

**INTERMITTENT SUCKLING  
AND EXTENDED LACTATION:  
IMPROVING ADAPTATION OF PIGLETS  
TO POSTWEANING CHALLENGES**

**TIJDELIJK SPENEN IN COMBINATIE MET EEN VERLENGDE LACTATIE:  
EEN GRADUELE OVERGANG NAAR HET SPENEN VAN BIGGEN  
(met een samenvatting in het Nederlands)**

Proefschrift

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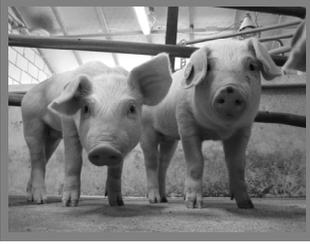
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## ABBREVIATIONS

<b>ACTH</b>	Adrenocorticotrophic hormone
<b>ADG</b>	Average daily gain
<b>ADFI</b>	Average daily feed intake
<b>BW</b>	Body weight
<b>CRF</b>	Corticotrophin-releasing factor
<b>CUMFI</b>	Cumulative feed intake
<b>EGF</b>	Epithelial growth factor
<b>ETEC</b>	Enterotoxigenic <i>Escherichia Coli</i>
<b>FABP</b>	Fatty acid binding protein
<b>GH</b>	Growth hormone
<b>GLP-2</b>	Glucagon-like peptide 2
<b>GIP</b>	Gastric inhibitory polypeptide
<b>GR</b>	Glucocorticoid receptor
<b>2-IB</b>	2-iminobiotin
<b>I-FABP</b>	Intestinal fatty acid binding protein
<b>IGF</b>	Insulin-like growth factor
<b>IS</b>	Intermittent suckling
<b>ODC</b>	Ornithine decarboxylase
<b>SISP</b>	Small intestinal segment perfusion (test)
<b>SMA</b>	Superior mesenteric artery
<b>SPF</b>	Specific pathogen free
<b>TPN</b>	Total parenteral nutrition





# CHAPTER 1

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## **GENERAL INTRODUCTION**

## BACKGROUND THESIS

Shortly after birth, piglets are completely dependent on the milk produced by the sow for their nutrient intake. During the postnatal period, however, piglets will become increasingly independent from their dam with respect to nutrient intake by the decreasing nursing frequency and the increasing amount of solid feed in their diets (Puppe and Tuchscherer, 2000). The duration of the lactation period has been shortened dramatically over the last decades. Currently, piglets are weaned at the early age of 3 to 4 weeks in conventional, European pig husbandry. Since this transition to independency is rather abrupt and takes place at a young age, piglets are physiologically not fully competent to deal with this weaning process. This becomes evident from the dramatically decreased postweaning nutrient intake (van Beers-Schreurs and Bruininx, 2002), a reduced piglet growth (Okai et al., 1976; Colson et al., 2006), display of piglet distress behavior (Dybkjaer, 1992), and the postweaning changes in gut integrity and function that increase the susceptibility for diarrhoea (van Beers-Schreurs et al., 1992; Nabuurs, 1998a). In herds with a history of postweaning diarrhoea, the effects of weaning on intestinal dysfunction were more severe and even resulted in piglet loss (Nabuurs et al., 1993a). Considering the detrimental effects of conventional weaning on postweaning piglet performance, it is desirable to alleviate the constraints of piglets in their adaptation to weaning.

In view of piglets weaned under natural conditions (Jensen, 1988), a possible way to get closer to the natural weaning process would be to extend the suckling period. An older age at weaning has been shown to improve the piglet's ability to cope with weaning, in terms of dry feed intake (Puppe and Tuchscherer, 2000), piglet growth (Colson et al., 2006), behavior (Weary and Fraser, 1997; Worobec et al., 1999), and intestinal structure and/or function (Cera et al., 1988). However, the inhibitory effect of suckling (Stevenson et al., 1981) and the metabolic constraints (for review see Prunier et al., 2003) on ovarian activity and ovulation during a prolonged continuous lactation will postpone the establishment of the next pregnancy of the sow. Improving piglet postweaning performance by extending lactation length can only be profitable if reproductive performance of sows is not compromised. In the past few years, a management strategy, called intermittent suckling (IS), has been studied, in which piglets are temporarily separated from

their sow on each day, during the last 11 days of a 4-week suckling period (Kuller, 2008). This IS strategy mimics the increasing amount of time spent away from the piglets during the lactation period, when sows themselves can control contact with their piglets (Bøe, 1991; Weary et al., 2002). It has been shown that subjecting sows to IS induces ovulations during lactation in a number of sows (Matte et al., 1992; Kuller et al., 2004), resulting in the establishment of the next pregnancy during the ongoing lactation period (Gerritsen, 2007). In addition, the limited presence of the sow during IS stimulated solid feed intake of the piglets during the suckling period and, concomitantly, resulted in a greater postweaning feed intake and growth compared to conventionally weaned piglets (Kuller et al., 2004).

Combining IS with an extended lactation seems to be a promising strategy to encourage piglets to ingest solid feed during the suckling period and to create, also associated with the older age at weaning, a more gradual adaptation to weaning without compromising sow reproductive performance. The studies of the current thesis focus on the effects of an extended lactation in combination with IS on piglet's adaptation to weaning. In the following paragraphs of this chapter, literature data on the effects of weaning on piglet nutrient intake, growth, behavior and gut characteristics will be reviewed. Thereafter, the influence of several types of management strategies on piglet postweaning performance will be described. Finally, the aims and the outline (of the studies) of the present thesis are presented.

### **WEANING OF PIGS UNDER NATURAL OR SEMI-NATURAL CONDITIONS**

Shortly before parturition a sow isolates herself from the other sows of the herd and subsequently starts building a nest. Within the first 3 to 4 days after farrowing, piglets learn to recognize their dam, forming a bond through suckling, nose contacts and vocalisations. Around 10 days after farrowing, the litter integrates with other sows of the herd and their litters (Fraser et al., 1998). From this time onward, nursing frequency and the number of sow-initiated nursings declined, whereas the number of sow-ended nursings increased and the period of time that the dam spends away from her piglets prolonged with increasing piglet age (Jensen, 1988; Jensen and Recen, 1989; Bøe, 1991; Fraser et al., 1998). Moreover, the total number of nursings declined from an average of 41 nursings at day 4 after farrowing to only 19 nursings at 2 weeks of age (Jensen, 1988). Supervised by the sow, piglets become acquainted with nutrient sources other than sow milk and, concomitantly with the decreasing nursing frequency, they start consuming an

increasing amount of solid feed. Sows may continue to nurse their litter until piglets are around 12 up to 17 weeks of age (Jensen, 1986; Stolba and Wood-Gush, 1989; Bøe, 1991). Therefore, under these (semi-) natural conditions, weaning is considered to be a long, gradual process rather than an event that takes place abruptly at a specific moment.

## **WEANING OF PIGLETS IN PIG HUSBANDRY**

In contrast to (semi-) natural conditions, piglets in European conventional pig husbandry are weaned abruptly at 3 to 4 weeks of age, when the piglet is still largely depending on sow's milk for its nutrient intake (Pajor et al., 1991; Puppe and Tuchscherer, 2000). Conventional weaning is associated with an many changes; an abrupt change from a diet based on sow's milk to a nonmilk diet (most often dry feed), reallocation from the farrowing pen to a nursery pen, maternal separation, and often mixing with unfamiliar pen mates. As a result, it has profound effects on piglet nutrient intake, growth, behavior and small intestinal function in the postweaning period, which will be described in the following paragraphs.

### **Piglet preweaning nutrient intake**

Early in life, piglets are completely depending on colostrum and milk, produced by the sow, for their nutrient intake. Both nutrient sources are perfectly adapted to the young piglet's digestive and metabolic requirements, and contain immunoglobulins to protect it against microbes (Coalson and Lecce, 1973). Moreover, sow's colostrum and milk contain polyamines (or precursors) that stimulate cell division and differentiation in the small intestine (Wu and Knabe, 1994), and several growth factors like epidermal growth factor (EGF; Jaeger et al., 1987) and insulin-like growth factor (IGF; Cera et al., 1987; Odle et al., 1996). In conventional housing systems, nursing frequency increases during the first week after birth, reaches a maximum (on average 31.4 nursings per day at 8.5 days postpartum, with a litter size of 8-10 piglets), and slowly decreases thereafter until weaning (Puppe and Tuchscherer, 2000). In contrast to the drastic decrease in daily number of sucklings observed in the first two weeks postpartum under (semi-)natural conditions (see before; Jensen, 1988), nursing frequency remains rather high at the end of the suckling period under conventional housing conditions (> 24 nursings per day; Puppe and Tuchscherer, 2000). This is most probably due to the impossibility for sows housed in farrowing crates to escape from the demanding litter.

To familiarize piglets with creep feed before they become completely dependent on it for their nutrient intake after weaning, creep feed is often already provided during the suckling period. Although the decreasing nursing frequency was found to be accompanied by an increase in creep feed intake, uptake was relatively low in the first three weeks, and a considerable increase was only found in the fourth to fifth week of age (Pajor et al., 1991; Puppe and Tuchscherer, 2000; Bruininx et al., 2004). Moreover, both the amount and the onset of solid feed intake is highly variable between and within litters (Pajor et al., 1991; Fraser et al., 1994; Bøe and Jensen, 1995).

### **Piglet postweaning nutrient intake**

As mentioned above, weaning is associated with a rather abrupt dietary change. As a result, piglets often respond to weaning by a period of fasting (Bark et al., 1986). A nutrient intake level equal to a metabolizable energy requirement for maintenance is commonly not met until 1 week after weaning (Le Dividich and Seve, 2000; van Beers-Schreurs and Bruininx, 2002). Monitoring of individual feed intake after weaning by means of feedings stations revealed a great variation in postweaning feed intake of group housed piglets (Bruininx et al., 2001). The latter study indicated that approximately 50% of the group housed weanling piglets had their first feed intake within the first 4 h postweaning, whereas for the remaining 50% of the piglets it lasted up to 50 h postweaning before all piglets had started eating.

Of all the factors that affect nutrient intake of piglets after weaning, the removal of milk and its replacement by an alternative nutrient source is assumed to be of great(est) importance. In a study of Van Beers-Schreurs et al. (1998) piglets received a weaner diet, or sow milk at a high level (comparable to that of unweaned piglets) or low level (comparable to that of piglets on the weaner diet) after they were weaned. Compared to the amount of metabolizable energy intake of piglets fed a high level of sow's milk, the mean voluntary daily intake of metabolizable energy in piglets provided with a commercial weaning diet (or low level of milk) was only 15% of that on day 1 postweaning and reached 52% of that on day 6 postweaning (van Beers-Schreurs et al., 1998). Since feeding of sow milk to newly-weaned piglets to prevent a period of low energy intake postweaning is not feasible in practice, it is of great interest to identify factors with a possible beneficial effect on voluntary postweaning feed intake.

Time spent on eating behavior directly after weaning was greater with a higher piglet age (7, 14 or 28 days) at weaning (Worobec et al., 1999). Moreover, feed intake of piglets exposed to creep feed during lactation increased more rapidly after weaning when age at weaning increased from 2 to 4 weeks (Leibbrandt et al., 1975). It is beyond dispute that age at weaning has a substantial impact on postweaning feed intake.

Using feed stations to monitor individual feed intake after weaning, it was found by Bruininx et al. (2001) that during the first 2 days postweaning, only few piglets started eating in the periods when lights were off (1% during the first dark period and 5% during the second dark period postweaning), whereas these percentages were considerably higher during the periods with lighting (52% and 33% for the first and second light period postweaning, respectively). This suggested a positive effect of lighting on stimulation of dry feed intake (Bruininx et al., 2001). Indeed, prolonging the photoperiod stimulated the postweaning feed intake and resulted in higher feed intake levels during the second week postweaning, although the moment of first feed intake was not affected by the lighting schedule (Bruininx et al., 2002b).

Addition of a flavour to the diet of weanling pigs, almost never resulted in a consistent improvement of postweaning feed intake (Pluske, 1993). Moreover, if the flavouring agent was not added to the sow's lactational diet, effects of flavour on postweaning feed intake were often poor (King, 1979). Indeed, feeding flavoured creep feed to piglets during the suckling period without adding the same flavour to the sow's diet, did not result in a higher postweaning feed intake of the flavoured weaner diet (Millet et al., 2008). In a recent study of Langendijk et al. (2007), feed intake at day 10 postweaning was stimulated by the addition of garlic and aniseed both to the sow's diet during lactation and to the solid feed of the piglets both pre- and postweaning, when piglets were weaned at 6 weeks, but not at 4 weeks of age (Langendijk et al., 2007).

Due to its high water content, sow milk ingested by piglets during the suckling period provides them with both necessary nutrients and water (Fraser et al., 1998). Shortly after weaning, water intake of piglets is often low, and failure to drink water may reduce the postweaning dry matter intake (Fraser et al., 1998). Providing liquid feeding to newly-weaned piglets avoids the immediate need to establish separate drinking and feeding behavior, thereby reducing the risk of food and water deprivation commonly seen shortly after weaning (Gill, 2007). Indeed, liquid feeding was found to stimulate postweaning feed intake substantially with

greater levels compared to dry feeding (Gill, 2007). However, due to difficulties with feed hygiene, fermentation, feed refusal or wastage, and the fact that liquid feeding is rather laborious, it is currently not widely used for weaned piglets on commercial farms (Gill, 2007).

As mentioned above, although creep feed is commonly provided during the suckling period, intake is rather low and highly variable during the suckling period. However, a number of studies have shown that feed intake early postweaning is improved by (a substantial) creep feed intake during lactation (Bruininx et al., 2002a; English, 1981). The beneficial effects of (a substantial) solid feed intake during lactation on postweaning piglet performance will be discussed in more detail in one of the following paragraphs.

### **Piglet growth during lactation and after weaning**

During the first week of life, a stable teat-order is established which helps to reduce fighting and competition between littermates (Hemsworth et al., 1976; Rossillon-Warnier and Paquay, 1984). Since newborn piglets completely depend on milk for their nutrient intake, relative weight gain of a piglet depends on the productivity of the teat suckled (Algers et al., 1990). Large differences in milk production by different teats have been observed, sometimes up to a two-fold difference in adjacent teats (Algers and Jensen, 1991). Therefore, the variation in weaning weight is predominantly a result of difference in milk production by each mammary gland. Piglets nursing the first 4 or 5 anterior pairs of mammary glands had a higher growth compared with piglets nursing the remaining glands (Rossillon-Warnier and Paquay, 1984; Kim et al., 2000). In general, piglets gain some 180 to 240 g per day between birth and weaning at 3 to 4 weeks of age, increasing their birth weight up to 5-fold at the time they are weaned. However, despite this impressive growth observed in suckled piglets, their growth potential is even greater. Artificially reared piglets fed with a milk replacer after weaning at the age of 2 to 3 days, showed an increased growth rate from day 8 onward compared to piglets suckled by their sow (Pluske et al., 1995).

