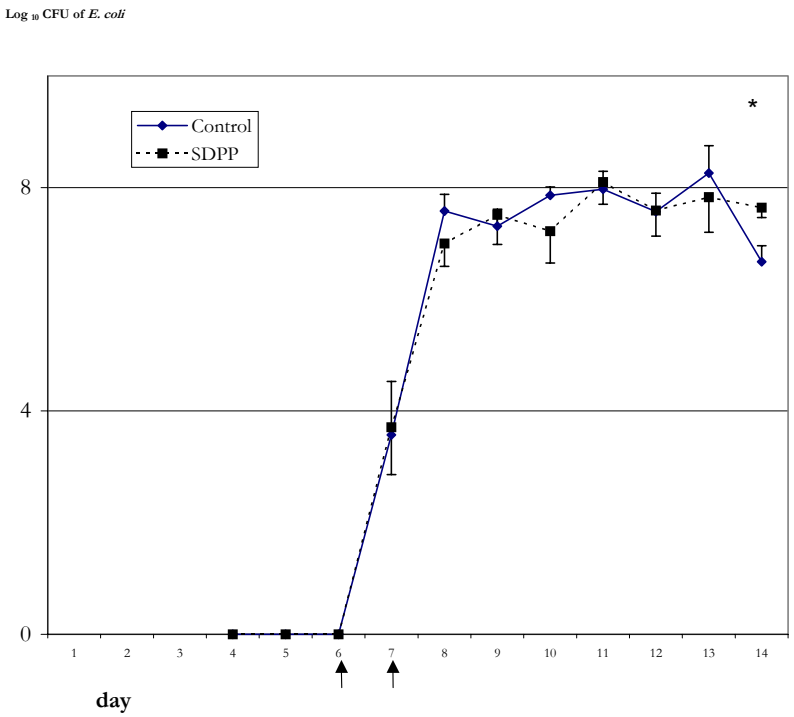

Fig 5: Faecal *E. coli* O139:K82 counts (means ± SEM) during the experimental period. ★ = significant difference ($P < 0.05$). See also legend to Fig. 1.

Table 4.

E. coli O139:K82 counts ($\log_{10}$ CFU/g intestinal contents) for different sites of the gastro-intestinal tract in piglets fed either a control diet or a diet containing SDPP and challenged with *E. coli*.

	Control diet		SDPP diet		
		n		n	SEM
Spontaneously died					
Jejunum	8.70	5	8.30	7	0.26
Caecum	7.81	6	7.55	7	0.40
Rectum	8.27	6	8.51	6	0.32
Euthanised on day 15					
Jejunum	2.06	4	2.88	3	1.08
Caecum	4.48	4	6.12	3	0.75
Rectum	4.90	4	6.89	3	1.15

See footnote to Table 3.
n = number of piglets

Discussion

This study shows that SDPP instead of soybean meal plus whey powder in the diet of weaned piglets challenged with *E. coli* had beneficial effects on feed intake, weight gain and condition score. The immunoglobulins in the SDPP diet (Table 2) may have played a role in the SDPP-induced improvement of health and growth performance in the present experiment. There is evidence for beneficial effects of immunoglobulins of plasma origin orally administered to new-born, colostrum-deprived piglets, but for weaned piglets there is no such information (Van Dijk et al. 2001a). In any event, the immunoglobulins present in the SDPP diet did not affect intestinal proliferation and faecal excretion of the *E. coli* strain the piglets were challenged with.

The experimental model of PWD produced clear intestinal colonisation by the challenge *E. coli* and produced diarrhoea. From the clinical findings and the post mortem examinations it was concluded that piglets died because of the diarrhoea. No apparent signs of OD were seen, except for in one piglet that showed nervous signs before it died. In our challenge model, mortality (65 %) was substantially higher than that seen normally in the field (2 % or more according to Nabuurs et al. 1993). In practice, there are often antimicrobial growth promoters in the diet and medication is started immediately after the initial signs of an outbreak of PWD or OD. *E. coli* counts in the jejunum, caecum and rectum collected post mortem were higher in piglets that died during the experiment than in piglets that were euthanised on day 15. Possibly, this difference is caused by a rapid post mortem bacterial proliferation. Linear regression analysis revealed that there were no statistically significant relationships between faecal or condition scores and either *E. coli* counts in faeces or intestinal contents. This is in accordance with the findings of Nabuurs et al. (1993), who found that in PWD field cases, the onset of diarrhoea did not invariably coincide with the beginning of shedding of a particular ETEC. The faecal excretion pattern of the *E. coli* strain in this experiment (Fig 5) showed a rapid increase in the first two days after infection followed by a plateau. The *E. coli* counts of the rectum contents from the piglets that were euthanised on day 15 were much lower than those in faeces on day 14. Nabuurs et al. (1993) found that in clinical PWD cases ETEC strains appeared in the faeces for a period of five to seven days after which they disappeared. In a challenge experiment, Nollet et al. (1999) found a rapid increase in faecal ETEEC counts followed by a plateau for about 10 days and then a gradual decrease in faecal excretion of the strain challenged with. It would appear that it takes at least 10 days after infection for the pathogen to be quenched. There was no systematic effect of dietary SDPP on the excretion pattern. Nollet et al. (1999) did find a reduction in faecal ETEEC excretions induced by feeding non-immune SDPP. However, those authors used a 3-6 times higher level of SDPP than we did in our challenge experiment.

The finding that after challenge there was no pronounced rise in rectal temperature agrees with the statement of Van Beers-Schreurs et al. (1992) that in cases of PWD rectal temperature is normal. An explanation for the lower rectal temperature in the control piglets, when compared to the SDPP-fed piglets, may lie in their lower metabolic rate due to their lower ADFI and ADG. The depressed feed consumption after infection in the control piglets is in accordance with natural cases of PWD (Van Beers-Schreurs et al. 1992).

The substantially higher ADFI and ADG in the SDPP group, when compared to the control group, corroborate the literature (Van Dijk et al. 2001a). The higher feed intake by the SDPP-fed piglets on day 3 (Fig 1) could be caused by their higher initial weight. However, the feed intake / body weight ratio also was higher for the SDPP-fed piglets. The higher ADFI on day 3 in the SDPP-fed piglets can be explained by a superior palatability of the SDPP diet (Ermer et al. 1994). It is interesting to note that in earlier experiments with SDPP feeding on the same experimental farm with normal piglets, i.e. non-fasted, non-cold stressed, non-colistin pre-treated and non-infected piglets (Van Dijk et al. 2001b, c), the response of ADFI and ADG was either absent or less pronounced than in the present experiment. This supports the common suggestion (Van Dijk et al. 2001a) that dietary SDPP has a greater stimulatory effect on ADFI and ADG when the piglets are in less favourable circumstances. On swine farms with sub-optimal conditions and an infection pressure by pathogenic *E. coli*, a marked positive effect of non-immune SDPP on ADG and ADFI might be expected. The positive effect of SDPP may be extended to later periods of fattening when SDPP is not included in the diet.

