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Increasing weaning age of piglets from 4 to 7 weeks reduces stress, increases post-weaning feed intake but does not improve intestinal functionality

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This study tested the hypothesis that late weaning and the availability of creep feed during the suckling period compared with early weaning, improves feed intake, decreases stress and improves the integrity of the intestinal tract. In this study with 160 piglets of 16 litters, late weaning at 7 weeks of age was compared with early weaning at 4 weeks, with or without creep feeding during the suckling period, on post-weaning feed intake, plasma cortisol (as an indicator of stress) and plasma intestinal fatty acid binding protein (I-FABP; a marker for mild intestinal injury) concentrations, intestinal morphology, intestinal (macro)molecular permeability and intestinal fluid absorption as indicators of small intestinal integrity. Post-weaning feed intake was similar in piglets weaned at 4 weeks and offered creep feed or not, but higher ($P < 0.001$) in piglets weaned at 7 weeks with a higher ($P < 0.05$) intake for piglets offered creep feed compared with piglets from whom creep feed was witheld. Plasma cortisol response at the day of weaning was lower in piglets weaned at 7 weeks compared with piglets weaned at 4 weeks, and creep feed did not affect cortisol concentration. Plasma I-FABP concentration was not affected by the age of weaning and creep feeding. Intestinal (macro)molecular permeability was not affected by the age of weaning and creep feeding. Both in uninfected and enterotoxigenic Escherichia coli*-infected small intestinal segments net fluid absorption was not affected by the age of weaning or creep feeding. Creep feeding, but not the age of weaning, resulted in higher villi and increased crypt depth. In conclusion, weaning at 7 weeks of age in combination with creep feeding improves post-weaning feed intake and reduces weaning stress but does not improve functional characteristics of the small intestinal mucosa.*

Keywords: pig, weaning age, creep feed, stress, intestine

Implications

Abrupt weaning at 4 weeks of age or earlier is a considerable stressor for piglets resulting in impaired intestinal integrity and growth depression. To overcome these problems weaning at an older age seems self-evident. In this study, it is shown that stress at the time of weaning is lower and feed intake is improved when piglets are weaned at 7 weeks and fed creep feed. Intestinal integrity, however, is not or only partly improved by weaning at 7 weeks. Therefore, increasing weaning age to 7 weeks does not improve intestinal functionality.

Introduction

Under natural conditions weaning is a gradual process and the average weaning time of piglets is more than 17 weeks (Jensen and Recén, 1989). In modern pig husbandry, weaning occurs abruptly by separating the piglets from the sow at a much younger age of 7 to 35 days (within the EU a minimum of 28 days; Colson *et al*., 2006). Besides the abrupt separation from the sow and consequently a change in diet, additional stressors, such as changes in the physical (moving to an unfamiliar pen or barn) and social (mixing with unfamiliar pen mates) environments have to be faced (Weary *et al*., 2008). As a consequence, feed intake during the first days post-weaning does not meet the energy requirement and the integrity of the intestinal tract is impaired (van Beers-Schreurs and Bruininx, 2002). Therefore, post-weaning problems such as growth depression and the occurrence of diarrhoea are still widespread in the modern pig husbandry (van Beers-Schreurs and Bruininx, 2002).

To overcome these problems weaning at an older age seems self-evident (Everts *et al*., 1999). For modern pig production this is not appropriate in view of economic sustainability, but the

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situation may be different when relevant characteristics, such as health and welfare of the piglets, are also valued (Everts *et al*., 1999). In organic pig husbandry, weaning age is 6 to 7 weeks based on the purported beneficial impact on health and welfare (Hovi *et al*., 2003).

In a pilot experiment, in which piglets were weaned at an age of 4 or 10 weeks and not given creep feed, weaning at 4 weeks resulted in a limited food intake and an arrest of growth, while after weaning at 10 weeks food intake started directly and body weight (BW) gain was not impaired. However, in piglets weaned at 10 weeks and not given creep feed, a growth reduction at 6 to 7 weeks of age occurred. Therefore, it is hypothesized that weaning at an age of 7 weeks causes less stress, improves feed intake after weaning and, as a consequence, the integrity of the intestinal tract will be maintained.

The objectives of this study were to compare weaning at an age of 4 weeks with weaning at an age of 7 weeks with or without creep feeding with respect to feed intake and BW gain, cortisol levels as an indicator of stress and intestinal morphology, fluid absorption in non-infected and enterotoxigenic *Escherichia coli* (ETEC)-infected intestinal segments and intestinal (macro)molecular permeability as indicators of small intestinal integrity.

Material and methods

All procedures involving animal handling and testing were reviewed and approved by the ASG-Lelystad Animal Care and Ethics Committee (Lelystad, The Netherlands).