The reduced voluntary feed intake directly after weaning, associated with the withdrawal of milk and provision of solid feed, results in a so-called 'postweaning growth check'. Most piglets loose around 100 to 250 g on the first day after weaning, but the preweaning bodyweight is often restored within 2 to 4 days after weaning (Le Dividich and Seve, 2000). Usually, weight gain of piglets during the first week postweaning is lower than before weaning, but linear growth

recommences afterwards. However, the duration of the growth check can be quite variable. In around 20% of the observed litters under practical conditions, piglets gained less than 200 g per piglet per day during the first 2 weeks postweaning (Madec et al., 1998). Despite the low weight gain or even weight loss after weaning, however, the gastrointestinal tract undergoes a dramatic growth in the period shortly after weaning (Le Dividich and Seve, 2000). The relative small intestinal weight of piglets weaned at 21 days of age was found to increase with 15%, 37%, and 82% on day 3, 7 and 14 postweaning, respectively, compared to preweaning values (~ 3% of total body weight; Cera et al., 1988).

The severity and duration of the postweaning growth check of piglets is influenced by the age of piglets at weaning. Piglets were found to have a shorter, and less severe period of growth reduction when weaning age increased from 2 to 4 weeks of age (Leibbrandt et al., 1975). Weaning of piglets at 3 weeks of age markedly reduced piglet growth during the first week postweaning (55% lower compared to preweaning values), and growth was restored to preweaning levels only at day 9 postweaning (Colson et al., 2006). Postponing weaning for 1 week still resulted in a profound reduction in piglet growth during the first two days after weaning (45% lower compared to preweaning values), but preweaning growth levels were restored between day 2 and 7 postweaning (Colson et al., 2006). Similarly, body weight of piglets weaned at 3 weeks was reduced from 5.9 to 5.0 kg at day 3 postweaning, and was restored again to 5.8 kg at day 7 postweaning, whereas no decrease in body weight was found in piglets weaned at 5 weeks of age (Cera et al., 1988). In addition, weaning at 3 weeks of age resulted in a growth check in the first 2 days after weaning and an increasing growth thereafter reaching preweaning levels around 1 week postweaning (Okai et al., 1976). Weaning at 5 weeks of age did not result in a growth check, but piglets managed to gain weight preventing a decline in bodyweight after weaning (Okai et al., 1976). Similarly, in a recent study of Jarvis et al. (2008) weaning of piglets at 42 days of age, kept loose with the sow from d 21 to 42, did not result in a decreased growth in the postweaning period at all, which contrasted to the growth reduction of piglets weaned at 12 or 21 days observed in the same study (Jarvis et al., 2008). This effect of age at weaning on postweaning performance is obviously associated with the previously mentioned increasing voluntary feed intake with at an older age (Leibbrandt et al., 1975). Based on an epidemiological study, a weaning age and a weaning weight of at least 30 days and 7.9 kg, respectively, were found to optimise postweaning performance (Skirrow et al., 1997). Hence, when piglets are older and concomitantly more

matured at the time of weaning, they have a greater capability to cope with weaning as judged by growth and dry feed intake.

### **Physiological and behavioral responses to maternal separation**

In commercially reared animals, weaning occurs at a younger age than under natural conditions, since a fast reproductive turnover is profitable. The offspring is confronted with a sudden need to adapt from a milk diet and maternal care to a situation with a solid diet and loss of maternal contact, coinciding with different housing and changes in social environment. Maternal deprivation causes behavioral and physiological changes, which often contribute to the survival of the offspring. The first response of infant rats to maternal separation is an increase in ultrasonic vocalizations, often resulting in reunification with the mother (Noirot, 1972) and an increase in exploratory behavior to retrieve the nest (Kuhn and Schanberg, 1998). A prolonged period of maternal separation subsequently induces several physiological changes, such as an inhibition of growth hormone (GH) secretion and reduction of ornithine decarboxylase (ODC) levels in brain and peripheral tissues, which is the rate limiting enzyme in the production of polyamines (regulating nucleic acid synthesis). These physiological changes are believed to enable the weanling to cope with the transient period of reduced nutrient intake during longer periods of maternal separation (Kuhn and Schanberg, 1998). They have also been observed in suckling pigs after a 16-h period of maternal separation (Pond et al., 1986).

The above mentioned increase in vocalizations shortly after maternal separation has been observed in many mammalian species (Kuhn and Schanberg, 1998), including the pig (Fraser, 1975). The call rate of isolated piglets was found to be doubled when piglets were exposed to playback of sow calls, indicating that the piglet calls are directed to the sow (Weary et al., 1997). Moreover, sows show a stronger response (increased vocalization, more locomotion and more time near the speaker) to playback isolation calls of piglets with a higher rate, higher frequency (Hz) and longer duration, which are all indicators for a greater level of piglet need (Weary et al., 1996). During lactation piglets rarely vocalize without specific context, but weaning of piglets resulted in a high rate of calling, with the highest rate on the day of weaning (8.2 calls / min) and a gradual decrease thereafter (1.6 calls/min at day 4 postweaning; Weary and Fraser, 1997). Moreover, the call rate of weaned piglets decreased with an increasing piglet age at weaning from 3 to 5 weeks of age (Weary and Fraser, 1997).

A single overnight separation of piglets from their dam resulted in a 4-fold increase in their plasma cortisol concentrations, but did not affect adrenal adrenocorticotrophic hormone (ACTH) receptors (Klemcke and Pond, 1991). Moreover, repeated daily maternal separation (by social isolation) for 2 h between 3 and 11 days of age resulted in increased basal plasma cortisol concentrations on day 12, and higher plasma ACTH levels at 56 days of age (Kanitz et al., 2004). Weaning at 28 days of age was found to induce a non-significant increase in plasma cortisol at day 2 postweaning (from 55 to 78 nmol/l; Kanitz et al., 1998). Furthermore, a decreased binding of the glucocorticoid receptor (GR) in both the hippocampus and the amygdala was observed at day 4 postweaning, whereas no changes were observed in the hypothalamus (Kanitz et al., 1998). No differences in serum cortisol concentrations were found in piglets weaned at 3 or 4 weeks of age at the fourth day postweaning compared to preweaning values (Carroll et al., 1998). It was anticipated this was an indication for piglet's being adapted to the stress of maternal separation only after several days postweaning. This seems to be in line with the observation in early-weaned piglets (6 days of age), which showed a transient increase in urinary cortisol at the first day after weaning, with values at day 5 postweaning similar again to preweaning values (Worsaae and Schmidt, 1980; Hay et al., 2001). Caution has to be used, however, in attributing the increased cortisol levels to the psychological stress associated with maternal separation at weaning, since they may also, and possibly even to a greater extent, be affected by nutritional stressors (like fasting; Funderburke and Seerley, 1990; Hay et al., 2001). In addition, early weaning (6 days of age) resulted in a fast and profound decrease in urinary noradrenalin levels, most probably as a consequence of starvation (saving heat production), and a more gradual and long-lasting decrease in urinary adrenalin levels (Hay et al., 2001).

In a study of Salfen et al. (2003) the effect of short-term feed deprivation (for 72 h) on endocrine responses of weaner pigs was studied. Feed deprivation resulted in increased serum cortisol concentrations from 12 to 72 h (re-feeding). Serum leptin and insulin-like growth factor-I (IGF-I) were decreased during feed deprivation and increased again after refeeding. Ghrelin was lower shortly after feed deprivation (12 h), but increase thereafter between 12 h and 36 h of the fasting period. Ghrelin, a GH releasing agent and regulator of appetite, stimulates gastric acid secretion and intestinal motility. A decrease in serum ghrelin during feed deprivation was suggested to prevent acid-induced gastrointestinal disease and may represent an adjustment to the stressor. Moreover, expression of ghrelin mRNA in the stomach,

pituitary glands, and hypothalamus tended to be lower in feed deprived compared to control piglets. It was concluded by these authors that feed deprivation is accompanied by multiple (neuro)endocrine changes, which influence feed intake and the somatotrophic response to fasting (Salfen et al., 2003).

The maternal deprivation associated with weaning at a young age, can cause all sorts of stereotypic behavior in captive animals (Latham and Mason, 2008). In pigs, the most striking example of stereotypic behavior induced by weaning is the development of belly-nosing, involving repeated rhythmic up-and-down massage movements with the snout at the flanks or undersides of penmates (Latham and Mason, 2008). Belly-nosing can result in injuries of the skin in the flank area, which might become a focus for manipulation by penmates, and can ultimately result in a significant injury (Fraser et al., 1998). This behavior is rarely seen in the first days after weaning, but typically develops several days after weaning (Fraser, 1978; Worsaae and Schmidt, 1980) and reaches a peak between 2 and 4 weeks postweaning (Gonyou et al., 1998). The frequency of belly-nosing behavior was found to be inversely related to the age of piglets at weaning (Metz and Gonyou, 1990; Gonyou et al., 1998; Worobec et al., 1999; Jarvis et al., 2008). Although the motivation of piglets to express belly-nosing behavior is still under investigation, nutritional, social and environmental stressors were suggested to play a part in the development of this behaviour (Dybkjaer, 1992; Gardner et al., 2001; Li and Gonyou, 2002; Jarvis et al., 2008; Widowski et al., 2008).

Separation of piglets from their dam results in an immediate response of piglets in demonstrating restless, agitated behavior and, as mentioned above, isolation calls. Weaning by removal of the sow at 3 weeks of age induced an immediate increase in overall activity, and piglets appeared to have difficulty lying down together comfortably (much shifting of position when huddled together) during the first few days after weaning (Fraser, 1978). This restlessness of newly weaned piglets can also be induced by inadequate environmental conditions, like cold, crowding or lack of stimuli in the housing conditions (Dybkjaer, 1992; Fraser et al., 1998). In contrast to the previously reported restlessness of piglets immediately after separation (Fraser, 1978), Colson et al. (2006) observed increased resting after weaning and an increase in litter cohesion during the first days after weaning, both in piglets weaned at 3 and at 4 weeks of age. In the latter study, however, piglets were moved from their farrowing pen to a weaner pen. Since piglets moved at weaning were found to spend more time lying than piglets weaned in their farrowing pen (Bøe, 1993), the new environment might have contributed to the

displaying of this behavior. Moreover, the author suggested that the observed lack of activity might have been an indication of impaired thermoregulation (lying in litter cohesion), or might have been induced by stress, since it is a typical symptom of chronic stress situations (Colson et al., 2006).

Under practical conditions, weaning is often associated with mixing of piglets from unfamiliar litters. Mixing of piglets at weaning can lead to elevated plasma cortisol levels (Blecha et al., 1985) and aggressive behavior (Friend et al., 1983), commonly resulting in wounding of piglets (McGlone and Curtis, 1985). Besides, even in the absence of social mixing, weaning can induce an increase in aggressive behavior (Fraser, 1978). The sudden separation from the dam most probably leads to changes in social relations and the need to re-establish each piglet's rank within the litter. This leads to more postweaning aggression in (unmixed) piglets weaned at 2 to 3 weeks of age compared to (unmixed) piglets weaned at 6 weeks of age (Jarvis et al., 2008). No differences in aggressive behavior shortly after weaning were observed in piglets weaned at 7, 14 or 28 days of age (Worobec et al., 1999), but litters were mixed at weaning in this study, making it hard to point out the possible contribution of weaning age in the development of aggressive behavior. In a study of Yuan et al. (2004) weaning was dissociated from mixing; piglets were either weaned between 9 and 12 days of age or at 21 days of age, and piglets of both treatments were mixed at 9 weeks of age. Piglets weaned at a younger age were found to fight longer and loose more initiated fights at the day of mixing compared with piglets weaned at older age (Yuan et al., 2004). This suggests that weaning at a young age results, besides greater difficulties in (behavioral) coping with weaning (Jarvis et al., 2008), in poorer social skills later in life (Yuan et al., 2004). Besides the age at weaning, the environment in which the piglets are housed after weaning can influence the expression of aggressive behavior. Housing piglets in pens equipped with hide areas reduced the number of fights within the first 30 min after mixing and the amount of wounding (McGlone and Curtis, 1985). Moreover, housing piglets under poor conditions (mixed, no straw, higher density), resulted in an increased amount of time spent on belly-nosing, manipulating pen mates, chewing chain and inactive behavior (Dybkjaer, 1992).

The increased aggression after weaning (unmixed litters) at 3 weeks of age was accompanied by a decrease in body scratching (comfort behavior) and scampering (play behavior; Fraser, 1978). Both individual and social play behavior was found to be markedly depressed on the first day after weaning, but increased again in the days thereafter (Donaldson et al., 2002). Housing piglets under poor conditions

(mixed, no straw, higher density) decreased the percentage of litters displaying play behavior at 2 and 4 weeks postweaning (Dybkjaer, 1992).

In conclusion, weaning of piglets at a young age is associated with the development of altered behavioral patterns, like aggression, manipulation of penmates, belly-nosing, lack of play behavior and higher vocalisation rate (Fraser, 1975; Dybkjaer, 1992; Donaldson et al., 2002). Moreover, weaning induces profound physiological changes, some of them predisposing the piglet to cope with the transient period of decreased nutrient intake associated with maternal deprivation (Pond et al., 1986; Kanitz et al., 1998).

### **Small intestinal development of suckling piglets**

Immediately after birth, the gastrointestinal tract becomes responsible for the provision of metabolites and protective substances (by endocytosis of immunoglobulins) to the newborn piglet (Cranwell, 1995; Mellor et al., 1995). The small intestinal weight and length increased profoundly (72% and 24%) during the first day(s) after birth (Widowson et al., 1976; Xu et al., 1992). These changes are most probably induced for the larger part by the ingestion of colostrum, since food deprivation or ingestion of water or other milk products diminishes the observed intestinal growth rate (Widowson et al., 1976; Zhang et al., 1997). The increased intestinal weight after ingestion of colostrum is believed to be mainly caused by cell swelling and hyperplasia of enterocytes, most probably induced by growth factors (EGF, IGF-I and IGF-II) present in the colostrum (Mellor et al., 1995). The small intestinal weight gradually increased during lactation from 68 g at day 2 postpartum to 177 g and 429 g at 3 and 5 weeks of age, respectively (Cera et al., 1988). The relative weight of the small intestine first showed a decline between day 2 and 21 of age, but increased thereafter between day 21 and 35 of age (Cera et al., 1988). Shortly after birth, the small intestine is lined with long, slender villi, but during the suckling period (day 10 and 21 of age) villi become shorter with larger diameters, and crypts become deeper (Cera et al., 1988; Cranwell, 1995). A further reduction in villus height in suckling piglets (without access to creep feed) was found between day 21 and 35 of age, which was suggested to be induced by a change in luminal content, most probably by the ingestion of sow feed or bedding material (Cera et al., 1988). However, this might also have been an effect of the older age, since Hall et al. (1989) demonstrated that villous height decreased as milk-fed gnotobiotic piglets aged from 24 to 44 days.