In this experiment, the feeding of non-immune SDPP at an economically acceptable percentage in the diet could not prevent piglet losses caused by the challenge with pathogenic *E. coli*. Moreover, SDPP feeding could not reduce the colonisation and excretion of a pathogenic *E. coli*. SDPP-fed piglets had a more favourable faecal score and had a healthier appearance than did control piglets, which is in accordance with the findings of Gatnau (1990) and Van der Peet-Schwering and Binnendijk (1995). Based on this experiment it can be suggested that on swine farms with a history of PWD, the addition of non-immune SDPP to the weaner piglets' diet at a level of 8% may improve post-weaning growth performance. It should be noted, however, that the design of the present experiment without non-infected control and SDPP groups does not allow a solid conclusion as to SDPP providing protection against *E. coli*. Further research is needed to reveal optimal dietary inclusion levels of SDPP and to identify more effective preparations of SDPP.

Acknowledgements

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CHAPTER 6

GENERAL DISCUSSION

The aim of the research described in this thesis was to learn more about the mode of action of the growth and health promoting properties of spray-dried animal plasma (SDAP) in diets for weaned piglets. In a meta analysis (Chapter 2) it was calculated from 68 comparisons between SDAP containing diets and control diets that the SDAP-induced change in average daily gain (ADG) and average daily feed intake (ADFI) in the first two weeks after weaning was +27 % and +25 %, respectively. There are no other feed ingredients or additives, that have such large effects. For comparison, the growth promoting effect of anti microbial growth promoters (AMGP) in piglets was estimated to be in the range of +4% (Visek 1978) to +16% (Kamphues 1999). In two experiments, less diarrhoea was found in SDAP-fed piglets (Gatnau 1990, Van der Peet-Schwering and Binnendijk 1995). Knowledge about the mode of action of the unique feed ingredient SDAP may help to optimise its application from a point of view of effectiveness and economics. Moreover, bioactive components present in SDAP may be isolated from other, less expensive raw materials. Concepts that evolved from this research project may extend to human nutrition and health as piglets may serve as a model for humans as to gastro-intestinal function (Moughan et al. 1992).

The application of SDAP in weaned piglets' diets has been studied from the late eighties. During more recent years experiments have been carried out to unravel its mode of action, this research being reviewed in Chapter 2. Most experiments with SDAP in weaned piglets that were published in the scientific literature, were conducted in Northern America rather than Northern Europe. An important conclusion from the literature review was that the positive effect of SDAP on ADG and ADFI was much more pronounced in the first than in the second week after weaning. This observation may indicate that SDAP contains certain growth factors that stimulate the functional development of the neonatal intestine which would be analogous to milkborne growth factors (Odle et al. 1996). Immunoglobulins (Ig) could be the bioactive component of SDAP. The hypothesis was formulated in that dietary SDAP would induce changes in intestinal morphology, mitotic activity and function (Chapters 4.1 and 4.2). Another possibility is that the gastric pH is relatively high in the first week after weaning, leading to a decreased proteolysis of Ig by pepsin, which is optimally active at an acidic pH (Cranwell et al. 1976, Reilly et al. 1997). Although SDAP has proven to be an excellent protein source in weaned piglets' diets, its amino acids digestibility

is low relative to casein. This may be explained by the high content of Ig in SDAP, because Ig are rather resistant to digestion in the intestine (Reilly et al. 1997).

Health promoting aspects of SDAP only had been demonstrated in two experiments (Gatnau 1990, Van der Peet-Schwering and Binnendijk 1995). Unfortunately, health parameters were not registered in other published experiments. As a consequence, there was not much documented evidence for health promoting properties of SDAP. However, it can be speculated that the SDAP-induced increase in feed intake in the first week after weaning will prevent, at least partly, the villus atrophy that is generally seen in this period. This preventive effect would relate to the concept of luminal nutrition (Pluske et al. 1997). A reduction in villus atrophy after weaning will lead to less loss of digestive and absorptive capacity of the intestine and hence less post-weaning diarrhoea (Pluske et al. 1997). Moreover, the SDAP-induced increase in feed intake in the first week after weaning could lead to a decreased paracellular permeability (Spreeuwenberg et al. 2001), which may prevent bacterial toxins in the lumen to pass the gut wall. The literature review showed that SDAP has a more pronounced growth-promoting effect under conditions where piglets have a low base line growth performance. This observation may indicate that SDAP has health improving properties because low growth performance is associated with a higher degree of clinical or subclinical disease (Elbers 1991, Williams et al. 1997). Indeed, Coffey and Cromwell (1995) and Bergström et al. (1997) suggested that SDAP has a more pronounced effect under conditions with high infection pressure.

The literature review revealed that the mode of action of SDAP was unclear. However, the meta analysis did provide directions to unravel the mode of action of SDAP. In the studies described in this thesis mostly spray dried porcine plasma (SDPP) was used because the literature review showed that porcine plasma has greater beneficial effects than bovine plasma. The following studies were performed.

- Two experiments with SDPP were carried out under typical Northern-European conditions (Chapter 3.1) to learn if piglets' growth performance and health would be also improved under these conditions.
- An attempt was made to find out if there would be an interaction of SDAP with anti microbial growth promoters (AMGP) (Chapter 3.2). The information obtained would provide clues as to the question whether or not the mode of action of SDAP is comparable to that of AMGP. Another goal was to find out if SDAP could be an alternative for AMGP in weaned piglets' diets. In Chapter 3.2, the interaction of SDAP with diet composition also is described.
- SDPP was fed to piglets before weaning (Chapter 3.3). It was speculated that SDPP would induce a considerable improvement in daily gain during lactation because piglets' growth performance potency during this period is not reached.
- To test the hypothesis that SDPP would have trophic or protective properties on the small intestinal mucosa, hereby preventing post weaning villus atrophy,

two experiments were conducted (Chapter 4.1 and 4.2). In those experiments, the effect of SDPP was investigated on post weaning villus length, crypt depth, enterocyt mitotic activity and brush-border disaccharidase activity.