Animals and treatments

Four groups of four sows (Dalland) each were weighed and housed in individual farrowing crates in the Lelystad Centre for Pig Husbandry. After farrowing a few piglets were cross-fostered to ensure that each sow nursed 10 piglets and that each group contained 40 piglets. Piglets had access to nipple waters, were checked twice daily by animal care technicians and were weighed once a week. At 2 days of age each of the 160 piglets were subjected to a back test to measure the resistant score (Hessing *et al*., 1994). The classification of the resistant score (44% resistant, 48% intermediate and 8% non-resistant piglets) was used for mixing at the day of weaning. From 12 days of age onwards piglets from two groups of sows were offered creep feed in plastic creep feeders. On 10, 6 and 3 days before weaning faecal samples were taken and observed for colour (Bruininx *et al*., 2002). Piglets of two groups of sows (one offered creep feed and one not creep fed) were weaned at 4 weeks of age (4+ and 4−, respectively) and piglets of the two other groups of sows at 7 weeks (7+ and 7−, respectively). The creep feed offered was a pelleted barley-based starter diet with 1% chromic oxide (Table 1). This diet was appropriate for the piglets that were weaned at 7 weeks of age. As it was not the aim to study different creep feeds at different weaning ages, the same diet was used for the piglets weaned at 4 weeks although creep feed intake in 4 weeks weaned piglets is known to be affected by diet composition (Fraser *et al*., 1994).

At weaning (day 0) the piglets were removed from the sows and the sows and the piglets were weighed. For each treatment, the piglets were mixed and housed in four crates containing 10 piglets each. In each crate two or three piglets of one sow were housed. In the four crates the number of male and female piglets, the average BW of the piglets and the average resistant score of the piglets were equalized. After weaning all piglets were offered the same diet as the creep feed.

Feed intake per crate was recorded daily. BW was measured on the day of weaning, on days 5, 7 and 11 after weaning and at 16 weeks of age. Blood samples were taken by vena puncture the day before weaning, on the day of weaning 2 h after mixing and on days 1, 4, 7 and 11 after weaning. On days −1, 3 and 6, seven piglets from each group were selected, taking into account that from the creep feed-offered groups only those piglets were selected that showed that they had eaten creep feed. The piglets were transported from the farm to the institute. Taking into account that the piglets were not mixed, they were housed in boxes and fasted overnight. On days 0, 4 and 7, three piglets of each group were anaesthetized with a mixture of nitrous oxide, oxygen and sevoflurane administered through a facemask and a midline abdominal ventral incision was made. Intestinal samples were taken to measure (macro)-molecular transport and villous height and crypt depth. Samples to measure (macro)molecular transport and villous height and crypt depth were also taken from the other four piglets, but in these piglets this was combined with measuring the intestinal fluid absorption.

Table 1 *Ingredients and calculated chemical analysis of the creep feed*

Ingredients (g/kg)		Calculated analysis (g/kg)	
Barley	464.2	Dry matter	89.1
Maize heat treated	100.0	Ash	55.9
Whey powder	100.0	CP	188.0
Soya beans extracted	75.0	Crude fibre	33.2
Fish meal	50.0	Starch	341.5
Milk powder skimmed	50.0	Sugar	116.7
Wheat middlings	40.0	Digestible phosphor	4.0
Maize starch	30.0	Digestible lysine	10.3
Linseed	25.0	Digestible amino acids	150.0
Potato protein	20.0	Net energy (MJ/kg)	9.8
Vegetable oil	19.0		
Limestone	8.5		
Mono calcium phosphate	7.0		
Premix	6.0		
$NaHCO_3$	2.0		
Salt	1.0		
L-lysine-HCl	1.4		
DL-methionine	0.7		
L-threonine	0.1		
L-tryptophan	0.1		

Cortisol and intestinal fatty acid binding protein (I-FABP)
On days −1, 0, 1, 4, 7 and 11 blood samples were taken; on day 11 in each group blood samples were taken from the 19 remaining animals. For sampling, special care was taken to minimize pain and stress. In order to perform the blood samplings as softly and quickly as possible, a trained personnel gently cradled the piglet in its arms in dorsal recumbence position while remaining close to the pen. A blood sample was obtained via jugular vein puncture. The whole procedure, including handling and sampling, took <2 min. The blood samples were immediately chilled on ice. The blood was centrifuged for 10 min at 2000 × **g** and aliquots of plasma were stored at −70°C for later analysis of cortisol and I-FABP, a marker for mild intestinal injury (Niewold *et al.*, 2004). Plasma cortisol concentration was measured using a time-resolved fluoroimmunoassay (Ruis *et al.*, 2001) and plasma I-FABP concentration was measured using a human commercial ELISA test kit (Niewold *et al.*, 2004).

Villous height and crypt depth
On days 0, 4 and 7 from seven piglets of each group five samples of intestinal tissue were taken along the small intestine at 10%, 25%, 50%, 75% and 95% of the length. The tissue was cut on the mesenteric side, pinned with the serosal side to a piece of cork and placed in a formalin solution with the mucosal side down to fix the villi vertically. After fixation, a piece of each sample was embedded in paraffin wax. Each sample was stained with haematoxylin and eosin and the villous height was measured from the tip of villous to the villous–crypt junction and the crypt depth from this junction to the base of the crypt (Nabuurs *et al.*, 1993a).