Besides changes in intestinal structure, digestive capacity of suckling pigs changes profoundly with increasing piglet age. Small intestinal lactase enzyme activity, responsible for the digestion of lactose in sow's milk, is high at birth, and was found to decline with age (Manners and Stevens, 1972; Kidder and Manners, 1980). In contrast, enzyme activity of sucrase and threhalase in the small intestine were negligible at birth, but were detectable at 1 week of age and continued to rise until adulthood (Manners and Stevens, 1972; Manners, 1976). Similarly, a rise in isomaltase, maltase 2 and maltase 3 enzyme activity was observed in the small intestine with increasing age of suckling piglets (Kidder and Manners, 1980). Most of the peptidases present in the enterocytes reach peak levels at birth and decline thereafter, to reach adult levels at around 8 weeks of age (Cranwell, 1995). Evidently, the small intestine of suckling pigs undergoes a steady maturation in the first 2 months of a piglet's life, with profound changes in intestinal structure and digestive capacity. In addition, between 3 and 4 weeks of age (at a time that conventional weaning commonly occurs), the piglet's degree of intestinal maturity is assumed to be around two-third of the final digestive ability (Colson et al., 2006).

### **Effects of weaning on the small intestine in piglets**

Weaning at day 21 was associated with a marked increase in absolute (from 177 g to 610 g) and relative intestinal weight (from 32 to 62 g/kg bodyweight) during the first 2 weeks postweaning (Cera et al., 1988). Weaning induces a dramatic effect on small intestinal structure, with profound changes in villus height and crypt depth. In piglets weaned at 21 days of age, villus height was reduced with 20 to 35% in the first 24 h after weaning, and continued to decrease to ~50% around five days after weaning, with increasing values in the days thereafter (Hampson, 1986). The observed effects on villous height were more pronounced in the proximal part of the small intestine (first half) than in the distal part of the small intestine (Hampson, 1986). Similar reductions in villous height have been observed in other studies, with a minimal villous height between 2 and 5 days postweaning, and increasing thereafter (Cera et al., 1988; Kelly et al., 1991; van Beers-Schreurs et al., 1998; Hedemann et al., 2003). Piglet age at weaning influences the severity of the detrimental effects of weaning on intestinal structure. The reduction in villous height at day 3 postweaning was found to be less severe when piglets were weaned at 5 weeks of age instead of 3 weeks of age (27% versus 65%; Cera et al., 1988). Besides the shortening of villi, weaning was found to induce a profound increase in

crypt depth from about 3 to 4 days after weaning onward reaching a plateau around 1 week postweaning (Hampson, 1986; Kelly et al., 1991; Boudry et al., 2004). Moreover, mitotic counts pointed to an increased proliferative activity in the crypts from the third day postweaning onwards (Hedemann et al., 2003). The reduction in villous height, or so-called villous atrophy, is caused by a reduced cell division at the base of the villi while the shedding at the extrusion zone proceeds, resulting in a reduction in the number of enterocytes lining the villous (Hall and Byrne, 1989). Weaning induces a rather abrupt change in the source of nutrients for the young piglets. During the suckling period, piglets depend on sow's colostrum and milk for their nutrient intake, whereas dry feed is most often the only nutrient supplied after weaning. The sow's milk contain polyamines (or precursors) that stimulate cell division and differentiation in the small intestine, and it was hypothesized that the intestinal atrophy commonly occurring in early-weaned piglets (21 d of age) may result in part from a lack of milk-borne free taurine, glutamine, glycine and histidine (Wu and Knabe, 1994). One might also hypothesize that weaning-associated villous atrophy is (partly) caused by intestinal hypoperfusion. On the one hand, intestinal hypoperfusion could occur as an adaptation to a stressor in order to maintain the perfusion of organs essential to cope with this stressor (fight or flight response). Under experimental conditions in anesthetized pigs, severe reduction of the intestinal blood flow (to only 25% of normal values) by partial occlusion of the superior mesenteric artery (SMA) for 1 h was found to increase intramucosal pH and to result in histologic lesions (Pargger et al., 1997). On the other hand, intestinal hypoperfusion might occur as a result of fasting. Portal blood flow was found to be reduced in 18-d old piglets fed total parenterally nutrition (TPN) for 6 days compared to enterally fed piglets (Burrin et al., 2003). Moreover, piglets subjected to TPN were found to have a lower intestinal weight, lower intestinal DNA content, shorter villi and lower lactase activity compared to enterally fed piglets (Burrin et al., 2003). Results of Niinikoski et al (2004) indicated that TPN reduced portal and SMA blood flow reaching a plateau of 30% reduction compared to enteral feeding after 8 h, which maintained for 2 d (end of experiment). Besides the reduced intestinal blood flow, intestinal weight and jejunal villous height were reduced after 24 h of TPN compared with enterally fed pigs (Niinikoski et al., 2004). Another day of TPN (48 h) was found to decrease intestinal crypt cell proliferation and jejunal DNA mass and to increase the number of apoptotic villi cells. It was hypothesized by these authors that the observed rapid decrease in intestinal blood flow precedes the villous atrophy and suppression of

cell proliferation and cell survival in the small intestine (Niinikoski et al., 2004). Since weaning is associated with multiple stressors and a period of low feed intake or fasting, one might hypothesize that splanchnic hypoperfusion plays a part in the occurrence of intestinal dysfunction after weaning.

Weaning resulted in a sharp and rapid reduction in lactase and sucrase activity, reaching lowest values at day 4 to 5 postweaning (Hampson and Kidder, 1986; Boudry et al., 2004). Sucrase activity recovered by 11 days after weaning, whereas lactase activity continued to decline in the postweaning period (Hampson and Kidder, 1986). Similarly, a transient decrease in maltase activity was observed after weaning (Boudry et al., 2004). Besides effects on carbohydrase activity, weaning also resulted in a transient decline of aminopeptidase N and dipeptidylpeptidase IV activity in the first 3 days postweaning, but levels returned to preweaning values again at day 9 postweaning (Hedemann et al., 2003).

Besides, and most likely associated with, the described changes in intestinal morphology and digestive capacity, weaning affects small intestinal absorption. Weaning resulted in a marked malabsorption of orally dosed D-xylose in 3-week old piglets, with lowest absorption values around day 8 postweaning, and increasing thereafter (Miller et al., 1984; Hampson and Smith, 1986). In a study of Nabuurs et al. (1994) net absorption of a solution, containing glucose and electrolytes, during a small intestinal segment perfusion test (SISP) was found to decrease after weaning, with lower absorption values in weaned piglets up to 14 days postweaning (Nabuurs et al., 1994). These changes in net absorption were related to the shortening of villi and deepening of crypts after weaning (Nabuurs et al., 1994). Net absorption decreased even more when the perfused segments were exposed to enterotoxigenic *Escherichia coli* (ETEC). Interestingly, the net absorption in segments exposed to ETEC was not related to intestinal morphology after weaning when villi were recovered (day 11 and 14 postweaning). These findings were corroborated in a subsequent study of these authors (Nabuurs et al., 1996). The authors suggested that the lack of a relationship might be explained by the presence of young, immature enterocytes in the intestine and the concomitant enhanced susceptibility to bacterial toxins of these enterocytes (Nabuurs et al., 1994; Nabuurs et al., 1996).

Stress and starvation both are factors influencing the mucosal integrity by an increased paracellular and/or transcellular permeability (Kiliaan et al., 1998; van der Hulst et al., 1998; Wiren et al., 1999). Total parenteral nutrition was found to result in an impairment of gut barrier function (increased permeability) in neonatal

piglets (Kansagra et al., 2003). Since weaning of piglets is associated with stress, temporary underfeeding and profound effects on intestinal structure, it was postulated that weaning might also affect small intestinal permeability. Indeed, Spreeuwenberg et al (2001) demonstrated that paracellular permeability was increased at day 2 and 4 postweaning compared to values at weaning or 1 day after weaning. Similarly, intestinal permeability of 19-day old weaned piglets was increased compared with age-matched unweaned piglets (Moesser et al., 2007a). In addition, the expression of receptors for corticotrophin-releasing factor (CRF) was found to be increased in intestinal tissues of weaned piglets, suggesting that stress signalling pathways are involved in the weaning-associated intestinal function. Indeed, treatment of piglets with a CRF receptor antagonist before weaning abolished the stress-induced mucosal changes (Moesser et al., 2007a). Moreover, extending the suckling period to 28 days of age also prevented the reductions in intestinal barrier function as observed in early-weaned, 19-day old piglets (Moesser et al., 2007b).

The changes in intestinal morphology and function associated with weaning result in a higher susceptibility of piglets to diarrhoea (van Beers-Schreurs et al., 1992; Nabuurs, 1998b). Although the presence of rotaviruses and *Escherichia coli* are important in the aetiology of diarrhoea in weaned piglets, other factors most probably contribute as well, since both agents were also detected in faeces of weaned piglets without signs of diarrhoea (Hampson and Smith, 1986; Nabuurs et al., 1993b). The incidence of postweaning diarrhoea increased with occurrence of diarrhoea during the suckling period and with a larger litter size (11 to 12 piglets) at weaning, whereas the risk on diarrhoea decreased with increasing weaning age and increasing weight at weaning (Svensmark et al., 1989; Skirrow et al., 1997). Interestingly, piglets from herds with a history of postweaning diarrhoea had shorter villi and deeper crypts at the time of weaning and 11 and 14 days thereafter, compared to specific-pathogen-free (SPF) piglets, although the latter piglets also showed villus shortening and crypt deepening after weaning (Nabuurs et al., 1993a). Moreover, piglets from herds with a history of postweaning diarrhoea and mortality had shorter villi at weaning and during 2 weeks after weaning compared to piglets from herds without mortality and SPF piglets (Nabuurs et al., 1993a). Although villous height in SPF piglets was lower at day 4 postweaning compared to preweaning values, it was restored to and exceeded the preweaning values thereafter. In contrast, villous height of piglets from postweaning diarrhoea herds did not alter over the postweaning period. Crypts of piglets from postweaning

diarrhoea herds were deeper during the postweaning period compared to SPF piglets. Piglets from all herds had deeper crypts at 7 days postweaning compared to preweaning values.

Postweaning nutrient intake on itself has a profound effect on the above mentioned, weaning-associated changes in intestinal structure and function. At day 5 postweaning, villous height and crypt depth of 14-day old weaned piglets subjected to a restricted nutrient supply were found to be reduced compared with piglets with a continuous nutrient supply (Kelly et al., 1991). Ad libitum feeding of cow's milk to weaned piglets every 2 h has been shown to result in a villous height and crypt depth at day 5 postweaning comparable with the values observed preweaning (Pluske et al., 1996a). Piglets fed a high amount of sow milk (comparable with unweaned piglets) every 4 h were found to have slightly shorter villi compared to unweaned piglets, but higher villi compared to piglets weaned on a commercial weaner diet or on a low level of sow milk (comparable with piglets receiving the weaner diet; van Beers-Schreurs et al., 1998). Since no differences in postweaning villous height were observed between piglets offered the latter two diets, it was suggested that villous atrophy was more due to the amount of feed intake than to the composition of the diet (van Beers-Schreurs et al., 1998). This also became evident in a study of Pluske et al. (1996b), who found a significant linear relationship between the amount of postweaning energy intake and small intestinal villous height or crypt depth.

In conclusion, weaning of piglets is associated with profound changes in small intestinal morphology and function, and, as a result, a higher susceptibility for diarrhoea. These effects are more pronounced in herds with a history of postweaning diarrhoea (with or without mortality) and might be (partly) prevented by a sufficiently high level of nutrient intake after weaning. An older age at weaning was found to diminish the detrimental effects of weaning on small intestinal villous atrophy and permeability, and reduced the risk on diarrhoea. It is anticipated that this beneficial effect of an older age at weaning is partly mediated both by the more matured gastrointestinal tract and the higher level of postweaning feed intake by older piglets. Given the high variation in the ability of piglets to cope with weaning, e.g. illustrated by the highly variable moment of first postweaning feed intake (Bruininx et al., 2001), it is of great interest to monitor postweaning intestinal function of individual piglets over time. Unfortunately, most parameters of small intestinal function are end-point measurements (Montagne et al., 2007), making longitudinal measurements currently impossible.

**EFFECT OF MANAGEMENT STRATEGIES ON POSTWEANING PIG PERFORMANCE****Provision of creep feed during lactation**

To familiarize piglets with dry feed before weaning, both behaviourally and physiologically, creep feed is often provided during the suckling period. Consumption of creep feed, however, is often relatively low in the first 3 weeks of age, and a considerable increase was only found in the fourth to fifth week of age (Pajor et al., 1991; Puppe and Tuchscherer, 2000; Bruininx et al., 2004). Moreover, both the amount and the onset of solid feed intake is highly variable between and within litters (Aherne et al., 1982; Pajor et al., 1991; Fraser et al., 1994; Bøe and Jensen, 1995). Piglets consuming a substantial amount of creep feed (on average 610 g/piglet) before weaning showed a 6% and 15% improvement of piglet feed intake and weight gain, respectively, in the first 3 weeks postweaning (weaned at day 28 of age; English, 1981). However, there are also studies in which the supplemented creep feed had no beneficial effect on postweaning performance (Okai et al., 1976; Barnett et al., 1989; Appleby et al., 1992; Fraser et al., 1994), or only tended to (Pajor et al., 1991; Fraser et al., 1994). In a study of Aherne et al. (1982) a similar amount of creep feed intake before weaning (680 g/piglet on average) did not result in an improved feed intake or growth after weaning. In the latter study, the large variation in creep feed intake between and within litters, e.g. ranging from only 50 g up to 2850 g per litter, might have played a role. Interestingly, in the same study there was a strong correlation between creep feed intake during lactation and postweaning feed intake and growth (Aherne et al., 1982), suggesting that litters with a greater feed intake before weaning are better adapted to weaning, in terms of dry feed intake and growth. Bruininx et al. (2002a) found that piglets eating creep feed during the suckling period needed less time after weaning to obtain their first feed intake than non-eating piglets or piglets not offered any creep feed. Moreover, feed intake and growth in the first week postweaning were greater for eaters than for non-eaters or piglets not offered any creep feed during the suckling period (Bruininx et al., 2002a). Despite the beneficial effects of creep feed on postweaning feed intake and growth, it was not found to prevent the weaning-associated villous atrophy or the reduction in small intestinal sugar absorption (Hampson, 1986; Hampson and Smith, 1986; Bruininx et al., 2004), nor did it change postweaning digestive enzyme activity (Hampson and Kidder, 1986). In contrast, in a study of Nabuurs et al. (1993a) creep feed supplementation during the suckling period resulted in deeper crypts at weaning

and higher villi at day 7 postweaning. Moreover, piglets eating creep feed during lactation had a higher net absorption in the small intestines during a SISF test compared to piglets not eating creep feed (Kuller et al., 2007b). Furthermore, high creep feed intakes were found to reduce the risk of postweaning diarrhoea (Skirrow et al., 1997). The low creep feed intake and high variation in creep feed intake between piglets and litters (Pajor et al., 1991) might have resulted in less distinct results in some of the studies described above. Moreover, feed intake was mostly determined on litter level (Okai et al., 1976; Aherne et al., 1982; Barnett et al., 1989), instead on individual piglet level (Pajor et al., 1991; Fraser et al., 1994; Bruininx et al., 2002a; Kuller et al., 2007b). It is anticipated that a considerable stimulation of feed intake during lactation will improve postweaning piglet growth, feed intake and small intestinal function.