- To test if the growth performance and health improving properties of SDPP could be explained by actions on the gastro-intestinal microflora (normal and pathogenic bacteria) four experiments were conducted. The influence of SDPP was tested on selected intestinal microflora of weaned piglets *in vitro* and *in vivo* (Chapter 5.1). Moreover, an experiment was conducted in which piglets that were fed diets with or without SDPP were challenged with a pathogenic *E. coli* (Chapter 5.2).

Below, the results of the experiments are discussed.

1. Zootechnical results.

Four experiments were conducted, comprising in total 6 comparisons between weanling piglets diets that either contained relatively low levels of SDPP or control protein sources (Chapters 3.1 and 3.2). Low levels of SDPP in the diets (4 and 3%) were used because higher inclusion levels would not be economic and because the meta analysis (Chapter 2) indicated that with low levels considerable effects could be expected. In 3 out of the 6 SDPP-control comparisons, no difference in post weaning growth performance was found. In the other 3 cases, significant SDPP-induced improvements in ADG were seen, the increases being 7, 8 and 18% for the first three weeks after weaning. The magnitude of the SDPP effects on growth performance was not as high as that found in most experiments conducted in the USA. Possibly, the high hygiene status of the research stations used may have caused the observed, relatively small effects. It has been suggested that SDPP has a more pronounced effect under conditions with high infection pressure (Coffey and Cromwell 1995, Bergström et al. 1997). Indeed, in the challenge experiment (Chapter 5.2) when piglets were kept under sub-optimal conditions and high infection pressure, the SDPP-induced increase in ADFI and ADG was more pronounced than in the feeding trials described in Chapter 3. However, the literature review indicates that higher responses can be expected when using higher inclusion levels of SDPP in the diet than those used in the experiments described here.

In Chapter 3.2 an experiment is described with the objective to determine a possible interaction between diet complexity (high versus low value ingredients in a complex and simple diet, respectively) and SDAP in diets without AMGP. There were significant interactions between SDAP and diet composition: SDAP-induced effects on growth performance were most pronounced for a complex versus a simple diet.

The objective of the second experiment described in Chapter 3.2 was to determine a possible interaction between the inclusion of SDAP and AMGP. SDAP tended ($P < 0.1$) to have more effect on ADG and ADFI for diets without AMGP. It can be concluded from this experiment that the inclusion of SDAP in weaning diets has a positive effect on growth performance, especially when diets without AMGP are fed. This could point at SDAP as an alternative for AMGP, that may be banned in the near future (Health Council 1998).

Chapter 3.3 describes the effect of the addition of SDPP to creep feed offered to piglets during the lactation period. During the pre-weaning period there were no significant effects of SDPP on daily gain. During the first week after weaning, the piglets that had been fed the SDPP diet before weaning, had a significantly higher ADFI than did the piglets that were fed a control diet before weaning. During the fourth week after weaning, the piglets given the creep feed with SDPP before weaning had a significantly higher ADG and lower FCR than the piglets that were fed a control creep feed. It was concluded that SDPP in creep feed can have positive carry-over effects on post weaning growth performance. Especially the finding that ADFI in the first week after weaning is stimulated by SDAP in creep feed can be regarded as positive. In practice, feed intake during the first week after weaning often is low, while increasing the feed intake in this period will diminish small intestinal villus atrophy and associated disease problems (Pluske et al. 1997) and may prevent that the small intestinal epithelial barrier function is compromised (Spreeuwenberg et al. 2001).

Based on the meta analysis (Chapter 2) and the experiments, the following conclusions can be drawn.

- The positive effect of SDAP on ADG and ADFI is much more pronounced in the first than the second week after weaning.
- Baseline growth rate is an important determinant of the effect of SDAP on ADG, with high baseline growth rate being associated with small effects of SDAP.
- SDPP can have positive effects on piglets growth performance under Northern European conditions and the magnitude of these effects will be greater with higher diet inclusion levels of SDPP and on farms with sub-optimal hygienic conditions.
- When using complex diets with high quality feed ingredients, the addition of SDAP can have positive effects on growth performance, that are more pronounced than when using simple diets.
- The positive effects of SDAP on growth performance are more pronounced for diets without AMGP than for diets with AMGP.
- SDAP in creep feeds does not have positive effects on growth performance during lactation, but it can have positive carry-over effects into the weeks thereafter.

2. Intestinal morphology and function

Two experiments were conducted to investigate the influence of SDAP on intestinal morphology and function. In the first experiment 24 day old weaned piglets were used (Chapter 4.1). There were no significant effects of SDPP on villus length and crypt depth. On average, there was less mitotic activity of small intestinal enterocytes in the SDPP-fed piglets than in those fed casein on days 4 and 7 after weaning. Because less mitotic activity leads to less immature enterocytes, this may provide a mechanism for the reported beneficial effects of SDPP on growth performance and health. In the second experiment (Chapter 4.2) the piglets were weaned earlier (18 days of age) because it was speculated that the effects of SDPP would be more pronounced as villus atrophy is more severe in early weaned piglets. Moreover, a lower, more practical inclusion level of SDPP in the diet (8% in stead of 15%) was used. There were no significant effects of dietary SDPP on small intestinal villus length, crypt depth and enterocyt mitotic activity. This indicates that SDPP has no trophic effect on the small intestinal mucosa and that it does not protect against the damaging effect on the small intestinal villi that is associated with the process of weaning. However, it must be noted that these experiments were conducted under research station conditions, that are more optimal than in practice. There was no effect of SDPP on lactase, sucrase or maltase specific activities that are a measure of the digestive function of the small intestine. It can be concluded that SDPP versus casein has no effect on small intestinal morphology and disaccharidase activities in early-weaned piglets kept under low infection pressure. The fact that in the first experiment an effect on enterocyt mitotic activity was found whereas in the second experiment it was not, may relate to the SDPP inclusion level in the diet being too low in the second experiment. In both experiments casein was used as a control protein source. Casein is generally considered to be a high value protein source for piglets. As a control protein for SDPP, casein may not enhance the contrast, but it does exclude possible confounding effects on small intestinal morphology because, unlike plant protein sources, casein would be expected not to damage the intestinal epithelium (Hall and Byrne 1989). This reasoning implies that SDPP could have a trophic or protective effect on small intestinal villi when it is incorporated in standard rations. In experiments described here, there were no differences in feed intake between SDPP and control diet fed piglets. On practical farms however, a SDPP- induced increase in feed intake can be expected, which will lead to less villus atrophy, because an increase in feed intake would by itself increase villus height (Kelly et al. 1991, Pluske et al. 1996, Van Beers et al. 1998).