(Macro)molecular permeability
On days 0, 4 and 7 an intestinal tissue sample from seven piglets was taken from about 50% of the length of the small intestine to measure (macro)molecular transport. It was cut along the mesenteric border and stripped of muscle layers. Flat sheets of tissue free of Payer's patches were mounted in Ussing-type chambers. The exposed area of the intestine was 0.7 cm^2. Both sides of the epithelium were perfused with Ringer solution and gassed with 5% CO_2 and 95% O_2. Solutions were maintained at 37°C with water jackets and circulated (total volume 4.7 ml on each side). Sodium fluorescein isothiocyanate (Na-FITC, 376 Da, Sigma, St. Louis, MO, USA) and horseradish peroxidase (HRP, 40 kD; type VI, Sigma) dissolved in Ringer solution were added mucosally at a final concentration of 10^{-3} and 10^{-5} M, respectively. The small molecule Na-FITC was transported both trans- and paracellularly and the large molecule HRP was transported paracellularly (Verdonk *et al.*, 2007). Serosal samples of 400 μl were taken at 30, 60, 90 and 120 min and were replaced by 400 μl of fresh Ringer solution to keep the volume constant. An arithmetical correction for the dilution of the buffer was applied to calculate the amount of Na-FITC and HRP at the serosal side correctly. In the serosal samples Na-FITC was determined by measuring the fluorescence and HRP was measured enzymatically (Bijlsma *et al.*, 1996).

The apparent permeability coefficient (P_{app}) was determined on the basis of the appearance at the serosal side according to the following equation: $P_{app} = P/(A \times C_0)$, where P_{app} = permeability coefficient from the mucosal to the serosal side (cm/s), P = permeability rate (net passage per unit of time in mol/s), A = exposed intestinal area (cm^2) and C_0 = initial mucosal concentration (mol/ml).

Fluid absorption in non-infected and ETEC-infected intestinal segments
Net intestinal fluid absorption on days 0, 4 and 7 of four piglets of each group was measured in the small intestinal segment perfusion (SISP) test as described before (Nabuurs *et al.*, 1993b). The piglets were tranquillized with azaperone (2 mg/kg BW) and anaesthesia was induced and maintained with sevoflurane and nitrous oxide. The piglets were placed in dorsal recumbence and an incision was made in the abdomen lateral to the linea alba. The abdominal cavity was opened and the first intestinal segment was prepared approximately 40 cm caudal to the ligament of Treitz. A thin cranial tube (inflow) was placed and a wide tube (outflow) was placed about 20 cm distal to the first. Caudal from and adjacent to this tube a 2-cm piece of intestine was removed and cut on the mesenteric side to measure the circumference and for the measurement of villous height and crypt depth. A second segment was prepared in the same way adjacent to the first and these two segments formed a pair (at 10% site). Paired segments were prepared at the 25%, 50%, 75% and 95% sites. Moreover, a 5-cm piece of the intestine was removed to determine the receptor status to bind ETEC (Sellwood *et al.*, 1975).

Fifteen minutes before perfusion started the cranial segment of each pair was injected with 5 ml ETEC (CIDC culture 1000 : O149 : K91 : K88^{ac}; 10^9 CFU/ml phosphate buffered saline (PBS), producing heat-labile toxin and heat-stable toxin b) whereas the caudal segment of each pair was injected with 5 ml PBS (Nabuurs *et al.*, 1993b). Simultaneously, the 10 segments were perfused with 64 ml of the perfusion fluid (saline supplemented with 1 g/l glucose and 1 g/l amino acids to assure bacterial growth; Nabuurs *et al.*, 1993b) over a period of 8 h. The non-absorbed fluid drained freely into the corresponding drainage bottles. At the end of the experiment the fluid remaining in the segments was blown out into the drainage bottles (outflow). The piglets were killed by the injection of sodium pentobarbital (200 mg/kg BW) and the length of the small intestine and the segments was measured. Net fluid absorption in each segment was calculated from the difference between the volume of inflow and the volume of outflow, divided by the surface area (length × circumference) of the segment.