### **Communal piglet area**

Litters are often mixed at weaning, resulting in increased fighting, injuries, and more restlessness probably disrupting postweaning adaptation of piglets. It was hypothesized that mixing of piglets at a younger age and in the presence of the sow (as occurs in nature) might alleviate the stress (due to exposure to unfamiliar pigs) associated with weaning, and reduce aggressive behavior associated with weaning. Weary and co-authors (1999) investigated this hypothesis by allowing piglets to mingle in a common area, created by removing partitions of adjacent pens. During the suckling period, piglets spent an increasing amount of time in the common area with increasing age (20% and 45% at day 12 and 26 of age, respectively) which was associated with a decreasing suckling frequency. The time spent on aggressive behavior when piglets were mixed after weaning was reduced in previously mingled litters compared to conventional weaned litters (Weary et al., 1999). Although no effects were found on preweaning feed intake, piglets housed with a common area had a higher postweaning feed intake and tended to have a greater postweaning growth compared to conventionally weaned piglets (Weary et al., 1999). Results from other studies in which piglets were socialized before weaning, corroborated the above described effects on aggressive behavior (D'Eath, 2006; Parratt et al., 2006) and postweaning growth (Hessel et al., 2006). Although mixing during the suckling period also resulted in some aggressive behavior, the socialized piglets seemed to have learnt social skills which benefited them later in life, becoming evident from the more rapid formation of a stable hierarchy after mixing 20 days postweaning (Parratt et al., 2006).

**Sow controlled housing**

In a conventional housing system, sows are often confined by a farrowing crate, which prevents the dam from crushing her piglets, but also providing piglets with an undiminished access to her udder during the lactation period. As a result, a high nursing frequency is maintained until piglets are weaned (often more than 24 nursings per day; Puppe and Tuchscherer, 2000) and sows commonly lose a substantial amount of weight during the suckling period. Moreover, the abrupt separation of sows and their litters coincides with social stress and nutritional stress for the piglets by the removal of milk as nutrient source. Hence, Rantzer et al. (1995a,b) studied an alternative weaning method considered to be better biologically adapted, the so-called sow controlled housing system. In this system, sows can leave the farrowing pens to mingle with other sows and/or eat in a common pen, whereas the piglets can not leave the farrowing pen. Sows in the sow controlled housing decreased their time spent in the farrowing pen (from 1008 min/day to 355 min/day) and the number of nursings (from 22.5 to 13 per day) considerably from 2 to 4 weeks of lactation (Rantzer et al., 1995b). Piglets in sow controlled housing had a lower preweaning growth and a greater preweaning feed intake compared to piglets in a conventional system (Rantzer et al., 1995a). During the suckling period, no changes in piglet behavior were observed during sow controlled housing compared to conventional housing (without farrowing crate). Early after weaning (removing the sow at d 33), piglets in sow controlled housing tended to spent more time on eating behavior compared to conventionally housed piglets (Rantzer et al., 1995b), and they had (if corrected for the lower weaning weight) a greater postweaning feed intake (Rantzer et al., 1995a). In the latter study, intervals between consecutive weighings were rather long, which might have concealed possible short-term effects of the housing system on postweaning performance. In subsequent studies on this alternative housing system more frequent observations on postweaning piglet growth and feed intake were described and the above mentioned results of Rantzer et al. (1995a,b) were corroborated. Pajor (1998) found that sow controlled housing improved piglet feed intake to such an extend that it compensated for the reduced nursing frequency, ultimately resulting in similar piglet growth at weaning (d 35) observed in conventionally housed litters. Moreover, piglets in a sow controlled housing gained 52% and 14% more weight in the first and second week postweaning, respectively (weaned by moving to weaner pens at day 35; Pajor et al., 1999). Feed intake of piglets in sow controlled housing was greater on the first day postweaning compared to piglets in

a conventional housing system (381 g/piglet versus 211 g/piglet). Moreover, they consumed 31% more food in the first two weeks after weaning than conventional piglets (Pajor et al., 1999). Shortening the suckling period with one week to weaning 28 days of age, still resulted in an 65% increase in preweaning feed intake compared to conventional housing, but weaning weight was 11% lower (Pajor et al., 2002). Piglets in a sow controlled housing had a greater feed intake and growth after weaning, but this effect was only short-lived and did not compensate for the lower weight at weaning, resulting in a lower body weight at the end of the experiment (7 weeks of age) than for conventional weaned piglets (14.5 versus 15.8 kg; Pajor et al., 2002). Combining the sow controlled housing system with a communal piglet area resulted in a decrease in nursing frequency of 30% at 4 weeks, and sows consumed less feed during lactation with a similar weight loss and weaning-to-oestrus interval compared to sows in conventional housing (Weary et al., 2002). Piglets in this alternative housing had a greater feed intake before weaning (day 28), but tended to gain less weight during lactation than conventionally housed piglets. The alternative housing of piglets resulted in less aggressive behavior due to mixing at weaning (d 28) and a greater postweaning feed intake and growth, leading to a similar body weight at day 42 compared to conventional housed piglets (Weary et al., 2002). It was suggested that a sow controlled housing system combined with a piglet area allowing both piglets and sows to mingle can provide advantages for both sows (regulate number of nursings, social interaction with other sows) and piglets (better postweaning performance and less aggression at weaning), without compromising the high levels of production in conventional systems (Weary et al., 2002).

### **Limited nursing and intermittent suckling**

The above described sow controlled housing system seems to be a more ‘natural’ weaning method given the increasing time the sow spends away and the improved postweaning performance of the piglets. However, in the sow controlled housing system the time spent away from the piglets was highly variable between sows and increased with increasing piglet age (between 4% and 100% at day 27; Pajor et al., 2000; Pitts et al., 2002). Some sows did not increase their time spent away during the suckling period and still spent over 85% of their time in the farrowing pen in the fifth week of lactation (Pajor et al., 2000). In general, sows in sow controlled housing that spent more time away from the piglets, had a lower number of nursings, a lower feed intake and less weight loss during the lactation period (Pajor

et al., 2002; Pitts et al., 2002). Piglets of the latter sows had a greater preweaning feed intake, a reduced postweaning growth check and a higher weight gain in the first week postweaning (Pajor et al., 2002). Hence, a housing system that could regulate the time sows spent away from the piglets, would most probably reduce the variation between litters in terms of nursing frequency, preweaning creep feed intake and growth of piglets. A possible way to regulate this might be to impose the period of time spent away from the litter on the sows instead of letting the sows decide the timing and duration of their absence. One method described to limit the nursing time was to allow piglets to nurse for only a short period of time (30 to 60 min) 3 to 4 times daily in the last week(s) of lactation (Thompson et al., 1981; Chapple et al., 1989). This drastic reduction in nursing time increased creep feed intake 2 to 3 fold, but also resulted in a markedly reduced preweaning piglet growth with only 55 to 60% of that observed in continuously suckled piglets (Thompson et al., 1981). The limited nursing did improve postweaning feed intake and growth of piglets, most probably due to the fact that they were used to consumption of dry feed and the absence of the sow (Thompson et al., 1981; Chapple et al., 1989).

Another approach used to limit the nursing time is intermittent suckling (IS), a management strategy in which piglets are temporarily separated from their sow for several hours on each day from a certain age until weaning. Intermittent suckling was originally developed as an alternative management system to improve the efficiency of pig production. The limited nursing time was suggested to result in an increased sow productivity (inducing oestrus during late lactation) and an increased overall feed efficiency (feed consumed directly by piglets has a more efficient conversion rate to piglet weight gain than that fed through the sow). In one of the first studies on IS, sows and piglets were separated daily for 12 h from 3 or 5 weeks of age onward during an 8-week suckling period (Smith, 1960). Results of the latter study indicated that IS induced lactational oestrus in sows, increased creep feed intake of piglets and their proportion of creep feed derived energy intake compared to continuously suckled piglets (IS piglets had an energy intake from creep feed at 6 weeks of age comparable to that of 8-week old normally suckled piglets; Smith, 1960). Onset of IS at 3 weeks of age was associated with an obvious, but transient reduction in piglet growth, whereas onset at 5 weeks of age resulted in slightly lower piglet growth rate compared to continuously suckled piglets (Smith, 1960). In conclusion, it was hypothesized that this management strategy could result in 1) a higher number of litters per sow per year, 2) a decrease

in feed energy requirement for piglet gain during the suckling period, 3) a decrease in weaning age (6 weeks instead of 8 weeks), 4) a possible prevention of weaning-associated weight loss (Smith, 1960).

In subsequent studies, IS was often applied during a shorter lactation period (3 to 5 weeks), with variations in the separation interval (3 to 12 h) and duration (1 to 2 weeks of IS). In line with the findings of Smith (1960), IS was found to induce a lactational oestrus in sows in quite some studies (for review see Matte et al., 1992) and, consequently, to establish the next pregnancy during the ongoing lactation (Gerritsen, 2007). Subjecting piglets to IS with a separation time between 3 to 12 h and a duration of 1 to 2 weeks in the second part of a 4-week suckling period was found to result in similar piglet weaning weights compared to continuously suckled piglets (Kirkwood et al., 1983; Newton et al., 1987a; Newton et al., 1987b). Moreover, in line with Smith (1960), piglets separated from their sow during IS (12 h/d) had a greater creep feed intake compared to normally suckled piglets (Kirkwood et al., 1983). In more recent studies, Kuller et al. (2004, 2007) reported an 218% and 157% increase in creep feed intake during the suckling period in litters subjected to IS (12 h/d) during the last 11 days of a 4-week suckling period compared to conventional housed litters. In contradiction to previous findings of Newton et al. (1987a,b) and Kirkwood et al. (1983), but in line with Smith (1960), the onset of IS resulted in a reduced piglet growth, ultimately leading to lower weaning weights of IS litters compared to continuously suckled litters (Kuller et al., 2004; Kuller et al., 2007a). However, these lower weaning weights were compensated for by the higher feed intake and growth of IS litters in the first week after weaning (Kuller et al., 2004; Kuller et al., 2007a).

Intermittent suckling with a separation period of 4 h from 10 days of age until weaning (day 21; creep feed provided), improved piglet feed intake in the first week postweaning compared to conventionally weaned piglets without creep feed during lactation (Hampson and Smith, 1986). However, this difference in postweaning feed intake disappeared if conventionally weaned piglets were also exposed to creep feed during the suckling period. These findings seemed to suggest that not the 4-h separation period during IS, but the exposure to creep feed during the lactation period itself realized the beneficial effects on postweaning feed intake. Moreover, in the latter study, IS did not affect xylose absorption in the small intestines (Hampson and Smith, 1986). In a study of Nabuurs et al. (1996), IS with a separation period of 8 h (last 2 weeks before weaning at day 30-32, creep feed provided) resulted in deeper crypts at weaning and higher villi at day 4

postweaning compared to conventionally weaned piglets (Nabuurs et al., 1996). Moreover, it prevented the decrease in small intestinal net absorption observed in conventionally weaned piglets at day 4 after weaning (Nabuurs et al., 1996). Since the above described effects of IS were only observed if supplementary feed was provided, it was suggested that these effects might have been mainly mediated by the increased creep feed intake by IS before weaning (Nabuurs et al., 1996).

During IS, piglets are involuntarily separated from their sow. Enforced (repeated) maternal deprivation can cause behavioral and physiological changes (Kuhn and Schanberg, 1998; also see above paragraph). In piglets, a single overnight maternal separation results in greater basal plasma cortisol concentrations (Klemcke and Pond, 1991). Therefore, the extent to which repeated maternal separation (as applied in an IS regimen) affects piglet behavior and induces behavioral patterns associated with postseparation distress (Dybkjaer, 1992) might be questioned. However, since to our knowledge no data is available describing possible effects of IS on piglet behavior (or physiology), this question remains currently unanswered.

In conclusion, IS during the suckling period increased preweaning feed intake of piglets, ultimately resulting in an improved postweaning performance, in terms of feed intake, growth and small intestinal function compared to conventionally weaned piglets. The extent of the effects of IS on piglet performance before and after weaning seemed to be rather variable between different studies. This was assumed to be related to the variation in age at onset, separation interval, and duration of IS and weaning age applied in these studies. Moreover, the commencement of IS was found induce of lactational oestrus, ultimately resulting in the next pregnancy of the sow during the ongoing lactation.

#### **WHY STUDY EXTENDED LACTATION COMBINED WITH INTERMITTENT SUCKLING IN PIGS?**

As mentioned previously, weaning of piglets at a young age of 3 to 4 weeks, as currently applied in European pig husbandry, exposes them to both nutritional (withdrawal of milk) and social (absence of sow, mixing with unfamiliar piglets) stressors. As a result, it results in various detrimental effects on postweaning piglet performance, like a postweaning growth check, reduced nutrient intake, distress behavior, and an impaired intestinal function. Under (semi-)natural conditions, the complete weaning of piglets takes place around 12 to 17 weeks of age, providing the piglets time to mature and to become increasingly independent from the sow

for their nutrient intake (Stolba and Wood-Gush, 1989). In pig husbandry, an older age at weaning was found to increase the postweaning feed intake (Leibbrandt et al., 1975), attenuate the postweaning growth reduction (Colson et al., 2006; Leibbrandt et al., 1975), reduce the development of stereotype behavior (Metz and Gonyou, 1990), improve social skills later in life (Yuan et al., 2004), and attenuate the weaning-associated villous atrophy (Cera et al., 1988) and detrimental effects on intestinal function (Moeser et al., 2007b). In view of this improved physiological and behavioral adaptation of older piglets to weaning, it seems to be possible to alleviate the constraints of piglets at weaning by extending the lactation length. However, if the lactation is extended, the persisting suckling stimulus will prevent the onset of cyclicity, and hence result in a reduced reproductive performance of the sow. A possible way to retain the sow reproductive performance during an extended lactation period is to induce lactational oestrus. As described above, subjecting litters to IS was found to induce oestrus and ovulation in sows, establishing the next pregnancy during the ongoing lactation (Smith, 1960; Gerritsen, 2007). Moreover, IS was found to improve piglet preweaning creep feed intake and to result in an improved postweaning piglet performance (Smith, 1960; Nabuurs et al., 1996; Kuller et al., 2004). The success rate of IS in the improvement of postweaning performance varied considerably between different studies, which was ascribed to differences in applied IS regimens in these studies (see above). In more recent studies on the investigation of IS as an alternative weaning strategy to prevent postweaning disorders, IS was applied during a conventional lactation period (Kuller, 2008). To our knowledge, data on combining IS with an extended lactation period is scarce and mainly focussed on sow reproductive performance and/or stimulation of preweaning piglet feed intake (Smith, 1960).