The two experiments also revealed that the process of weaning is associated with a decrease in enterocyte mitotic activity at day 2 after weaning, followed by an increase at day 7. This fits into the concept of hyper-regeneration following villus atrophy. An increased mitotic activity results in more immature enterocytes (Wild

and Murray 1992), leading to an impaired digestive and absorptive function (Dauncey et al. 1983, Smith 1984, Smith 1985, Wild and Murray 1992) and increased sensitivity to bacterial toxins and increased toxin migration through the enterocyte membrane (Mezoff et al 1991, Chu and Walker 1993, Nabuurs et al. 1994). As suggested by Hampson (1986) and Nabuurs et al. (1994), these effects explain the increased susceptibility of the piglet to diarrhoea, edema disease and growth depression in the post-weaning period.

The following can be concluded.

- SDAP at a high inclusion level in the diet (15%) reduces small intestinal enterocyt mitotic activity, leading to less immature enterocytes and hence a better function and health of the intestine.
- SDAP has no direct effect on villus height or crypt depth, so it does not protect against villus atrophy that is associated with the process of weaning. In practice an indirect villus atrophy preventive effect, mediated by a higher feed intake after weaning, may be expected.

3. Intestinal microflora and health.

In the feeding experiments (Chapter 3) only at certain time intervals a SDPP-induced better faecal score and better condition score was recorded, but further no consistent effects on the scores were found. This lack of effect of SDPP can be explained by the optimal hygienic and health conditions of the research facilities used. Under infected conditions however (Chapter 5.2), there were clear positive effects of SDPP on faecal and condition scores.

The interaction between AMGP and SDPP as to the effect on post weaning growth performance that is described in Chapter 3.2 could imply, that an influence on the intestinal microflora is involved in the mode of action of SDPP. In Chapter 5.1, the effect of SDPP on intestinal microflora was investigated. There were no clear bacterial effects of SDPP. It would thus appear that these experiments exclude that the beneficial effects of dietary SDPP on piglets post weaning growth performance and health are caused by antimicrobial properties of SDPP via the mechanism of nutrient sparing in favour of the host. However, when interpreting the data of the experiments, one must consider that the intestinal microbial ecosystem is very complex. Mackie et al. (1999) estimated that the human colon contains bacteria belonging to approximately 400 different species. Therefore it can not be excluded that SDPP has an effect on bacterial species, other than those investigated in the experiments described in Chapter 5.1.

In Chapter 5.2, an experiment is described in which the effect of dietary SDPP was determined on clinical response in weaned piglets challenged with a pathogenic *E. coli* O139K82. On basis of the literature, as described in Chapter 2, it was speculated that SDPP would reduce disease in the challenge model, as

mediated by protection of the intestine by immunoglobulins or inhibition of binding of pathogenic *E. coli* to the brush borders by glycoproteins. The ADFI and ADG were substantially higher in the SDPP group than in the control group. SDPP-fed piglets generally had a more favourable faecal score and a healthier appearance than did the control piglets. However, piglet losses and the faecal excretion of *E. coli* O139K82 were similar for control and test piglets and there were no diet effects on the *E. coli* O139K82 counts at different sites of the intestine. Nollet et al. (1999) conducted a comparable challenge experiment and demonstrated that the feeding of SDPP did reduce the excretion of pathogenic *E. coli* with faeces. However, Nollet et al. (1999) studied the effects of daily intakes of SDPP as high as 45 and 90 g per piglet. These intakes are extremely high, may have caused non-specific effects, and are not cost-effective.

To further investigate whether the mode of action of SDPP is associated with an effect on the intestinal microflora, experiments with germ-free piglets are of interest.

The following can be concluded.

- On swine farms with a history of post weaning diarrhoea, the addition of SDPP to the weaner piglets' diet can contribute to prevention of the disease and may improve post-weaning growth performance.

4. Immunoglobulins as component of SDPP.

Coffey and Cromwell (1995) have proposed that SDPP may enhance piglet performance by improving immunocompetence through Ig present in SDPP. The Ig would prevent viruses and bacteria from damaging the gut wall, resulting in a more functional intestinal wall. Dritz et al. (1996) have hypothesised that the Ig fraction decreases exposure of the immune system to antigens, leading to decreased production of inflammatory cytokines and, in turn, to increased feed intake. This is supported by recent data that point at a reduction in intestinal inflammation in weanling piglets fed diets with SDPP (Jiang et al. 2000). Further, there is only indirect evidence for these ideas. Immunoglobulins derived from processed blood and orally administered to colostrum-deprived newborn piglets have beneficial effects on health and performance (Drew and Owen 1988, Gatnau 1990, Gomez et al. 1998). Extrapolating these effects to non-colostrum-deprived weanling piglets is not justified, but it is clear that Ig in SDAP can have an effect on piglet health. The response to SDPP is higher than that to SDBP (Chapter 2) suggesting that a specific Ig effect could be involved.

Various researchers conducted experiments to identify the fraction of SDPP that improves piglet performance (Gatnau et al. 1995, Owen et al. 1995, Pierce et al. 1995, Weaver et al. 1995). Unfortunately, these experiments have only been described in abstract form and no details are given about the method of isolating

the various SDPP fractions and their purity. The overall conclusion was that the beneficial effects of SDPP are associated with the IgG fraction.

Positive results in animals and humans have been obtained after oral administration of Ig from other sources than SDAP. Ig can serve as a prophylaxis or treatment against gastro-intestinal disease. The suggested underlying mechanism is that the antibodies could passively immunise the gastro-intestinal tract and protect it from infection by bacteria or viruses and that they could inactivate toxins (Reilly et al. 1997). Guardino et al. (1994) demonstrated that oral administration of non-specific Ig prepared from human serum is associated with a faster recovery from acute gastro-enteritis in children. Hilpert et al. (1987) successfully treated children suffering from gastro-enteritis with rotavirus-specific Ig isolated from bovine colostrum. Korhonen et al. (2000) summarise experiments in which non-specific Ig isolated from bovine colostrum or whey has been used in calves. The authors also summarise experiments in which colostrum is used from cows hyperimmunised prepartum with vaccines against certain pathogens and they state that the antibacterial activity of milk can be increased by systemic immunisation of cows against a defined pathogen. Other experiments with orally administered antibodies to human infants and in HIV-infected patients have been reviewed by Reilly et al. (1997).