Statistical analysis
Data were subjected to analysis of variance (ANOVA) using the GenStat statistical package for Windows (release 6.1, 6th edition, VSN International Ltd, Oxford, UK). The experimental unit for assessing parameters of feed intake and BW

gain was the crate, and the data were analysed according the following model:

$$Y_{ij} = \mu + W_i + C_j + e_{ij},$$

where μ is overall mean, W_i is the weaning age with $i = 1, 2$; C_j is creep feed with $j = 1, 2$; and e_{ij} is the residual error. The experimental unit for assessing the parameters of (macro)-molecular permeability, villous height and crypt depth and intestinal fluid absorption was the piglet and data were analysed according the following model:

$$Y_{ijk} = \mu + W_i + C_j + D_k + e_{ijk},$$

where μ is overall mean, W_i is weaning age with $i = 1, 2$; C_j is creep feed with $j = 1, 2$; D_k is the day after weaning with $k = 1 \ldots 3$ and e_{ijk} is the residual error. Including the receptor status as covariate in the ANOVA model to analyse intestinal fluid absorption in ETEC-infected segments did not affect the levels of significance. Therefore, for intestinal fluid absorption in ETEC-infected segments the reported estimated means are also the result of ANOVA according to the model described. The experimental unit for assessing blood parameters was piglet and data were analysed as repeated measurements (AREPMEASURES) according to the following model:

$$Y_{ijk} = \mu + W_i + C_j + D_k + e_{ijk},$$

where μ is overall mean, W_i is weaning age with $i = 1, 2$; C_j is creep feed with $j = 1, 2$; D_k is day with $k = 1 \ldots 6$ and e_{ijk} is the residual error, with log transformation of data on cortisol before analysis. The results are given as estimated means.

Results

Feed intake and BW gain

On the basis of the colour of the faeces a limited number of 4+ piglets consumed creep feed before weaning at 4 weeks: 10 and 3 days before weaning only 5% and 41%, respectively, of 4+ piglets consumed creep feed. On 10 and 3 days before weaning at 7 weeks, respectively, 68% and 90% of 7+ piglets consumed creep feed. The day before weaning 4+ piglets consumed 104 ± 88 g/day creep feed and at the same time, 21 days before weaning, 7+ piglets consumed 85 ± 48 g/day. The day before weaning, the intake of creep feed by 7+ piglets was 580 ± 138 g/day. The day after weaning, the feed intake by 4+ piglets was not different compared with 4− piglets (Table 2). Thereafter, in both groups, feed intake increased gradually and was not different for 4− and 4+ piglets. Feed intake after weaning was higher ($P < 0.001$) in 7 weeks than in 4-week-weaned piglets. During 9 days after weaning at 7 weeks, feed intake of 7− piglets was lower ($P < 0.05$) than that of 7+ piglets.

At weaning BW of 4− and 4+ piglets was not different and lower ($P < 0.001$) than in piglets weaned at 7 weeks, with a higher ($P < 0.001$) BW for 7+ compared with 7− piglets (Table 2). In the first 5 days after weaning, BW gain in 4-week-weaned piglets was <150 g/day and lower ($P < 0.001$) than in

Table 2 *Effect of the age of weaning and creep feeding on feed intake, BW and BW gain after weaning*

		Weaning							
		4 weeks Creep feeding		7 weeks Creep feeding			*P*-value		
	Day	−	+	−	+	s.e.d.[3]	W^1	C^2	$W \times C$
Feed intake (g/day)	1	98^a	140^a	383^b	750^c	49.2	***	***	***
	2	205^a	221^a	374^b	656^c	29.7	***	***	***
	3	242^a	248^a	417^b	746^c	22.4	***	***	***
	4	226^a	243^a	529^b	841^c	80.9	***	*	*
	5	249^a	250^a	645^b	1026^c	79.3	***	**	**
	6	274^a	274^a	614^b	1071^c	87.8	***	**	**
	7	315^a	318^a	769^b	1061^c	74.8	***	*	*
	8	354^a	371^a	807^b	1198^c	63.4	***	***	**
	9	438^a	390^a	1040^b	1349^c	49.5	***	**	***
	10	468^a	394^a	996^b	1152^c	65.7	***	ns	*
	11	471^a	416^a	1114^b	1325^c	61.1	***	ns	*
BW (kg)	−12	5.8^a	6.0^a	11.7^b	12.8^c	0.30	***	**	*
	−6	7.5^a	7.9^a	13.1^b	15.1^c	0.33	***	***	***
	0	9.3^a	9.8^a	14.3^b	18.8^c	0.29	***	***	***
BW gain (g/day)	1–5	134	134	454	386	39.2	***	ns	ns
	6–11	348^b	237^a	680^c	846^d	50.5	***	ns	**
	1–11	251^a	191^a	590^b	662^c	28.5	***	ns	**

ns = non-significant.
[1]W = age of weaning.
[2]C = creep feeding.
[3]s.e.d. for the $W \times C$ interaction.
[a–d]Values in the same row not sharing a common superscript are significantly different ($P < 0.05$).
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

7-week-weaned piglets. For 6 to 11 days after weaning there was a weaning age by creep feeding interaction ($P < 0.01$), with for 4-week-weaned piglets offered creep feed a lower BW gain than for 4-week-weaned piglets withheld creep feed and a higher BW gain for 7+ compared with 7− piglets. After weaning BW gain of 7+ piglets was not different compared with the BW gain of these piglets in the last 2 weeks before weaning (681 ± 158 g/day). In 7− piglets BW gain in the first 10 days after weaning was higher than before weaning (197 ± 70 g/day). At 16 weeks of age BW of 7+, 4− and 4+ piglets (69.7 ± 5.7, 69.3 ± 7.9 and 70.3 ± 7.2 kg) did not differ but was lower ($P < 0.05$) for 7− piglets (61.1 ± 6.4 kg). From weaning to 16 weeks of age BW gain of 4+, 4−, 7+ and 7− piglets was on average 753 ± 86, 744 ± 76, 882 ± 106 and 809 ± 150 g/day.