### **AIMS AND OUTLINE OF THE THESIS**

The general objective of this thesis is to determine whether an extended lactation combined with intermittent suckling improves preweaning feed intake and consequently creates a more gradual adaptation to weaning. We will investigate how several intermittent suckling regimens, varying in separation interval, piglet age at onset and duration affect piglet performance, both during lactation and after weaning. Parameters of interest will be piglet feed intake and growth, piglet

behavior and postweaning small intestinal function. More specific research questions addressed in this thesis are:

- a) Do separation interval, timing and duration affect the impact of intermittent suckling regimens on pre- and postweaning piglet feed intake and growth?
- b) Is the repeated maternal separation during an intermittent suckling regime associated with piglet distress behavior?
- c) Can we monitor postweaning intestinal function longitudinally in pigs?
- d) Are postweaning small intestinal characteristics affected in piglets subjected to an intermittent suckling regimen during lactation?

At first, the effect of IS (from day 14 of age) during an extended, 6-week lactation period on piglet pre- and postweaning feed intake and growth was investigated (Chapter 2). Piglets were imposed to either one 12-h separation interval or two 6-h separation intervals each day. It was anticipated that dividing the total separation time over 2 intervals might prevent the reduced growth commonly seen after the onset of IS, while still stimulating creep feed intake. Besides piglet feed intake and growth, the effect of the latter IS regimens on piglet behavior were studied and these results are presented in Chapter 3.

Since feed intake is normally rather low in the first weeks of age, it was questioned whether it is sensible to subject piglets to intermittent suckling at a young age (d 14). It was postulated that piglets have a higher feed intake when first submitted to intermittent suckling at a later age. If so, the time needed to reach a level of preweaning feed intake that ensures a continued substantial nutrient intake postweaning, ultimately preventing a postweaning growth check, might be shorter. Therefore besides the effect of separation interval (Chapter 2), the effect of piglet age at onset of IS and duration of IS on piglet pre- and postweaning feed intake and growth was studied (Chapter 4 and 7).

At present, most parameters used to investigate the effects of weaning on piglet gut characteristics are end-point measurements, require sacrificing of piglets (Montagne et al., 2007). However, we aimed to monitor postweaning intestinal function of piglets, whether or not subjected to a IS regimen, longitudinally. In this thesis two experiments were conducted to study parameters of interest that might enable us to monitor postweaning intestinal function in the same piglets for a prolonged period of time. In chapter 5 of the present thesis we investigated whether the *Physiogear*<sup>TM</sup> I wireless flowmeter is a suitable system to measure blood flow in group-housed conscious pigs (Chapter 5). If so, it might be a useful tool to

determine the role of hypoperfusion in intestinal dysfunction after weaning. Moreover, in the next chapter, the effect of weaning on plasma citrulline and intestinal fatty acid binding protein (I-FABP), both possible markers of postweaning intestinal function, was determined (Chapter 6).

In chapter 7 of the current thesis, results of the preceding chapters were integrated and the effect of IS regimens, differing in timing and duration, on piglet growth and feed intake as well as piglet postweaning intestinal function were investigated (Chapter 7).

Finally, results of the previous chapters were integrated and discussed in the general discussion (Chapter 8). In this chapter, the effects of separation interval, timing and duration of IS regimens on piglet growth, feed intake and gut characteristics are addressed. Also, the effect of extended lactation combined with IS on piglet behavior will be discussed. Finally, some perspectives of IS and suggestions for future research are presented.

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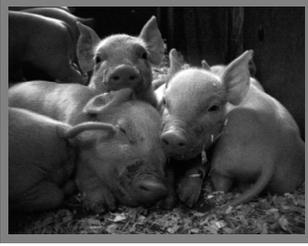
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## CHAPTER 2

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**POSTWEANING GROWTH CHECK IN PIGS IS MARKEDLY  
REDUCED BY INTERMITTENT SUCKLING AND  
EXTENDED LACTATION**

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## ABSTRACT

The objective of this study was to determine whether intermittent suckling (IS) combined with an extended lactation can reduce postweaning growth check in pigs. Three weaning regimens [conventional weaning (CW), IS with 6-h separation intervals (IS6), and IS with 12-h separation intervals (IS12)] were compared. In CW (n = 17 litters), litters had continuous access to the sow until weaning (d 21, d 0 = farrowing). In IS6 and IS12, litters were separated from the sow for 12h/d, starting at d 14 and lasting until weaning (d 41 to d 45). Litters were with the sow from 1400 to 2000 and from 0200 to 0800 (IS6, n = 14) or between 2000 and 0800 (IS12, n = 14). Litter size was standardized within 2 d after farrowing by crossfostering, resulting in an average litter size of  $10.9 \pm 1.8$  piglets. Piglets had ad libitum access to creep feed from d 7 onward. One week after the onset of IS (d 20), creep feed intake was increased in litters from both IS groups compared with CW litters ( $P < 0.05$ ). Both IS groups consumed considerable amounts of creep feed before weaning (d 41 to d 45). Total feed intake before weaning was greater ( $P = 0.004$ ) in IS12 ( $3,808 \pm 469$  g/piglet) than in IS6 ( $2,717 \pm 404$  g/piglet). In comparison, CW litters consumed  $18 \pm 9$  g/piglet before weaning (d 21). Irrespective of weaning regimen, total feed intake of litters before weaning was highly correlated with postweaning feed intake ( $P < 0.001$ ). Furthermore, in all treatment groups, total preweaning feed intake correlated with postweaning growth ( $P < 0.10$ ). Irrespective of treatment, piglets suckling anterior teats grew faster than piglets suckling middle or posterior teats during the first 2 wk of lactation. Body weights at the end of the experiment (d 55) were similar among weaning regimens. Onset of IS induced a growth check in both IS groups (34% for IS12 and 22% for IS6). Only a mild growth check was observed after weaning of IS litters (14% for both IS groups). However, a serious growth check (98%) was observed after weaning of CW litters. Results of the current study indicate that IS stimulated feed intake during lactation, providing a more gradual transition to weaning. Because the IS6 regimen did not prevent the growth check after the onset of IS and is rather laborious, we suggest that IS12 might be preferable for a practical implementation of IS.

## INTRODUCTION

In European, conventional pig husbandry, piglets are usually weaned at 3 to 4 wk of age. Weaning is associated with an abrupt dietary change, transport, altered housing, and mixing with unfamiliar pen mates. As a result, weaning is associated with reduced nutrient intake, reduced growth, and a greater susceptibility to diarrhea (van Beers-Schreurs et al., 1992; Nabuurs, 1998).

To familiarize piglets with creep feed before weaning, it is often provided during lactation. Piglets consuming an average amount of 610 g creep feed during lactation had an improved performance after weaning (d 28) compared with control piglets without creep feed supplementation during lactation (English et al., 1980). However, creep feed intake during lactation is usually low and highly variable between and within litters (Pajor et al., 1991).

Intermittent suckling (IS, separation of sow and piglets during a fixed period of the day) stimulates creep feed intake (Kuller et al., 2004a). In addition, IS mimics the increasing amount of time spent away from the piglets, when sows themselves can control contact with their piglets (Boe, 1991; Weary et al., 2002). Although creep feed intake during lactation is increased by IS, a total feed intake at weaning (d 25) of 600 g/piglet is accomplished in only a proportion (60%) of the litters (Kuller et al., 2004a). Onset of IS reduces piglet growth and results in lower weaning weights (Kuller et al., 2004a). In addition to IS, extending the lactation length might increase preweaning feed intake.

The aim of the current study was to determine whether combining IS with an extended lactation results in a less severe growth check at weaning. Intermittent suckling was applied in 2 regimens, with separation intervals of 6 h or 12 h, both with a total separation time of 12 h/d, and compared with a conventional weaning regimen. It was anticipated that dividing the total separation time over 2 intervals might prevent the reduced growth normally seen after the onset of IS, while still stimulating creep feed intake.

## MATERIALS AND METHODS

### **Animals, Housing and Diet**

The Ethical Committee of Wageningen University and Research Centre approved the experimental design, including all procedures involving animals.

The experiment was conducted in 3 batches, between March and December 2004, at De Haar Research Farm (Wageningen University and Research Centre, The Netherlands). Forty-seven multiparous sows (TOPIGS 40-line, TOPIGS, The Netherlands) were used in 3 batches consisting of 15, 15, and 17 sows. Sows were randomly selected from a breeding farm and had a parity ranging from 3 to 9. Sows were mated at the experimental farm and individually housed in the same room during gestation. One litter in batch 1 was omitted from data analysis due to the sow's low milk production, which resulted in severe weakness of the piglets in the second week of lactation. Another sow, in batch 2, was weaned within 2 d after farrowing, also because of low milk production, and her piglets were distributed across other litters of that batch. This resulted in a final number of 14, 14, and 17 sows in batches 1, 2, and 3, respectively.

Three weeks before farrowing, pregnant sows were individually housed in farrowing pens (2.15 m x 2.25 m), consisting of 4.39 m<sup>2</sup> of concrete floor and 0.45 m<sup>2</sup> of slatted floor. Two weeks before the expected day of farrowing, sows were provided with an intravascular catheter for another study under local (batch 1) or general (batch 2 and 3) anesthesia. From a few days before farrowing until weaning, sows were placed in farrowing crates (2.15 m x 1.00 m) within the farrowing pen to prevent crushing of the piglets. Each morning, the pen was cleaned and enriched with wood shavings. A heated piglet area was provided for the piglets by an infrared light from birth until d 10, and by floor heating. Artificial lighting was provided between 0800 and 2000 and was dimmed during the night.

Litter size at birth varied from 4 to 18 live piglets and was standardized within 2 d after farrowing by crossfostering within each batch, resulting in an average litter size of  $10.9 \pm 1.8$  piglets. One day after farrowing, piglets had their BW determined and were given an ear tattoo. Within 3 d of farrowing, piglets received an i.m. iron injection (Prevan 200, Eurovet Animal Health, Bladel, The Netherlands). Males were not castrated. Within each batch, the beginning of the experimental procedure (d 0) was designated as the day on which most litters were born. Litters were born from 2 d before to 3 d after d 0.

All piglets were offered creep feed ad libitum from d 7 onward. Creep feed was offered in a piglet feeder, with 2 feeding places (d 7 to d 13, 7 cm/feeding place; d 13 onwards, 15 cm/feeding place). From d 7 until 29, a milk-based commercial creep feed was offered (11.44 MJ NE/kg; 19% milk products; CP, 17.8%; 1.25% Lys, as-fed basis; Speen Select, Rijnvallei, The Netherlands). From d 31 until the end of the experiment (d 55), a commercial creep feed for weaner pigs was offered

(9.94 MJ NE/kg; 5% milk products; CP, 17.9%; 1.16% Lys, as-fed basis; Babybiggenkorrel<sup>†</sup>, Rijnvallei, The Netherlands). During a 2-d transition period (d 29 to 31), the 2 diets were mixed (1:1) to produce a gradual transition to the new diet. During the entire experiment, piglets had ad libitum access to drinking water, which was provided by 1 nipple per pen. Sows were fed an increasing amount of feed after farrowing until reaching the maximal allowance of 1% of BW plus 0.5 kg per piglet on d 11.

### Treatments

Sows in each batch were allocated according to parity and BW to 1 of 3 weaning regimens: conventional weaning (CW), IS with 12-h intervals (IS12) and IS with 6-h intervals (IS6); litters receiving different treatments were housed in separate rooms.

In CW litters (n = 17 litters), piglets had continuous access to the sow during a lactation period of 3 wk (d 0 to 21); CW litters were weaned on d 21, but the piglets remained in the farrowing pen until d 41. In IS litters, piglets had continuous access to the sow during the first 2 wk of lactation (d 0 to 14), and intermittent access from d 14 until weaning. In both IS groups, piglets had access to the sow for a total time of 12 h/d, but the distribution of these hours differed: in IS12 litters (n = 14), access to the sow was during a continuous period from 2000 to 0800; in IS6 litters (n = 14), access to the sow was from 1400 to 2000 and from 0200 to 0800. During separation from the litter, sows were housed individually in a different room. At night, during the return of IS6 sows to their litter, lights were on in all rooms. Weaning of IS litters occurred between d 41 and d 45 of lactation (d  $43 \pm 1$  on average).

At weaning (IS) or on d 41 (CW), each litter was moved from their farrowing pen to a fattening pen with a 2.63-m<sup>2</sup> slatted and 2.45-m<sup>2</sup> concrete floor. Litters remained in the fattening pens until the end of the experiment (d 55). Two IS litters were weaned earlier than scheduled; 1 IS12 litter due to a rectal prolapse of the sow on d 27 and 1 IS6 litter due to mastitis of the sow on d 37. For both of these litters, all data were included in data analysis, except for the data obtained after weaning in the litter weaned at 27 d.

### Measurements

All piglets were weighed 1 d after farrowing, and on d 7, 13, 16, 20, 23, 27, 34, 41, and 55 of the experiment. Piglets in IS treatments were also weighed at weaning

(W), and at 2 d (W2) and 6 d (W6) after weaning. Piglets of the CW treatment were moved to fattening pens at d 41, and weighed 2 (W2) and 6 d (W6) after moving. Creep feed residuals were determined simultaneously with BW of the piglets and on d 29 before transition of the creep feed diet. In case of moisture in creep feed residuals, DM percentage of the residuals was determined and creep feed intake was calculated.

During the first 2 wk of lactation, teat order was assessed for each litter because it might affect weight gain of piglets during lactation. Furthermore, we hypothesized that IS might have various effects on growth of piglets with a different teat order. Therefore, teat order was used as a covariate during statistical analysis. In accordance with Kim et al. (2000), teat order was defined as the specific teat (pair) nursed by each pig with respect to the anatomical location of the nursed mammary gland. Piglets were marked with a number on the back, and the position at the udder was recorded for each piglet during 3 suckling bouts on each of d 6 and 9 (6 observations total). Based on the preferred teat pair suckled by the piglets, a distribution of teat order in 3 classes was made: anterior (teat pairs 1 and 2), middle (teat pairs 3, 4, and 5), and posterior (teat pairs 6 and 7).