There also is evidence that laying hens are good producers of specific antibodies. After immunisation, specific antibodies are transported to the egg yolk from which the antibodies can be isolated and purified (Carlander et al. 2000). Yolk antibodies from hens that have been immunised against certain pathogens have been shown to prevent bacterial and viral infections in rabbits and piglets (Yoloyama et al. 1992, O'Farrelly et al. 1992.) The literature summarised above supports the hypothesis that the effect of SDPP is based on its Ig moiety. The mean Ig content of 7 SDPP samples used in experiments described in this thesis was 20.3 % ± 4.2 SD.

In two experiments, specific effects of SDPP could only be demonstrated in the proximal gastro-intestinal tract (Chapters 4.2 and 5.1). Similar effects have been reported by Zylsta et al. (1994) for orally administered Epidermal Growth Factor (EGF). The authors suggest poor survival of EGF in the GI tract. Possibly, this is also the case for immunoglobulins or other bioactive components in SDAP. This is supported by Reilly et al. (1997), who state that orally administered Ig are degraded by proteolytic enzymes to F(ab')2, Fab and Fc fragments. They also state that these fragments retain some of their neutralising activity locally in the gastrointestinal tract and that studies in humans have shown that 10-50 % of orally administered Ig can be recovered in the faeces. Further research is necessary to increase the stability of orally administered antibodies against proteolysis as suggested by Reilly et al. (1997). Possibly, plasma can be also applied in humans like bovine colostrum products that are already applied in human nutrition. Preliminary research

(Lembcke et al. 1997) revealed that a spray dried bovine serum product is well accepted as a dietary ingredient by children and that no adverse effects are found.

5. Concluding remarks

From this thesis it can be concluded that dietary SDPP has positive effects on piglets' post weaning growth performance and health. These effects are more pronounced when piglets are kept under sub-optimal conditions and/or high infection pressure and in piglets fed diets without AMGP. This thesis indicates that in relation to the mode of action of SDAP, effects on the gastro-intestinal microflora are involved. However, it seems that these effects comprise an influence on pathogenic bacteria rather than a general anti-bacterial effect leading to nutrient sparing for the host as described for AMGP. Future research is necessary to further clarify the mode of action and to optimise the application of SDAP in disease prevention with emphasis on increasing the Ig content and its stability in the GI tract. Perspectives for successful application can be expected especially in immunocompromized mammals, like e.g. neonates and in cases where antibiotic treatment is not possible, in the case of a ban on antibiotics or when dealing with multiresistant bacteria.

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SUMMARY

Spray-dried animal plasma (SDAP) is a by-product of slaughter plants. The plasma obtained from slaughtered pigs or ruminants is spray-dried and used for the production of both human foodstuffs and animal feeds. It has been demonstrated in many experiments described in the literature that SDAP in weaning piglets' diets can have considerable positive effects on piglets' growth performance. In a meta analysis (**Chapter 2**), it was calculated from 68 comparisons between SDAP containing diets and control diets that the SDAP-induced change in average daily gain (ADG) and average daily feed intake (ADFI) in the first two weeks after weaning was +26.8 % and +24.5 %, respectively. Two experiments described in the literature demonstrated that dietary SDAP can reduce post-weaning diarrhoea.

The aim of the research described in this thesis was to learn more about the mode of action of the growth and health promoting properties of SDAP in diets for weaned piglets. In the studies described in this thesis mostly spray dried porcine plasma (SDPP) was used.

- Two experiments with SDPP were carried out under typical Northern-European conditions (**Chapter 3.1**) to learn if piglets' growth performance and health would be also improved under these conditions.
- An attempt was made to find out if there would be an interaction of SDAP with anti microbial growth promoters (AMGP) (**Chapter 3.2**). The information obtained would provide clues as to the question whether or not the mode of action of SDAP is comparable to that of AMGP. Another goal was to find out if SDAP could be an alternative for AMGP in weaned piglets' diets. In **Chapter 3.2**, the interaction of SDAP with diet composition also is described.
- SDPP was fed to piglets before weaning (**Chapter 3.3**). It was speculated that SDPP would induce a considerable improvement in daily gain during lactation because piglets' growth performance potency during this period is not reached.
- To test the hypothesis that SDPP would have trophic or protective properties on the small intestinal mucosa, hereby preventing post weaning villus atrophy, two experiments were conducted (**Chapter 4.1 and 4.2**). In those experiments, the effect of SDPP was investigated on post weaning villus length, crypt depth, enterocyt mitotic activity and brush-border disaccharidase activity.
- To test if the growth performance and health improving properties of SDPP could be explained by actions on the gastro-intestinal microflora (normal and pathogenic bacteria) four experiments were conducted. The influence of SDPP was tested on selected intestinal microflora of weaned piglets *in vitro* and *in vivo* (**Chapter 5.1**). Moreover, an experiment was conducted in which piglets that

were fed diets with or without SDPP were challenged with a pathogenic *E. coli* (**Chapter 5.2**).

Below, the results of the experiments are summarised.

Zootechnical results

Four experiments were conducted, comprising in total 6 comparisons between weanling piglets' diets that either contained relatively low levels of SDPP or control protein sources (Chapters 3.1 and 3.2). In 3 out of the 6 SDPP-control comparisons, no difference in post weaning growth performance was found. In the other 3 cases, significant SDPP-induced improvements in ADG were seen, the increases being 7, 8 and 18% for the first three weeks after weaning.

In Chapter 3.2 an experiment is described with the objective to determine a possible interaction between diet complexity (high versus low value ingredients in a complex and simple diet, respectively) and SDAP in diets without AMGP. There were significant interactions between SDAP and diet composition: SDAP-induced effects on growth performance were most pronounced for a complex versus a simple diet. The objective of the second experiment described in Chapter 3.2 was to determine a possible interaction between the inclusion of SDAP and AMGP. SDAP tended ($P < 0.1$) to have more effect on ADG and ADFI for diets without AMGP. It can be concluded from this experiment that the inclusion of SDAP in weaning diets has a positive effect on growth performance, especially when diets without AMGP are fed.

Chapter 3.3 describes the effect of the addition of SDPP to creep feed offered to piglets during the lactation period. During the pre-weaning period there were no significant effects of SDPP on daily gain. During the first week after weaning, the piglets that had been fed the SDPP diet before weaning, had a significantly higher ADFI than did the piglets that were fed a control diet before weaning. During the fourth week after weaning, the piglets given the creep feed with SDPP before weaning had a significantly higher ADG and lower feed conversion ratio (FCR) than the piglets that were fed a control creep feed. It was concluded that SDPP in creep feed can have positive carry-over effects on post weaning growth performance.

Based on the meta analysis (Chapter 2) and the experiments, the following conclusions can be drawn.