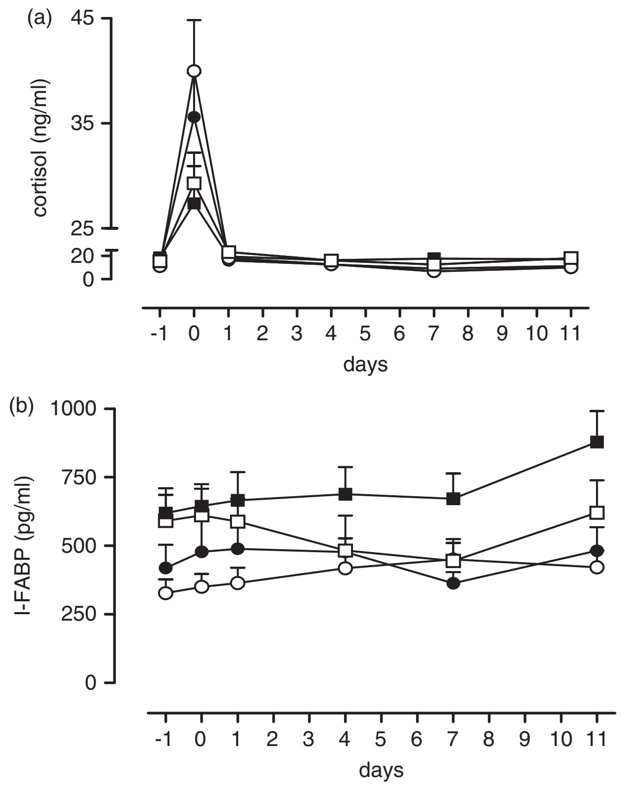

Figure 1 Plasma cortisol (a) and intestinal fatty acid-binding protein (I-FABP) (b) concentrations (mean ± s.e.m.) in piglets weaned at 4 weeks and offered creep (●) or not (○) in the suckling period and weaned at 7 weeks and offered creep feed (■) or not (□) in the suckling period.

Cortisol and I-FABP

There was no effect of creep feeding on plasma cortisol (Figure 1a). There was a weaning age by day interaction ($P < 0.001$). At the day of weaning plasma cortisol was increased and was higher ($P < 0.001$) for the piglets weaned at 4 weeks compared with the piglets weaned at 7 weeks. After weaning, plasma cortisol concentration was lowered in all four groups.

There was no effect of day after weaning, weaning age and creep feeding on plasma I-FABP concentrations (Figure 1b).

Villous height and crypt depth

Both villous height and crypt depth reduced in size from the cranial to the caudal part of the small intestine. On day 4 after weaning villous height was reduced compared with the day of weaning and recovered on day 7, whereas crypt depth was not affected by day after weaning (Table 3). Compared with weaning at 4 weeks, villi were higher after weaning at 7 weeks but this was only significant ($P < 0.05$) at 25% and 50% of the site along the small intestine (data not shown). There was no effect of age of weaning on villous height and crypt depth and creep feeding resulted in higher ($P < 0.01$)

Table 3 *The effect of the age of weaning and creep feeding on intestinal villous height, crypt depth and villous/crypt ratio at days 0, 4 and 7*

		Weaning					P-value						
		4 weeks Creep feeding		7 weeks Creep feeding									
	Day	−	+	−	+	s.e.d.[4]	W[1]	C[2]	D[3]	$W \times C$	$C \times D$	$W \times D$	$W \times C \times D$
Villous height (μm)	0	413[a]	538[f]	499[cdef]	518[def]								
	4	479[bcde]	475[bcd]	428[ab]	466[abcd]								
	7	456[abc]	479[bcde]	519[def]	533[ef]	27.9	ns	**	*	ns	ns	**	*
Crypt depth (μm)	0	104	119	93	101								
	4	109	108	103	116								
	7	109	106	112	118	6.5	ns	*	ns	ns	ns	**	ns
Villous/crypt ratio	0	3.93	4.51	5.34	5.13								
	4	4.37	4.39	4.19	4.33								
	7	4.16	4.51	4.66	4.50	0.21	***	ns	***	*	ns	***	ns

ns = non-significant.
[1] W = age of weaning.
[2] C = creep feeding.
[3] D = day.
[4] s.e.d. for the $W \times C \times D$ interaction.
[a–f] Values not sharing a common superscript are significantly different ($P < 0.05$).
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

villi and increased ($P < 0.05$) crypt depth. Villous/crypt ratio was highest on day 4 and recovered on day 7. Villous/crypt ratio was greater for weaning at 7 weeks compared with 4 weeks ($P < 0.001$).