### **Calculations**

Cumulative feed intake at each time was calculated by summing the total feed intake of previous times. Missing values (< 2.5% of observations, and all before d 29) were estimated based on interpolation of the ADFI of the litter at the previous and next time. This estimation resulted in a deviation of  $4.3 \pm 3.2\%$  when differences between estimated and observed values on d 27 were expressed as percentage of the total feed intake on d 29. Cumulative feed intake was analyzed using the procedures described below for daily feed intake.

Growth check was defined as the reduction in ADG directly after the start of IS (d 14) or weaning. The growth check was calculated 2 d after the start of IS (d 14) and after weaning (d 41 to 45) in IS litters, and 2 d after weaning (d 21) in CW litters. The relative growth check (%) was calculated as:  $100[(ADG_{\text{before}} - ADG_{\text{after}})/ADG_{\text{before}}]$ .

### **Statistics**

Unless stated otherwise, data are presented as means  $\pm$  SE. Normally distributed data were analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC). Although litter was considered to be the experimental unit, all data were expressed per piglet.

Effects were considered significant if  $P < 0.05$ ; for posthoc testing, Bonferroni's procedure was used.

Because feed intake data in the first 3 wk (i.e., until d 23) were not normally distributed, they were analyzed using a nonparametric Kruskal-Wallis test. When this test detected an overall treatment effect, treatments were compared pairwise. From d 27 onward, feed intake was normally distributed and analyzed using the following model:

$$Y_{ijk} = \mu + T_i + B_j + A_k + BW1 + LS27 + e_{ijk},$$

where  $Y_{ijk}$  = ADFI per piglet in a weighing interval,  $\mu$  = the overall mean,  $T_i$  = treatment,  $B_j$  = batch,  $A_k$  = age on d 0,  $BW1$  = BW 1 d after farrowing,  $LS27$  = litter size on d 27, and  $e_{ijk}$  = residual error.

Data for BW and growth before weaning (IS) or moving (CW) were analyzed using the following model:

$$Y_{ijklmn} = \mu + T_i + B_j + S_k(T_i \times B_j) + A_L + G_m + TO_n + BW1 + e_{ijklmn},$$

where  $Y_{ijklmn}$  = piglet BW or ADG in a weighing interval,  $\mu$  = the overall mean,  $T_i$  = treatment,  $B_j$  = batch,  $S_k(T_i \times B_j)$  = sow nested within treatment and batch,  $A_L$  = age on d 0,  $G_m$  = sex,  $TO_n$  = teat order,  $BW1$  = weight 1 d after farrowing, and  $e_{ijklmn}$  = residual error. The effect of treatment was tested against  $S_k(T_i \times B_j)$  as the error-term.

Piglet BW and ADG from weaning (IS) or moving to fattening pens (CW) onward were analyzed using the following model:

$$Y_{ijkl} = \mu + T_i + B_j + S_k(T_i \times B_j) + G_l + WA + BW1 + e_{ijkl},$$

where  $Y_{ijkl}$  = piglet BW or ADG in a weighing interval,  $\mu$  = the overall mean,  $T_i$  = treatment,  $B_j$  = batch,  $S_k(T_i \times B_j)$  = sow nested within treatment and batch,  $G_l$  = sex,  $WA$  = age at weaning,  $BW1$  = weight 1 d after farrowing, and  $e_{ijkl}$  = residual error. The effect of treatment was tested against  $S_k(T_i \times B_j)$  as the error-term.

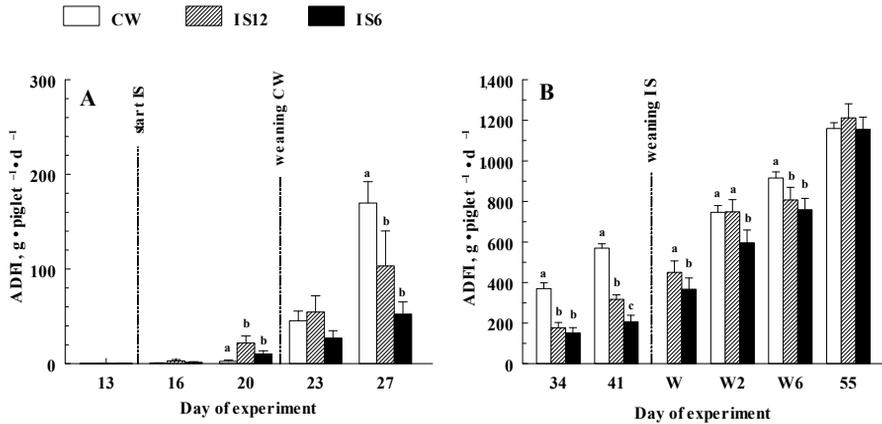
Correlations were calculated with Pearson correlation coefficients using SAS.

## RESULTS

### Feed Intake

Creep feed intake in the second week of lactation, before onset of IS, was negligible. One week after the beginning of IS (d 20), creep feed intake was greater in IS than in CW litters (Figure 2.1A;  $P < 0.05$ ). Weaning (d 21) increased feed intake in CW litters and resulted in a greater feed intake compared with IS litters from 1 wk after weaning CW (d 27) until weaning (d 41 to d 45) of IS litters

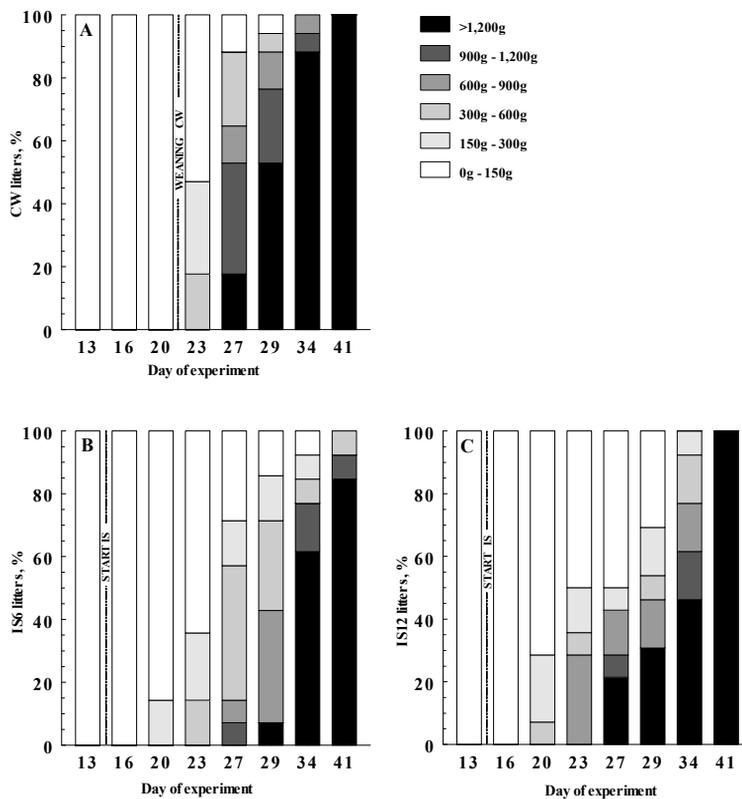
(Figure 2.1A and 2.1B;  $P < 0.01$ ). Feed intake was greater for IS12 litters than IS6 litters, but this difference only became significant just before weaning (d 41; Figure 2.1B;  $P < 0.001$ ) lasting until just after weaning (W2,  $P = 0.006$ ).



**Figure 2.1** Average daily feed intake of piglets per treatment until (A) and after (B) d 27 of the experiment. <sup>a-c</sup>Within each experimental period, differences between treatments ( $P < 0.05$ ) are indicated with different superscripts above the bars. The experiment began on d 0; intermittent suckling began on d 14. The conventional weaning litters (CW) were weaned on d 21, and intermittent suckling (IS) litters were weaned between d 41 and 45. IS12 = intermittent suckling with 12-h intervals; IS6 = intermittent suckling with 6-h intervals; W = Weaning of the IS treatments; W2 = 2 d after moving (CW, d 41) or weaning (IS); W6 = 6 d after moving (CW, d 41) or weaning (IS). Note differences between vertical scales between (A) and (B).

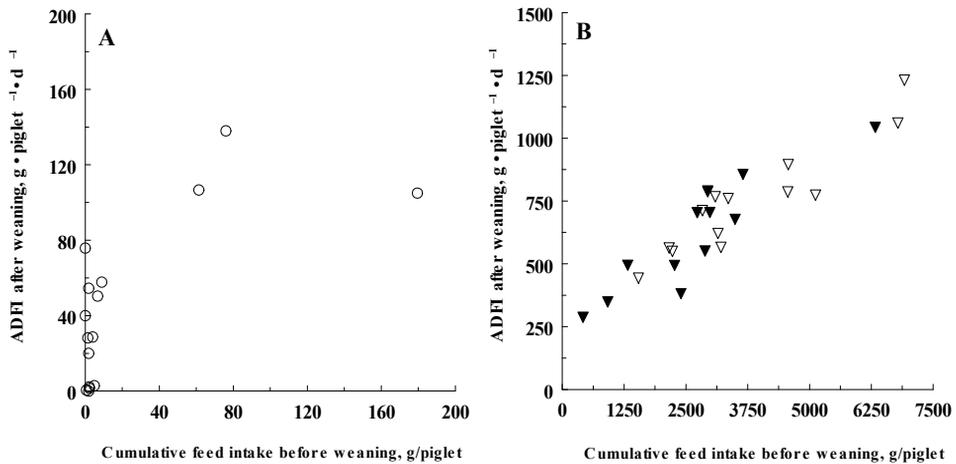
Cumulative feed intake was variable among litters, regardless of weaning regimen (Figure 2.2). In the CW treatment, cumulative feed intake before weaning (d 21;  $18 \pm 9$  g/piglet; maximum 140 g/piglet) in none of the litters exceeded 600 g/piglet, an amount demonstrated by English et al. (1980) to improve postweaning performance. Moreover, 82% of the CW litters consumed less than 10 g/piglet during lactation. Weaning (d 21) induced a sharp and rapid increase in feed intake in most CW litters within 2 d; and at 6 d postweaning, cumulative feed intake ranged from 8 to 1,568 g/piglet (d 27; Figure 2.2A). Despite their low feed intake levels during lactation, cumulative feed intake in CW litters before weaning (d 20) was correlated to feed intake directly (d 23) after weaning ( $r = 0.72$ ,  $P = 0.001$ ,

Figure 2.3A). In IS6 and IS12, IS induced a gradual increase in cumulative feed intake. Two weeks after onset of IS (d 27), cumulative feed intake of IS litters was highly variable, ranging from 6 to 1,992 g/piglet for IS12 ( $567 \pm 184$  g/piglet) and from 4 to 1,003 g/piglet for IS6 ( $355 \pm 72$  g/piglet). At this time (d 27), cumulative feed intake was greater in IS12 than in IS6 ( $P = 0.03$ ); 43% of the IS12 litters reached a cumulative feed intake above 600 g/piglet, compared with only 14% of the IS6 litters (Figure 2.2B and 2.2C).



**Figure 2.2** Percentage of litters distributed over 6 classes of cumulative feed intake (g/piglet) for conventional weaning (CW; A), intermittent suckling with 6-h intervals (IS6; B), and intermittent suckling with 12-h intervals (IS12; C) litters.

Cumulative feed intake at the end of lactation (d 41) was greater for IS12 litters ( $3,808 \pm 469$  g/piglet) than for IS6 litters ( $2,717 \pm 404$  g/piglet,  $P = 0.004$ ). At this time (d 41), cumulative feed intake of all IS12 litters (ranging from 1,526 to 6,918 g/piglet) and of 92% of the IS6 litters (ranging from 420 to 6,329 g/piglet) exceeded 600 g/piglet. In both IS treatments, cumulative feed intake at the end of lactation (d 41) was highly correlated to feed intake at 2 d postweaning ( $r = 0.93$  and  $r = 0.89$ , for IS12 and IS6,  $P < 0.001$ , Figure 2.3B). Although feed intake was highly variable between litters, feed intakes of consecutive experimental periods in general were highly correlated (data not shown).



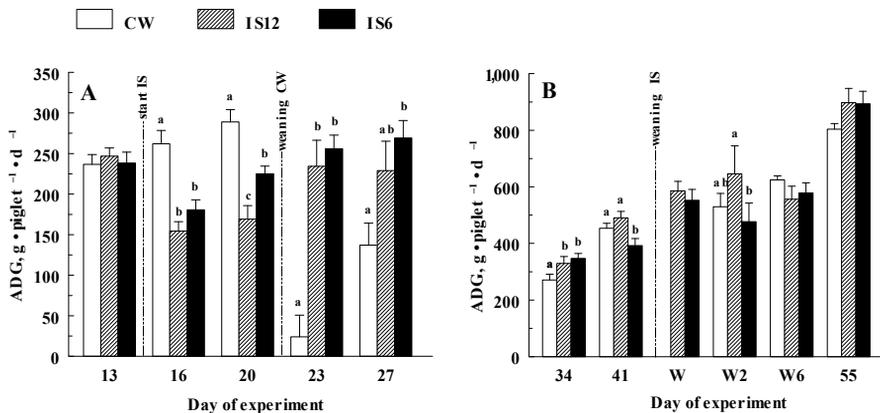
**Figure 2.3** Cumulative feed intake before weaning, in relation to average daily feed intake ( $\text{g} \cdot \text{piglet}^{-1} \cdot \text{d}^{-1}$ ) after weaning for conventional weaning (CW, A,  $\circ$ ) and IS (B,  $\blacktriangle$  for IS6 and  $\triangle$  for IS12) litters. IS12 = intermittent suckling with 12-h intervals; IS6 = intermittent suckling with 6-h intervals.

## Piglet Performance

The number of piglets treated for illness was similar in all treatments. Piglet mortality was greatest during the first 2 wk of the experiment, before the beginning of the treatments. A small percentage of the total piglet loss consisted of piglets, which were euthanized because of severe locomotor failure or BW loss. Piglet loss from d 14 until the end of the experiment (d 55) was greater in the CW treatment (9%) than in the IS6 treatment (2%;  $P = 0.01$ ); and intermediate in the IS12

treatment (4%). Most of these piglets died between d 13 and 20 with 73%, 100%, and 67% for CW, IS12, and IS6, respectively.

Piglet BW and growth were similar in all 3 treatments during the first 2 wk of the experiment. However, IS resulted in a reduced growth (d 16) in both IS treatments compared with the unweaned CW litters (Figure 2.4A;  $P < 0.001$ ). From d 16 to d 20, growth of IS litters was still lower than in the unweaned CW litters ( $P < 0.001$ ), and lower in IS12 than in IS6 litters ( $P = 0.04$ ). This decreased growth after IS resulted in a lower BW at d 20 for IS12 and IS6 than for CW (Table 2.1,  $P < 0.005$ ). However, the relative growth check after the beginning of IS (d 16) (34% for IS12; ranging from 4% to 78%, and 22% for IS6; ranging from -18% to 60%) was much less severe than that observed after (at d 23) weaning of CW litters (98%; ranging from 13% to 170%;  $P < 0.001$ ). In all treatments, the observed growth check was positively correlated with piglet growth in the preceding period ( $r = 0.57$ ,  $r = 0.53$ , and  $r = 0.48$  for IS12, IS6, and CW,  $P < 0.001$ ); piglets with a greater growth in the preceding period before IS or weaning (CW) suffered a more severe growth check than did piglets with a lower growth.