- The positive effect of SDAP on ADG and ADFI is much more pronounced in the first than the second week after weaning.
- Baseline growth rate is an important determinant of the effect of SDAP on ADG, with high baseline growth rate being associated with small effects of SDAP.

- SDPP can have positive effects on piglets growth performance under Northern European conditions and the magnitude of these effects will be greater with higher diet inclusion levels of SDPP and on farms with sub-optimal hygienic conditions.
- When using complex diets with high quality feed ingredients, the addition of SDAP can have positive effects on growth performance, that are more pronounced than when using simple diets.
- The positive effects of SDAP on growth performance are more pronounced for diets without AMGP than for diets with AMGP.
- SDAP in creep feeds does not have positive effects on growth performance during lactation, but it can have positive carry-over effects into the weeks thereafter.

Intestinal morphology and function

Two experiments were conducted to investigate the influence of SDAP on intestinal morphology and function. In the first experiment 24 day old weaned piglets were used (Chapter 4.1). There were no significant effects of SDPP on villus length and crypt depth. On average, there was less mitotic activity of small intestinal enterocytes in the SDPP-fed piglets than in those fed casein on days 4 and 7 after weaning. Because less mitotic activity leads to less immature enterocytes, this may provide a mechanism for the reported beneficial effects of SDPP on growth performance and health. In the second experiment (Chapter 4.2) the piglets were weaned earlier (18 days of age) because it was speculated that the effects of SDPP would be more pronounced as villus atrophy is more severe in early-weaned piglets. Moreover, a lower, more practical inclusion level of SDPP in the diet (8% in stead of 15%) was used. There were no significant effects of dietary SDPP on small intestinal villus length, crypt depth and enterocyt mitotic activity. This indicates that SDPP has no trophic effect on the small intestinal mucosa and that it does not protect against the damaging effect on the small intestinal villi that is associated with the process of weaning. There was no effect of SDPP on lactase, sucrase or maltase specific activities that are a measure of the digestive function of the small intestine. It can be concluded that SDPP versus casein has no effect on small intestinal morphology and disaccharidase activities in early-weaned piglets kept under low infection pressure.

The two experiments also revealed that the process of weaning is associated with a decrease in enterocyte mitotic activity at day 2 after weaning, followed by an increase at day 7. This fits into the concept of hyper-regeneration following villus atrophy. An increased mitotic activity results in more immature enterocytes, leading to an impaired digestive and absorptive function and increased sensitivity to bacterial toxins and increased toxin migration through the enterocyte

membrane. These effects may explain the increased susceptibility of the piglet to diarrhoea, edema disease and growth depression in the post-weaning period.
The following can be concluded.

- SDPP at a high inclusion level in the diet (15%) reduces small intestinal enterocyt mitotic activity, leading to less immature enterocytes and hence a better function and health of the intestine.
- SDPP has no direct effect on villus height or crypt depth, so it does not protect against villus atrophy that is associated with the process of weaning. In practice an indirect villus atrophy preventive effect, mediated by a higher feed intake after weaning, may be expected.

Intestinal microflora and health

In Chapter 5.1, the effect of SDPP on intestinal microflora was investigated. There were no clear bacterial effects of SDPP. It would thus appear that these experiments exclude that the beneficial effects of dietary SDPP on piglets' post weaning growth performance and health are caused by antimicrobial properties of SDPP via the mechanism of nutrient sparing in favour of the host. However, it can not be excluded that SDPP has an effect on bacterial species, other than those investigated in the experiments described in Chapter 5.1.

In Chapter 5.2, an experiment is described in which the effect of dietary SDPP was determined on clinical response in weaned piglets challenged with a pathogenic *E. coli* O139K82. The ADFI and ADG were substantially higher in the SDPP group than in the control group. SDPP-fed piglets generally had a more favourable faecal score and a healthier appearance than did the control piglets. However, piglets losses and the faecal excretion of *E. coli* O139K82 were similar for control and test piglets and there were no diet effects on the *E. coli* O139K82 counts at different sites of the intestine. It was concluded that on swine farms with a history of post weaning diarrhoea, the addition of SDPP to the weaner piglets' diet can contribute to prevention of the disease and that it may improve post-weaning growth performance.

From this thesis it can be concluded that dietary SDPP has positive effects on piglets' post weaning growth performance and health. These effects are more pronounced when piglets are kept under sub-optimal conditions and/or high infection pressure and in piglets fed diets without AMGP. This thesis indicates that in relation to the mode of action of SDAP, effects on the gastro-intestinal microflora are involved. However, it seems that these effects comprise an influence on pathogenic bacteria rather than a general anti-bacterial effect leading to nutrient sparing for the host as described for AMGP. Future research is necessary to further clarify the mode of action and to optimise the application of SDAP in

gastro-intestinal disease prevention with emphasis on increasing the Ig content and its stability in the GI tract. Perspectives for successful application can be expected especially in immunocompromized mammals, like e.g. neonates and in cases where antibiotic treatment is not possible, in the case of a ban on antibiotics or when dealing with multiresistant bacteria.

SAMENVATTING

Gesproeidroogd bloedplasma, spray-dried animal plasma (SDAP), is een bijproduct van slachterijen. Het plasma dat verkregen is van geslachte varkens of runderen wordt gesproeidroogd en kan gebruikt worden voor de productie van zowel humane voedingsmiddelen als diervoeders. In veel experimenten die beschreven staan in de literatuur is aangetoond dat SDAP in voeders voor gespeende biggen aanzienlijke positieve effecten kan hebben op de voeropname en groei na spenen. In een meta analyse (**Hoofdstuk 2**), werd uit 68 vergelijkingen tussen SDAP bevattende voeders en controle voeders geconcludeerd dat de door SDAP veroorzaakte groei- en voeropnameverbetering respectievelijk 26.8 % en 24.5 % bedroegen. Twee experimenten uit de literatuur gaven aan dat SDAP in speenvoer speendiarree kan verminderen.

Het doel van het onderzoek dat in dit proefschrift staat beschreven was om meer te weten te komen over het werkingsmechanisme van de groei- en gezondheidsbevorderende eigenschappen van SDAP in speenvoeders. In de proeven die in dit proefschrift staan beschreven is bloedplasma van varkens gebruikt (spray-dried porcine plasma, SDPP).