(Macro)molecular permeability
The mucosal-to-serosal permeability coefficient of Na-FITC was not affected by the time after weaning, the age of weaning or creep feeding (Table 4). The mucosal-to-serosal permeability coefficient of HRP increased with time after weaning. HRP permeability was not affected by the age of weaning and creep feeding.

SISP test: intestinal absorption
The small intestine was longer ($P < 0.001$) when the piglets were offered creep feed (Table 5). After weaning, the length of the small intestine increased with time. All along the small intestine the circumference was greater after weaning at 7 weeks compared with 4 weeks and when the piglets were offered creep feed.

Net fluid absorption was highest at the 75% and 95% site along the small intestine (data not shown). Net fluid absorption was not affected by the age of weaning and there was no difference in net fluid absorption between piglets offered creep feed and piglets withheld creep feed. In all four groups net fluid absorption decreased with time after weaning.

Piglets with receptors to bind ETEC were present in all four groups on every day. The number of 4+, 4−, 7+ and 7− piglets with receptors to bind ETEC was, respectively, 5 (42%), 10 (83%), 5 (42%) and 8 (67%) out of 12 piglets. As in non-infected segments net fluid absorption in ETEC-infected segments was highest at the 75% and 95% site along the small intestine. In all four groups and at all sites along the small intestine net fluid absorption in ETEC-infected segments was lower ($P < 0.001$) than in non-infected segments. Net fluid absorption in ETEC-infected segments was not affected by the age at weaning and creep feeding and therefore not significantly different for the four groups. In all four groups net fluid absorption in ETEC-infected segments decreased with time after weaning.

Sows
The BW of the sows was not different among the four groups at farrowing (246, 236, 221 and 242 kg for 4+, 4−, 7+ and 7− sows, respectively) and at the time of weaning (194, 188, 179 and 187 kg for 4+, 4−, 7+ and 7− sows, respectively) and loss of BW was not different for the four groups. All sows were inseminated 4 to 5 days after weaning and only one sow (weaned at 4 weeks and no creep feed for the piglets) returned to oestrus and had to be inseminated again.

Discussion

Comparing weaning at 4 weeks with weaning at 7 weeks implicates not only comparing an effect of the duration of suckling, but also an effect of a different physiological age. Since a change in duration of suckling is coupled with a change in physiological age, it has to be kept in mind that all measured parameters may be affected both by the duration of the suckling period and physiological age.

Feed intake and BW gain
To facilitate the transition from milk to a solid diet after weaning, creep feeding is often recommended (Hampson and Kidder, 1986; Appleby *et al*., 1991; Bruininx *et al*., 2002). The benefits of creep feeding for post-weaning performance and health, however, are still unclear. The high variability in creep feed intake within and between litters is one of the reasons for the inconsistent effect of creep feeding (Bruininx *et al*., 2002). In our study less than half of the piglets consumed creep feed (in an amount of not more than 100 g/day) 3 days before weaning at 4 weeks. Consequently, BW at weaning and feed intake after weaning were not different between piglets weaned at 4 weeks with or without creep feed, while the higher BW gain in piglets withheld creep feed

Table 4 *The effect of the age of weaning and creep feeding on intestinal permeability coefficients for Na-FITC and HRP at days 0, 4 and 7*

		Weaning											
		4 weeks Creep feeding		7 weeks Creep feeding			*P*-value						
	Day	−	+	−	+	s.e.d.[4]	W^1	C^2	D^3	$W \times C$	$C \times D$	$W \times D$	$W \times C \times D$
Na-FITC (10^{-7} cm/s)	0	18.3	10.0	8.4	7.1								
	4	11.4	11.2	11.3	10.0								
	7	9.9	9.2	7.8	12.8	2.89	ns	ns	ns	ns	ns	ns	ns
HRP (10^{-7} cm/s)	0	6.3	1.7	1.8	2.0								
	4	4.2	3.3	5.2	7.3								
	7	6.9	6.1	3.1	11.7	2.66	ns	ns	*	*	ns	ns	ns

Na-FITC = sodium fluorescein Isothiocyanate; HRP = horseradish peroxidise; ns = non-significant.
[1] *W* = age of weaning.
[2] *C* = creep feeding.
[3] *D* = day.
[4] s.e.d. for the $W \times C \times D$ interaction.
* $P < 0.05$.