**Figure 2.4** Average daily gain of piglets per treatment until (A) and after (B) d 27 of the experiment. <sup>a,b</sup>Within each experimental period, differences between treatments ( $P < 0.05$ ) are indicated with different superscripts above the bars. The experiment began on d 0, intermittent suckling began on d 14. The conventional weaning (CW) litters were weaned on d 21, IS litters were weaned between d 41 and 45. IS12 = intermittent suckling with 12-h intervals; IS6 = intermittent suckling with 6-h intervals; W = Weaning of the IS treatments; W2 = 2 d after moving (CW; d 41) or weaning (IS); W6 = 6 d after moving (CW; d 41) or weaning (IS). Note differences between vertical scales between (A) and (B).

**Table 2.1** Body weight of piglets during lactation and after weaning<sup>1</sup>

Day	Treatment <sup>2</sup>			SE <sup>3</sup>
	CW	IS12	IS6	
13	4,011	4,135	4,111	98
16	4,838	4,599	4,670	110
20	6,097 <sup>a</sup>	5,364 <sup>b</sup>	5,582 <sup>b</sup>	109
27	6,736	6,981	7,426	130
41	11,812	12,823	12,789	264
55	21,842	23,059	22,364	479

<sup>a,b</sup> Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

<sup>1</sup> Both IS regimens began on d 14, and IS litters were weaned between d 41 to d 45. The CW litters were weaned on d 21.

<sup>2</sup> CW = conventional weaning (n = 17); IS12 = intermittent suckling with 12-h intervals (n = 14); IS6 = intermittent suckling with 6-h intervals (n = 14).

<sup>3</sup> The SE of the mean BW until d 27 was based on 14 litters (IS treatments); on d 41 and d 55 it was based on 13 litters (IS12).

At the end of lactation (d 41), piglet BW of IS litters did not differ ( $P = 0.25$ ) from that of CW litters (Table 2.1). Weaning of IS litters resulted in a small growth check (14%) for both IS treatments directly (at W2, IS6) or shortly (at W6, IS12) after weaning (Figure 2.4B). By the end of the experiment (d 55), piglet BW were similar ( $P = 0.23$ ) for all treatments (Table 2.1).

Teat order affected piglet growth only until d 16 of the experiment. In general and regardless of treatment, piglets suckling anterior teats had a greater growth rate than piglets suckling the middle or posterior teats ( $P < 0.001$ ); piglets suckling the middle teats had a greater growth rate than piglets suckling the posterior teats ( $P < 0.02$ ). Piglet growth in the second week of lactation was  $263.4 \pm 6.3$ ,  $236.5 \pm 5.9$ , and  $211.3 \pm 10.8$  g·piglet<sup>-1</sup>·d<sup>-1</sup> for piglets suckling anterior, middle, or posterior teats, respectively. On d 16, piglet growth remained greater for piglets suckling

anterior teats ( $213.8 \pm 7.9 \text{ g}\cdot\text{piglet}^{-1}\cdot\text{d}^{-1}$ ) compared with piglets suckling posterior teats ( $185.0 \pm 14.2 \text{ g}\cdot\text{piglet}^{-1}\cdot\text{d}^{-1}$ ;  $P = 0.012$ ). Regardless of treatment, the growth check after IS or weaning (CW) of piglets suckling anterior teats was greater than that of piglets suckling middle teats ( $P = 0.04$ ).

### **Relation Between Piglet Feed Intake and Growth**

In both IS treatments, feed intake and growth were correlated after the beginning of IS ( $r = 0.81$ ,  $P < 0.001$  for IS12 and  $r = 0.54$ ,  $P = 0.05$  for IS6). Interestingly, after onset of IS, the correlation between feed intake and piglet growth was consistently high for IS12 litters ( $r$  ranging from 0.81 to 0.93), but lower for IS6 litters during the first 2 wk of IS ( $r$  ranging from 0.40 to 0.67) and increased thereafter ( $r$  ranging from 0.77 to 0.92). For piglets weaned conventionally, feed intake and growth were highly correlated immediately after weaning ( $r = 0.90$ ,  $P < 0.001$ ). Furthermore, the weaning induced growth check of CW litters was negatively correlated to feed intake immediately after weaning ( $r = -0.77$ ,  $P < 0.001$ ), whereas the IS-induced growth check in IS litters was not correlated to feed intake immediately after onset of IS.

Total cumulative feed intake during lactation was correlated to piglet growth directly after weaning both in CW litters ( $r = 0.59$ ,  $P = 0.02$ ) and in IS litters ( $r = 0.83$  and  $P = 0.01$  for IS12;  $r = 0.69$  and  $P = 0.06$  for IS6).

## **DISCUSSION**

The objective of this study was to determine whether IS during extended lactation reduces the postweaning growth check in pigs. This study demonstrates that IS treatment combined with extended lactation, using 6-h or 12-h separation intervals, increases feed intake within 1 wk, prevents a severe growth check at weaning, and results in piglet BW at 55 d similar to conventional weaned pigs. In the current study, effects of IS and extended lactation were compared with conventional weaned litters. However, CW litters might have been exposed to less severe stressors at weaning than under practical conditions. For instance, piglets were not mixed or transported at weaning, and transition to the weaner diet occurred only 1 wk after weaning. So, effects of weaning on piglet performance might be even more pronounced under practical conditions than observed in piglets of the CW litters.

Under natural conditions, the weaning process may take 8 to 12 wk. During this period, young piglets make a gradual transition from a diet based on sow's milk to a nonmilk diet, ultimately achieving nutritional independence from the sow (for review, see Miller and Slade, 2003). This transition coincides with profound changes in intestinal function (for review, see Cranwell, 1995), enabling digestion and absorption of novel food sources. In European conventional pig husbandry, weaning generally takes place around 3 to 4 wk of age. The abrupt change of diet, together with the stressful events associated with weaning, result in a reduced nutrient intake and growth. This leads to dramatic changes in intestinal morphology and function (Hampson and Kidder, 1986; Nabuurs et al., 1993; van Beers-Schreurs et al., 1998) and predisposes piglets to an increased morbidity and mortality. Increased feed intake levels before weaning partially prevented the degeneration of the structure (Nabuurs et al., 1993) and the impaired functioning of the gut (Nabuurs et al., 1996; Kuller et al., 2004b) that are normally associated with weaning. Consistent with previous studies, preweaning feed intake in all treatments of the current study was quite variable (Barnett et al., 1989; Pajor et al., 1991) and highly correlated to postweaning feed intake (Bruininx et al., 2002). Conventional weaned piglets, however, had a very low creep feed intake before weaning and they suffered from a severe growth check after weaning. In contrast, all IS litters consumed a considerable amount of creep feed before weaning and showed only a mild growth check after weaning. In fact, the preweaning feed intake level of IS litters was correlated to postweaning performance directly after weaning, which is in line with previous findings (Kuller et al., 2004a). The importance of preweaning feed intake has also been reported by English et al. (1980), who observed that a substantial creep feed intake (610 g/piglet) during a 28-d lactation improved growth and feed intake levels after weaning compared with control piglets without supplementary feeding during lactation. Indeed, in the current study this threshold was amply exceeded during lactation (d 41) by all IS litters, except for 1 IS6 litter. Our results suggest that IS during an extended lactation provides both a trigger to stimulate feed intake and a gradual transition to weaning.

Intermittent suckling had already increased feed intake 1 wk after onset of IS compared with continuously suckled litters, and diminished the number of low uptake litters, as demonstrated previously (Kuller et al., 2004a). The effect of IS on feed intake before weaning has been studied previously and resulted either in an increased (Smith, 1960; Thompson et al., 1981; Kuller et al., 2004a) or unaltered (Chapple et al., 1989) feed intake. The absence of a positive effect of IS in the

latter study might have resulted from low palatability of the creep feed diet (Chapple et al., 1989). Cumulative feed intake of IS12 litters observed in the first 2 wk after IS was in agreement with a previous study (Kuller et al., 2004a), in which IS (12 h/d) was applied during the last 2 wk of a 28-d lactation period. Although the 2 wk with IS increased preweaning total feed intake in the latter study, only 60% of the litters exceeded 600 g/piglet. Extending the lactation period in the current study resulted in a gradual increase of this percentage in IS12 litters to 79% at 5 wk and up to 100% at the end of lactation. Moreover, feed intake levels shortly after weaning (d 41 to d 45) were comparable to those of CW litters that had been weaned for 3 wk at that time. These results suggest that IS during a lactation period of 5 wk might be sufficient to improve adaptation to the post-weaning situation for the majority of IS12 litters.

In the current study, IS increased feed intake of litters within 1 wk after onset compared with continuously suckled litters. Furthermore, high levels of feed intake were observed in all IS litters before weaning. However, caution must be used to attribute the beneficial effects on post-weaning performance in the IS litters entirely to the limited time for suckling. Weaning age itself might also have affected performance after weaning. Pluske et al. (2003) demonstrated that piglets weaned at 2 wk of age have a less developed gastrointestinal tract compared with piglets weaned at 4 wk of age. Moreover, development of the gastrointestinal tract of 2-wk-old weaned piglets might proceed differently after weaning to that of piglets weaned at an older age. Furthermore, 3-wk-old weaned piglets appeared to have a greater reduction in villus height (65%) compared with 5-wk-old weaned piglets (27%), and post-weaning recovery to an equivalent villus height took much longer (21 vs 7 d; Cera et al., 1988). So, the older age at weaning of IS litters compared with CW litters might have resulted in a more advanced development of the gastrointestinal tract at weaning and might have diminished the detrimental effects of weaning on intestinal morphology and function, ultimately leading to a better post-weaning performance. Conversely, the beneficial effects observed in this study cannot solely be ascribed to a longer lactation. The difference in feed intake between IS6 and IS12 litters demonstrates that the IS regimen, and thus limited suckling, also contributed to increased feed intake.

Distributing the total separation time (12 h/d) over two 6-h periods (IS6) resulted in lower feed intake levels during lactation compared with a 12-h period of separation per day (IS12). Despite a lower feed intake, ADG of IS6 litters was greater compared with IS12 litters 1 wk after onset of IS (d 20). These differences were

maintained, although not significantly, for up to 3 wk after IS began. This finding suggests that IS6 litters had a greater milk intake compared with IS12 litters. Previous research (Newton et al., 1987a,b) already demonstrated that piglets subjected to IS are capable to compensate for the resulting milk intake deficit in the remaining hours with access to the sow. We estimated (data not presented above) the percentage of feed-derived and milk-derived ADG for IS litters in each weighing interval, using the average gain-to-feed ratio of CW litters between d 27 to d 55. Although the percentage of milk-derived ADG decreased gradually during lactation for both IS treatments, it remained higher for IS6 (64%) compared with IS12 (55%) at the end of lactation (d 41), suggesting a greater dependency on milk for IS6 litters compared with IS12 litters. This is also in line with the lower correlation between feed intake and growth in the IS6 litters during the first 2 wk after onset of IS. The correlation between feed intake and growth in IS6 litters became stronger and more comparable to IS12 litters later in lactation. This suggests that the IS6 litters gradually became less dependent on milk intake for their growth, concomitant with a decrease in milk production of the sow as seen in a natural weaning process. In conclusion, distributing the total separation time (12 h/d) over two 6-h periods per day attenuated but did not prevent the decreased growth after onset of IS. Furthermore, IS6 litters remained more dependent on milk intake for their growth, which probably caused the lower feed intake levels during lactation.

Weaning induces a disruption of nutrient intake and thus luminal nutrition, with some piglets beginning to eat dry creep feed not before 50 h post-weaning (Bruininx et al., 2001). A continuous supply of nutrients after weaning has been shown to prevent crypt hypertrophy and the decrease of villous height associated with weaning, but only in case this supply was sufficiently high, i.e., by gastric intubation every 3 h (Kelly et al., 1991a, b) or 4 h (van Beers-Schreurs et al., 1998). Because the onset of IS disrupts the continuous supply of nutrients to the small intestine as with weaning, one might suggest that IS also negatively affects intestinal structure or function. Hartke et al. (2005) observed no detrimental effects on intestinal morphology, after a single short-term (6 h or 12 h) fasting, but IS piglets in the current study were subjected to repeated nonsuckling periods. Nabuurs et al. (1996), however, demonstrated that 2 wk of IS (8 h/d) without supplementary feeding, did not induce changes of intestinal structure or net fluid absorption of the small intestine at weaning (around d 30) in the small intestinal segment perfusion test. Furthermore, in the current study, negative effects of IS (12

h/d) on growth were very mild and short lasting compared with conventional weaning. Interestingly, 2 wk of IS (8 h/d) with supplementary feeding (Nabuurs et al., 1996) resulted in a reduced villous height and net fluid absorption at weaning (around d 30) but partially protected piglets from the decrease in villus height and intestinal net absorption 4 d after weaning compared with piglets from continuously suckled or IS litters without supplementary feeding. Altogether, it appears that IS does not per se negatively affect intestinal structure or function. Moreover, IS with supplementary feeding partially prevents negative effects of weaning on intestinal structure and function.

Weaning age has been reduced to 3 or 4 wk in European conventional pig husbandry. As a result, weaning is associated with reduced nutrient intake, reduced growth and a greater susceptibility for diarrhea (van Beers-Schreurs et al., 1992; Nabuurs, 1998). We demonstrated that extending lactation length in combination with IS (12 h/d) resulted in increased feed intake before weaning, prevented a severe growth check at weaning, and resulted in similar piglet BW at d 55 compared with conventional weaned piglets. This suggests that the combination of intermittent suckling and extended lactation results in a more gradual transition to weaning. However, practical implication of an extended lactation can only be profitable when the number of litters per sow is not diminished, which requires the onset of cyclicity before weaning. Recent studies demonstrated that IS can induce lactational estrus (Gerritsen et al., 2005a; Langendijk et al., 2005) without affecting pregnancy rate and early embryonic survival at d 23 of gestation (Gerritsen et al., 2005b). Hence, combining IS and extended lactation might be a practical management strategy (improving piglet post-weaning performance without compromising sow reproductive performance). Distributing total separation time (12 h/d) over two 6-h separation intervals had no added value; and considering this together with the fact that the IS6 regimen is rather laborious, we conclude that IS12 is the most practical for implementation.