- Er werden twee experimenten met SDPP onder typisch Noord-Europese omstandigheden uitgevoerd (**Hoofdstuk 3.1**) om te bepalen of onder deze omstandigheden de groei en gezondheid van biggen eveneens verbeterd zouden worden. Alle experimenten die in de wetenschappelijke literatuur beschreven staan zijn namelijk uitgevoerd in de USA, waar de houderij-omstandigheden en voersamenstellingen aanzienlijk verschillen van die in Noord-Europa.
- Er werd een experiment uitgevoerd om the bepalen of er een interactie bestaat tussen SDAP en antimicrobiële groeibevorderaars (AMGB) (**Hoofdstuk 3.2**). AMGB zijn bepaalde stoffen met een antibacteriële groeibevorderende werking die toegelaten zijn om in diervoer te verwerken. Ze zullen waarschijnlijk om maatschappelijke en medische redenen verboden worden in de EU. Met uit dit experiment verkregen informatie zou meer inzicht verkregen kunnen worden over de vraag of het werkingsmechanisme van SDAP vergelijkbaar is met dat van AMGB. Een ander doel was te bepalen of SDAP een alternatief voor AMGB in biggenvoer zou kunnen zijn. In **Hoofdstuk 3.2** werd ook de interactie van SDAP met voersamenstelling beschreven.
- SDPP werd gevoerd aan biggen vóór spenen (**Hoofdstuk 3.3**). De achterliggende gedachte was dat SDPP zou resulteren in een aanzienlijke verbetering van de groei gedurende de lactatieperiode. Normaliter wordt namelijk de groeipotentie van de biggen gedurende deze periode niet gehaald, waarbij de melkproductie van de zeug de limiterende factor is.

- Om de hypothese te testen, dat SDPP trofische of beschermende invloeden zou hebben op de mucosa van de dunne darm, waarmee vlokatrofie voorkomen zou worden, werden twee experimenten met gespeende biggen verricht (**Hoofdstuk 4.1 and 4.2**). In deze experimenten werd het effect van SDPP onderzocht op villus lengte, crypt diepte, mitose activiteit van enterocyten en de activiteiten van brush-border disaccharidasen.
- Om te testen of de groei- en gezondheidsbevorderende eigenschappen van SDPP verklaard zouden kunnen worden uit invloeden op de gastro-intestinale microflora (normale en pathogene bacteriën) werden 4 experimenten uitgevoerd. De invloed van SDPP op bepaalde bacteriestammen werd *in vitro* en *in vivo* bepaald (**Hoofdstuk 5.1**). Bovendien werd er een experiment uitgevoerd waarin biggen, die voeders met of zonder SDPP kregen, besmet werden met een pathogene *E. coli* (**Hoofdstuk 5.2**).

Hieronder zijn de resultaten van de experimenten samengevat.

Zootechnische resultaten

Er werden 4 experimenten uitgevoerd, die in totaal 6 vergelijkingen omvatten tussen speenvoeders met ofwel relatief lage SDPP gehaltes, ofwel controle eiwitbronnen (**Hoofdstukken 3.1 and 3.2**). Bij 3 van de 6 vergelijkingen tussen SDPP en de controle, werden er geen verschillen in de groei na spenen gevonden. In de andere 3 gevallen werden in de SDPP-groepen significante groeiverbeteringen gedurende de eerste 3 weken na spenen gevonden van 7, 8 en 18%.

In **Hoofdstuk 3.2** wordt een experiment beschreven dat als doel had om een mogelijke interactie te bepalen tussen SDAP en de mate van complexiteit van voersamenstelling in voeders zonder AMGB. Er werden significante interacties gevonden tussen SDAP en voercomplexiteit: SDAP had het grootste groeistimulerende effect in een complexe voersamenstelling (met hoogkwalitatieve grondstoffen) in vergelijking met een simpele voersamenstelling (met grondstoffen van een lagere kwaliteit). Het doel van het tweede experiment dat in **Hoofdstuk 3.2** beschreven wordt was het vaststellen van een mogelijke interactie tussen SDAP en AMGB in speenvoer. Er was een tendens ($P < 0,1$) dat SDAP meer effect op groei en voeropname had in voeders zonder AMGB. Uit deze experimenten kan geconcludeerd worden dat SDAP in speenvoeders positieve effecten op groei heeft, in het bijzonder in voeders zonder AMGB.

Hoofdstuk 3.3 beschrijft het effect van toevoeging van SDPP aan voer dat tijdens de lactatieperiode aangeboden werd aan biggen. Gedurende de periode vóór spenen waren er geen significante effecten van SDPP op de groei van de biggen. In de eerste week na spenen hadden de biggen die vóór spenen het SDPP-voer gehad hadden, een significant hogere voeropname dan de biggen die het controle voer

gehad hadden vóór spenen. In de vierde week na spenen hadden de biggen die vóór spenen het SDPP-voer gehad hadden een significant betere groei en voederconversie dan de biggen die het controle voer gehad hadden vóór spenen. Er werd geconcludeerd dat SDPP in voeders die gevoerd worden vóór spenen positieve effecten kan hebben op de groei na spenen.
Op basis van de meta analyse (**Hoofdstuk 2**) en deze experimenten, werden de volgende conclusies getrokken.

- Het positieve effect van SDAP op groei en voeropname is veel groter in de eerste week na spenen dan in de tweede week na spenen.
- De groei die biggen realiseren op een bepaald bedrijf bepaalt in grote mate het effect van SDAP op groei, waarbij een hoge groei geassocieerd is met geringe effecten en vice versa.
- SDPP kan positieve effecten hebben op de groei na spenen onder Noord-Europese omstandigheden waarbij deze effecten groter zullen zijn bij hogere toevoegingpercentages van SDPP aan het voer en op bedrijven met een suboptimale hygiëne.
- Wanneer complexe voeders gevoerd worden met hoogkwalitatieve grondstoffen kan toevoeging van SDPP positieve effecten op groei hebben, die groter zijn dan wanneer simpele voeders gevoerd worden met grondstoffen van mindere kwaliteit.
- SDAP heeft een groter effect op groei in voeders zonder AMGB dan in voeders met AMGB.
- SDAP heeft in voeders die gevoerd worden vóór spenen geen positieve effecten op groei gedurende die periode, maar het kan positieve effecten hebben op de periode na spenen.

Darmmorfologie en functie

Er werden twee experimenten uitgevoerd om de invloed van SDPP op de morfologie en functie van de dunne darm te onderzoeken. Uit beide experimenten bleek dat het speenproces samengaat met een afname van mitose activiteit van de enterocyten op dag 2 na spenen, gevolgd door een toename hiervan op dag 7 na spenen. Dit past in het concept van hyperregeratieve vlokatrofie. Een verhoogde mitose activiteit leidt tot meer immature enterocyten, hetgeen tot gevolg heeft dat de verterings- en absorptiecapaciteit van de darm afneemt, dat de darm gevoeliger is voor bacteriële toxinen en dat deze toxinen beter door de mucosa kunnen migreren. Deze effecten kunnen een verklaring zijn van de verhoogde gevoeligheid van biggen na spenen voor diarree, oedeemziekte en groeivertraging.