Table 5 *The effect of the age of weaning and creep feeding on intestinal length and circumference and intestinal net fluid absorption in uninfected and ETEC-infected state at days 0, 4 and 7*

		Weaning											
		4 weeks Creep feeding		7 weeks Creep feeding			*P*-value						
	Day	−	+	−	+	s.e.d.[4]	W^1	C^2	D^3	$W \times C$	$C \times D$	$W \times D$	$W \times C \times D$
Circumference (cm)	0	2.3^{a}	2.3^{a}	3.1^{d}	3.6^{e}								
	4	2.5^{ab}	2.8^{bc}	3.6^{e}	4.0^{f}								
	7	2.9^{cd}	3.1^{cd}	3.7^{ef}	4.7^{g}	0.15	***	***	***	***	*	ns	*
Length (cm)	0	691	760	900	1061								
	4	764	914	1023	1167								
	7	900	1034	976	1192	73.5	***	***	***	ns	ns	ns	ns
Net fluid absorption uninfected ($\mu l/cm^2$)	0	818	912	576	660								
	4	681	540	557	689								
	7	473	373	408	566	94.5	ns	ns	***	ns	ns	ns	ns
Net fluid absorption ETEC-infected ($\mu l/cm^2$)	0	226	508	272	171								
	4	156	161	58	95								
	7	148	122	130	161	102.2	ns	ns	***	*	ns	**	ns

ETEC-infected = enterotoxigenic *Escherichia coli* infected; ns = non-significant.
[1] W = age of weaning.
[2] C = creep feeding.
[3] D = day.
[4] s.e.d. for the $W \times C \times D$ interaction.
[a–g] Values of the same parameter not sharing a common superscript are significantly different ($P < 0.05$).
* $P < 0.05$; *** $P < 0.001$.

6 to 11 days after weaning most likely was related to gut filling. When a more complex diet is used, instead of a standard starter diet, intake of creep feed by piglets weaned at 4 weeks might have been higher (Fraser *et al*., 1994). In addition, with a more complex creep feed, however, intake in 3- and 4-week-old piglets is <100 g/day and is variable within litters (Fraser *et al*., 1994). The piglets weaned at 7 weeks showed that intake of creep feed became substantial after 4 weeks of age. At the time of weaning at 7 weeks all piglets consumed a substantial amount (on average almost 600 g/day) of creep feed to fulfil their nutrient requirement. As a consequence piglets weaned at 7 weeks and offered creep feed were heavier at the time of weaning and feed intake after weaning was higher compared with piglets from whom creep feed was withheld. Offering creep feed to piglets weaned at 7 weeks of age is necessary as indicated by the BW at 16 weeks of age. Apart from that, BW at 16 weeks of piglets weaned at 7 weeks and offered creep feed is not different from piglets weaned at 4 weeks, as has been shown before for the organic piglets (Andersen *et al*., 2000). Although suckling did not fulfil their nutrient requirement, piglets weaned at 7 weeks without creep feed seemed not to have stripped the sows. At the time of weaning the BW of the sows nursing piglets 7 or 4 weeks was not significantly different nor the weaning-to-oestrus interval. In addition, in organic farming, BW and body condition of sows lactating their piglets for 7 weeks are not different compared with sows lactating 5 weeks (Andersen *et al*., 2000).

Stress

Weaning caused a significant increase in cortisol levels in all piglets. An increase in plasma cortisol at weaning is in accordance with earlier observations (Herskin and Jensen, 2002; van Erp-van der Kooij *et al*., 2003). The increase in cortisol levels in our study is not related to the way in which samples were taken, as samples were taken from all piglets in the same way and an increase in cortisol was also measured in stress-free collected saliva samples (van Erp-van der Kooij *et al*., 2003). As weaning is a known stressor for mammals, such an increase in cortisol at the time of weaning is not surprising (van Erp-van der Kooij *et al*., 2003). The increase in cortisol was not affected by creep feeding but when weaning took place at 7 instead of 4 weeks, the increase in cortisol levels was limited. Although increased cortisol levels may be possibly harmful for the piglets by its immunosuppressive action (van Erp-van der Kooij *et al*., 2003), increased cortisol levels around weaning have also been attributed to an increased synthesis of citrulline from glutamine in enterocytes (Wu *et al*., 2000b) and an enhanced intestinal polyamine synthesis (Wu *et al*., 2000a), which may be of physiological importance for intestinal adaptation.

Small intestinal integrity

As a consequence of the two functions of the small intestine, absorption of nutrients and exclusion of pathogenic compounds (antigens, toxins and bacteria), it is difficult to measure small intestinal integrity (van Beers-Schreurs and Bruininx, 2002). Commonly used indicators of small intestinal integrity are villous height, crypt depth, number of goblet cells, mucus production, permeability, inflammation and brush border enzyme activity (Vente-Spreeuwenberg and Beynen, 2003), whereas the more complex SISP test may also be used as an indicator of small intestinal integrity (van Beers-Schreurs and Bruininx, 2002).