In het eerste experiment werden biggen van 24 dagen oud gebruikt (**Hoofdstuk 4.1**). Er waren geen significante effecten van SDPP op vlokhoogte en cryptdiepte. Op dag 4 en dag 7 na spenen was er minder mitose activiteit van

enterocyten bij biggen die SDPP in hun voer gehad hadden dan bij biggen die caseïne in hun voer gehad hadden. Omdat een geringere mitose activiteit leidt tot minder immature enterocyten, kan dit een verklarend mechanisme zijn voor de positieve effecten van SDPP op groei en gezondheid. In het tweede experiment (Hoofdstuk 4.2) werden de biggen eerder gespeend (18 dagen leeftijd) omdat verwacht werd dat de effecten van SDPP dan groter zouden zijn aangezien vlokatrofie ernstiger is bij vroeg gespeende biggen. Bovendien werd er een lager, meer praktijkconform SDPP gehalte in het voer gehanteerd (8% in plaats van 15%). Er waren geen significante effecten van SDPP op vlokhoogte, cryptdiepte en mitose activiteit. Dit geeft aan dat SDPP geen trofische effecten op de mucosa van de dunne darm heeft en dat het geen bescherming geeft tegen de met spenen geassocieerde vlokatrofie. Er was geen effect van SDPP op de activiteiten van lactase, sucrase of maltase, die beschouwd worden als een maat voor de verteringsfunctie van de darm.

Het volgende kan geconcludeerd worden:

- Een hoog gehalte van SDPP in speenvoer (15%) verlaagt de mitose activiteit van enterocyten van de dunne darm hetgeen tot minder immature enterocyten leidt met als gevolg een betere darm functie en –gezondheid.
- SDPP heeft geen rechtstreeks effect op vlokhoogte of cryptdiepte waardoor het niet beschermt tegen de vlokatrofie die optreedt bij het speenproces.

Darmflora en gezondheid

In **Hoofdstuk 5.1** werd het effect van SDPP op de microflora van het maagdarmkanaal onderzocht. Er werden geen duidelijke antibacteriële effecten van SDPP gevonden. Hieruit kan geconcludeerd worden dat de positieve effecten van SDPP op de groei en gezondheid van gespeende biggen niet verklaard kunnen worden uit antimicrobiële eigenschappen van SDPP. Het kan echter niet uitgesloten worden dat SDPP invloed heeft op andere bacterie soorten, die niet in deze experimenten onderzocht werden.

In **Hoofdstuk 5.2** wordt een experiment beschreven waarin het effect van SDPP in voer bepaald werd op de klinische respons van biggen die geïnfecteerd werden met een pathogene *E. coli* O139K82. De groei en voeropname waren aanzienlijk hoger in de SDPP groep dan in de controle groep. De SDPP biggen hadden een betere mestconsistentie en zagen er gezonder uit dan de controle biggen. Er waren echter geen verschillen in uitval en in de aantallen *E. coli* O139K82 in de faeces en in de chymus van verschillende delen van het maag-darmkanaal. Er werd geconcludeerd dat SDPP een bijdrage kan leveren aan de preventie van speendiarree.

Uit dit proefschrift kan geconcludeerd worden dat SDPP positieve effecten heeft op de groei en gezondheid van gespeende biggen. Deze effecten zijn groter bij biggen die gehouden worden onder suboptimale omstandigheden en/of onder hoge infectiedruk en in voeders zonder AMGB. Dit proefschrift geeft aan dat bij het werkingsmechanisme van SDPP effecten op de gastro-intestinale microflora zijn betrokken. Het lijkt er echter op dat deze effecten meer een invloed op pathogene bacteriën betreffen dan dat het een algemeen antibacterieel effect betreft dat leidt tot nutriëntbesparing ten gunste van de gastheer, zoals dit beschreven is voor AMGB. Vervolgonderzoek is noodzakelijk om het werkingsmechanisme van SDAP verder op te helderen en de toepassing te optimaliseren met betrekking tot ziekte-preventie, waarbij de nadruk dient te liggen op verhoging van het immunoglobulinegehalte en het verhogen van de stabiliteit hiervan in de darm. Perspectieven voor succesvolle toepassing kunnen vooral verwacht worden op darmniveau bij zoogdieren met een niet optimaal functionerend immuunsysteem, zoals neonaten en in gevallen waarbij antibioticumbehandeling niet mogelijk is zoals bijvoorbeeld bij een verbod op AMGB of bij multiresistente bacteriën.

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CURRICULUM VITAE

Albert is in 1960 in Emmen geboren en op zijn vijfde verhuisd naar Terneuzen. Op zijn achttiende vertrok hij naar Utrecht voor de studie Diergeneeskunde waar hij zijn bul in 1987 behaalde. In Oldenzaal werd Albert practicus zoals hij het zich als student voorstelde, de allround dierenarts die alle dieren behandelt. Na enkele jaren realiseerde hij zich dat het teveel aan routinehandelingen bij de landbouwhuisdieren en ook bij de gezelschapsdieren hem ging tegenstaan. Van Dijk wilde zijn horizon verbreden en hij besefte dat zijn idee uit de studententijd als Dokter Vlimmen niet overeen kwam met zijn werkelijke gedachte over het beroep dierenarts zoals hij dat wilde invullen. Hij was altijd geïnteresseerd geweest in organisatie en de overgang naar het bedrijfsleven was niet onlogisch. Albert van Dijk werd aangenomen als Stafmedewerker Varkenshouderij bij Cehave in Veghel en in die functie gaf hij technische ondersteuning aan de buitendienst in de sector Varkenshouderij. Sinds drie jaar heeft Albert van Dijk meer mogelijkheden voor onderzoek en voor zijn promotie. Hij is nu werkzaam als nutritionist bij de onlangs gefuseerde Cehave-Landbouwbelang. Hij brengt een deel van zijn tijd door op de proefboerderij Laverdonk en heeft een bureau bij C.C.L.-Nutricontrol. (CCL = Coöperatief Centraal Laboratorium). Hij is Specialist Veterinaire Diervoeding en ondersteunt de verantwoordelijke voor de voersamenstelling.

Bovenstaande is een gedeelte van de tekst: 'Quo Vadis, Veterinair?' uit het blad 'Veterinaire Praktijk Informatie' van AUV, november 2000.