After weaning at 4 and 7 weeks net fluid absorption decreased with time after weaning as described before (Nabuurs *et al*., 1994). When creep feed intake is achieved by intermittent suckling, net fluid absorption improves (Nabuurs *et al*., 1996; Kuller *et al*., 2007). In our study, the net fluid intake was not improved by creep feed, probably as a consequence of the low level of creep feed intake compared with creep feed intake by intermittent suckling (Kuller *et al*., 2004). The reduced net fluid absorption in ETEC-infected segments, especially 4 and 7 days after weaning, represents the greater sensitivity of weaned piglets compared with suckling piglets to ETEC-induced diarrhoea (Nabuurs *et al*., 1994). This study shows that when piglets are weaned at 7 weeks an increased sensitivity for ETEC-induced diarrhoea also exists. This has been shown before for piglets weaned at 6 or 7 weeks and challenged with ETEC, with a longer-lasting ETEC excretion in 4-week-weaned piglets than in 6-week-weaned piglets (Wellock *et al*., 2008; Sørensen *et al*., 2009). That creep feeding did not diminish the reduction in net fluid absorption by ETEC in our study but creep feed intake achieved by intermittent suckling did (Nabuurs *et al*., 1996; Kuller *et al*., 2007), implies that either the amount of creep feed consumed and/or the way of weaning is important for diminished fluid absorption by ETEC after weaning.

The reduction in the size of both villous height and crypt depth from the cranial to the caudal part of the small intestine, the reduction in villous height on day 4 after weaning followed by a recovery on day 7 and the increase in crypt depth on day 7 after weaning correspond with earlier studies (Kelly *et al*., 1991; Nabuurs *et al*., 1993a; Pluske *et al*., 1996b). The higher villi in piglets offered creep feed is consistent with the higher feed intake of these piglets after weaning, as in underfed piglets villous height is decreased (Kelly *et al*., 1991; Pluske *et al*., 1996a; Vente-Spreeuwenberg *et al*., 2004). The increased crypt depth in creep-fed piglets is consistent with a decreased crypt depth on underfeeding (Kelly *et al*., 1991; Verdonk *et al*., 2007), although for underfed piglets also an increased crypt depth has been reported (Pluske *et al*., 1996a). Compared with weaning at 4 weeks of age, weaning at an older age resulted only in higher villi in the first half of the small intestine but not all over the small intestine. This is in agreement with an earlier study showing an increased villous height in the duodenum and the proximal part of the jejunum when weaning took place at an older age and an increased crypt depth all over the small intestine (Gu *et al*., 2002). Recently, wider villi and deeper crypts were reported for 6-week-weaned piglets compared with 4-week-weaned piglets (Miller *et al*., 2009).

The transepithelial permeability of Na-FITC is not affected by the age of weaning and creep feeding, which corresponds with the fact that post-weaning feed intake does not affect intestinal permeability of small molecules (Verdonk *et al*., 2007). The increased permeability of HRP on day 7 after weaning is not congruent with data of tube-fed weaned piglets in which the permeability of HRP dropped 2 days after weaning and stayed at that level for the following 14 days (Boudry *et al*., 2004), but is in agreement with an increase of permeability of mannitol after weaning as observed before (Spreeuwenberg *et al*., 2001; Verdonk *et al*., 2001). Increases in macromolecular permeability is associated with stress (Kiliaan *et al*., 1998; van Kalkeren, 2002) and underfeeding (Vente-Spreeuwenberg and Beynen, 2003) in rats and piglets. Although underfeeding may occur when piglets are weaned at 4 weeks, this is not the case for creep-fed piglets weaned at 7 weeks. At 7 weeks there is no difference in HRP permeability on the day of weaning between creep-fed and non-creep-fed piglets and after weaning the increase in HRP permeability is higher in creep-fed than in non-creep-fed piglets.

Plasma I-FABP concentration has been suggested as a sensitive marker of (mild) damage to the intestinal mucosa (Niewold *et al*., 2004). Two hours after mixing of the piglets on the day of weaning and on the following days after weaning no significant increased plasma I-FABP concentrations were measured, which is in agreement with a recent finding that plasma I-FABP concentrations were not different for weaned and suckling 3-week-old piglets (Berkeveld *et al*., 2008). This indicates that the process of weaning itself does not result in intestinal injury as occurs during ischaemia. Weaning-associated villous atrophy is believed to be caused primarily by reduced cell division at the base of the villi, while the shedding at the extrusion zone proceeds, ultimately resulting in the shortening of the villi (Berkeveld *et al*., 2008).

Conclusions

When piglets are weaned at 7 instead of 4 weeks of age, stress at the time of weaning is lower, and there is no affect of creep feed on the stress response at the time of weaning. At 7 weeks weaning, creep feed increases post-weaning feed intake but at 4 weeks weaning there is no effect of creep feed on post-weaning feed intake. On the other hand, creep feed improves villous height and crypt depth in piglets weaned at both 7 and 4 weeks of age.

Weaning at 7 *v*. 4 weeks does not affect villous height and crypt depth and functional parameters of intestinal integrity (macromolecular permeability and net fluid absorption in non- and ETEC-infected segments). This indicates that increasing weaning age of piglets from 4 to 7 weeks reduces stress, increases post-weaning feed intake but does not improve intestinal functionality.

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