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# CHAPTER 3

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## **INTERMITTENT SUCKLING DURING AN EXTENDED LACTATION PERIOD: EFFECTS ON PIGLET BEHAVIOR**

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## ABSTRACT

The objectives of the current study were to determine how intermittent suckling (IS) affects nursing behavior, litter activity, and general behavioral patterns during lactation, and whether IS during an extended lactation period results in behavioral patterns associated with piglet distress. Intermittent suckling was applied either with 6-h separation intervals (IS6) or with 12-h separation intervals (IS12) and was compared with the conventional treatment (CT). In the CT ( $n = 17$  litters), sows were continuously present until weaning (d 21, d 0 = farrowing). In both IS6 and IS12, sows were separated from their litter for 12 h/d, beginning at d 14 and lasting until weaning (d  $43 \pm 1$  d). In IS6, litters ( $n = 14$ ) and sows were separated from 0800 to 1400 and from 2000 to 0200; in IS12, litters ( $n = 14$ ) and sows were separated between 0800 and 2000. In IS litters, the activity pattern over the 24-h cycle was markedly changed by IS; litter activity was lower ( $P < 0.001$ ) during sow absence and greater ( $P < 0.001$ ) during sow presence compared with the unweaned CT litters. Moreover, both total nursing frequency ( $P < 0.001$ ) and the percentage ( $P < 0.002$ ) of successful nursings were reduced by IS. Although total nursing frequency was greater in IS6 compared with IS12 (on d 21 and d 28), no differences in the frequency of successful nursings existed between IS6 and IS12 from d 14 onward. Eating behavior was increased shortly after the onset of IS (d 17) in both IS6 ( $P = 0.059$ ) and IS12 ( $P < 0.001$ ) compared with the unweaned CT litters. The IS12 litters showed more eating behavior compared with IS6 and their exploratory behavior increased in time ( $P < 0.001$ ), whereas IS6 showed more nursing behavior. Aggressive or manipulative behavior of both IS treatments was similar compared with the unweaned CT, and remained relatively unaltered with time in IS12 and IS6. Weaning in the CT resulted in more manipulative ( $P < 0.001$ ) and aggressive ( $P = 0.004$ ) behavior compared with preweaning values. Intermittent suckling may contribute to adaptation to the postweaning state by stimulating eating behavior, without causing obvious behavioral distress.

## INTRODUCTION

The initial contact of piglets with nutrient sources other than milk often occurs through exploration of the environment (Fraser et al., 1998). To familiarize piglets with solid feed before weaning, the feed is often provided during lactation. However, in conventional housing, preweaning creep feed intake is usually low and highly variable between and within litters (Pajor et al., 1991). Compared with conventional housing, piglets kept in sow-controlled housing combined with a communal piglet area spend more than 40% of their time away from their dam, have a greater preweaning feed intake, and have a lower nursing frequency (Pajor et al., 2002). Similarly, intermittent suckling (IS; separating sow and piglets during a fixed period each day) increases preweaning feed intake (Berkeveld et al., 2007) and improves ADG immediately after weaning (Kuller et al., 2004). However, effects of IS on piglet behavior are currently unknown.

During IS, piglets are involuntarily separated from their sow. Enforced (repeated) maternal deprivation can cause behavioral and physiological changes, both in altricial mammals like rats (Kuhn and Schanberg, 1998) and precocious species like sheep (Napolitano et al., 2003) and calves (Haley et al., 2005). In piglets, a single overnight maternal separation results in greater basal plasma cortisol concentrations (Klemcke and Pond, 1991). Therefore, the extent to which repeated maternal separation (as applied in an IS regimen) affects piglet behavior and induces behavioral patterns associated with postseparation distress (Dybkjaer, 1992) might be questioned.

The objective was to study piglet behavior during IS by determining how IS affects nursing behavior, litter activity, and general behavioral patterns during lactation, and whether IS during an extended lactation results in behavioral patterns associated with piglet distress. In the current study, IS with separation intervals of 6 or 12 h was compared with a conventional treatment (CT).

## MATERIALS AND METHODS

### **Animals, Housing, and Diet**

The Animal Care and Use Committee of Wageningen University and Research Centre approved the following experimental animal use.

A total of 45 multiparous sows (TOPIGS 40-line, TOPIGS, Vught, the Netherlands) were used in 3 replicates consisting of 14, 14, and 17 sows,

respectively. Sows were randomly selected from a breeding farm, with parities ranging from 3 to 9. Sows were mated at the experimental farm and all were housed individually in the same room during gestation.

Three weeks before farrowing, pregnant sows were individually housed in farrowing pens (2.15 m x 2.25 m), consisting of 4.39 m<sup>2</sup> of concrete floor and 0.45 m<sup>2</sup> of slatted floor. From approximately 1 wk before the expected date of farrowing until weaning, sows were placed in farrowing crates (2.15 m x 1.00 m) within the farrowing pen to prevent crushing of piglets. Every morning, the pen was cleaned and the litters were provided with wood shavings. The piglet area was heated by an infrared light from birth until d 10, and by floor heating. Artificial lighting was provided between 0800 and 2000; no additional sunlight was present. During the night, light was dimmed to a level that enabled videorecording of sow and piglet behavior.

Litter size at birth varied from 4 to 18 live piglets and was standardized within 2 d after farrowing by cross-fostering, resulting in an average litter size of  $10.9 \pm 1.8$  piglets. Males were not castrated. In each replicate, the beginning of the experimental procedure (d 0), was designated as the day on which most litters were born. Litters were born from 2 d before to 3 d after d 0.

All piglets were offered creep feed ad libitum from d 7 onward in a piglet feeder with 2 feeding places. During the entire experiment, piglets had unlimited access to drinking water, which was provided by 1 drinking nipple per pen.

## **Treatments**

Before parturition, sows in each replicate were allocated, according to parity, BW, and backfat, to 1 of 3 treatments: 1) CT, 2) intermittent suckling with 12-h intervals (IS12), or 3) intermittent suckling with 6-h intervals (IS6). Litters receiving different treatments were housed in separate rooms.

In CT litters (n = 17), sows were continuously present in the farrowing pen with their litters throughout the 21-d lactation period; CT litters were weaned on d 21 by removal of the sow and the piglets remained in the farrowing pen until d 41. In the IS treatments, sows were separated from their litters for 12 h/d from d 14 of lactation onward. In both IS groups, sows were present for a total of 12 h/d, but the distribution of these hours differed. In IS12 litters (n = 14) this was a continuous period from 2000 to 0800; and in IS6 litters (n = 14) separation was from 1400 to 2000 and from 0200 to 0800. During separation from their litters, sows were housed individually in a different room. Weaning of IS litters occurred between d

41 and d 45 of lactation (mean, d  $43 \pm 1$ ). Litters were moved from their farrowing pen to a nursery pen at weaning (IS) or on d 41 (CT). The nursery pen consisted of 2.63 m<sup>2</sup> of slatted floor and 2.45 m<sup>2</sup> of concrete floor. Litters remained in the nursery pens until the end of the experiment at d 55. Two IS litters were weaned earlier than scheduled: 1 litter (IS12) because of a rectal prolapse of the sow (d 27) and 1 litter (IS6) because of mastitis of the sow (d 37). For both of these litters, all data gathered until weaning were included in the analysis.

## Measurements

*Piglet Performance and Feed Intake.* Individual piglets were weighed 1 d after farrowing and on d 7, 13, 16, 20, 23, 27, 34, 41, and 55. Creep feed residuals were determined per litter simultaneously with BW of the piglets and on d 29. Data on piglet performance and feed intake have been published previously (Berkeveld et al., 2007) but are briefly summarized in the following paragraph.

One week after onset of IS (d 20), creep feed intake was increased in both IS treatments compared with the CT. The ADFI was numerically greater in IS12 litters compared with IS6 litters throughout lactation, resulting in a greater cumulative feed intake in IS12 litters compared with IS6 litters at weaning. Onset of IS induced a growth check in both IS groups, resulting in lower BW at d 20 compared with the CT. A serious growth check was observed after weaning of CT litters (98%), but in IS litters only a mild growth check was observed (14%). Body weights at the end of the experiment were similar among treatments.

*Teat Order.* During the first 2 wk of lactation, teat order was assessed for each litter based on the methodology of Kim et al. (2000). In each litter, 2 piglets were selected from cranial (teat pairs 1 and 2), middle (teat pairs 3, 4, and 5) and caudal (teat pairs 6 and 7) teat order (6 in total) and observed during the scan-sampling recordings, as described below.

*Behavioral Measurements.* Continuous 24-h, time-lapse videotape recordings were obtained at d 10, 14, 16, 21, and 28. The 24-h recordings were continuously analysed for litter activity and nursing behavior. Litters were considered active if at least 50% of the piglets demonstrated behavior other than lying. Observations on nursing behavior included beginning time and duration of each nursing, termination by the sow or piglets, and position of the sow (lying or standing)

during nursing. A nursing was considered to begin when at least 50% of the piglets were actively massaging the udder and was considered to end when more than 50% of the litter had left the udder or were not actively manipulating the udder (Valros et al., 2002). The nursing was terminated by the sow if she moved from a left or right side lying position to sternal recumbancy or to a standing position. In case of nursing while standing (motionless), lying down, or walking up and down in the farrowing crate were defined as the end of nursing. The nursing was said to be terminated by the piglets if more than 50% of the litter was not actively manipulating the udder. In addition to the duration of nursings, the interval between nursings was calculated as the difference in beginning time between 2 consecutive nursings. Preliminary analyses revealed that the successive nursings shortly after the return of IS sows followed each other rapidly. Because previous research (Weary et al., 1999) indicated that successful nursings rarely occur within 20 min, a nursing in the current study was considered to be unsuccessful when the consecutive nursing began within a 20-min interval. Both total number of nursings and successful nursings were used for analysis of nursing behavior.

On d 17, 24, and 37, the behavior of 6 piglets per pen was observed live for 30 min every 2 h by using 2-min instantaneous scan sampling, thus providing 180 observations per piglet per day. Observations were recorded using a Psion Organiser II LZ64 data recorder installed with the Observer software package (Noldus Information Technology BV, Wageningen, the Netherlands). Piglets were uniquely marked with color spray for individual identification. The different types of behaviors recorded during scan sampling were based on an ethogram previously described (Bolhuis et al., 2005) and are presented in Table 3.1.

### **Calculations**

Litter activity and nursing behavior were summed per day, and are referred to hereafter as total litter activity and total nursing time or frequency. Furthermore, observations on litter activity were assigned and summed in 4 periods: morning, 0800 to 1400; afternoon, 1400 to 2000; evening, 2000 to 0200; and night, 0200 to 0800. After the onset of IS, sows of IS litters were present in only 2 periods: in the evening and night in IS12 litters, and in the afternoon and night in IS6 litters. In addition to summing activity per period, for IS litters activity was also summed during the periods of absence or presence of the sow. Litter activity during the sow's absence (or presence; min/12 h) was expressed as the percentage of total litter activity on that day (min/d).

**Table 3.1.** Ethogram used during 2-min scan sampling observations

Behavior	Description
Eating behavior	Eating from the food trough or chewing feed
Exploratory behavior	Sniffing, touching, or rooting (substrate on) the floor or part of the pen above floor level Scraping the floor with one of the forelegs Nibbling, chewing, or biting part of the pen above the floor level Chewing particles other than feed (e.g. wood shavings, feces) or vacuum chewing
Aggressive behavior	(Mutual) ramming or pushing pen mate with the head, with or without biting
Manipulative behavior	Belly nosing Nibbling, sucking, or chewing any part of a pen mate
Social behavior	Touching, sniffing, or mounting a pen mate
Play behavior	Running across the pen, occasionally accompanied by nudging a pen mate gently Jumping and turning around the body axis Lying on back and moving from side to side on the floor Shaking of head while holding material (e.g. wood shavings) that protrudes from mouth Pushing or gentle snout contact with no retaliation by the recipient
Nursing behavior	Suckling movements with teat in the mouth or with the nose in contact with the udder Attempting to find a teat by walking and pushing other piglets while most piglets are nursing
Sow-directed behavior	Touching, sniffing, nibbling, sucking or chewing any part of the sow, except for the udder
Inactivity	Lying on the side or belly with open or closed eyes without performing any other described behavior

Some of the video observations were missing because of inadequate recordings, mostly caused by technical problems. In the CT, 3 litters had missing values (1.9% of total observations). In the IS12 treatment, 1 litter had missing values (0.4% of total observations). In the IS6 treatment, 2 litters had missing values (1.5% of total observations).

Behavioral observations of each piglet were summed per day. The behavioral elements were summed per category per day (see Table 3.1). The proportion of total observations spent per behavioral category was calculated by dividing the number of observations in the behavioral category by the total observations per day.

### **Statistical Analysis**

Unless stated otherwise, data are presented as means  $\pm$  SE. Effects were considered significant if  $P < 0.05$ , and a tendency if  $0.05 \leq P < 0.10$ ; in post-hoc testing, the Bonferroni correction was applied. All statistical analyses were performed by using SAS (SAS Inst. Inc., Cary, NC). Normally distributed data were analyzed by using PROC MIXED (Littell et al., 1998). Correlations were calculated by using Pearson correlation coefficients for normally distributed data and Spearman correlation coefficients for skewed data. Correlations between behavioral observations and piglet ADFI or ADG were calculated. Because ADFI and ADG were not determined on a daily basis, the ADFI or ADG of the period closest to the day of behavioral observations was used.

Both data on total nursing time and total litter activity were analyzed by using PROC MIXED, with age on d 0, treatment, day, and their interaction as fixed factors, replicate as a random factor and sow (nested within treatment and replicate) as repeated measurement, with an autoregressive covariance structure. The effect of treatment was tested against sow as the error term. Data on litter activity over periods were analyzed by using the same model, but with period and its interaction with treatment and day as additional fixed factors.

Data on the proportion of total observations spent per behavioral category (scan sampling) were analyzed by using PROC MIXED, with age on d 0, treatment, day and their interaction as fixed factors, replicate and sow (nested within replicate and treatment) as random factors and piglet (nested within treatment, replicate and sow) as repeated measurement, with an autoregressive covariance structure. The effect of treatment was tested against the random sow effect. Because no significant effect of teat-order class on piglet behavior was observed, it was omitted from the

analyses. In case of skewed distribution of residuals, various proportions of behavior were arcsine square root-transformed to obtain homogeneity of variances. Transformation of proportions did not result in a normal distribution of residuals for eating, aggressive, and manipulative behavior. These behaviors and the percentage of standing nursings were analyzed by using a nonparametric Kruskal-Wallis test. If this test detected an overall treatment effect, data were subsequently tested pairwise.

## RESULTS

### Effect of IS on Litter Activity

Total litter activity on d 10, before onset of IS, was similar in all 3 treatments (Table 3.2).

**Table 3.2.** Total activity (min/d) of litters (means  $\pm$  SE) of continuously suckled pigs (CT) or intermittently suckled pigs with short (IS6) or long (IS12) separation intervals<sup>1</sup>

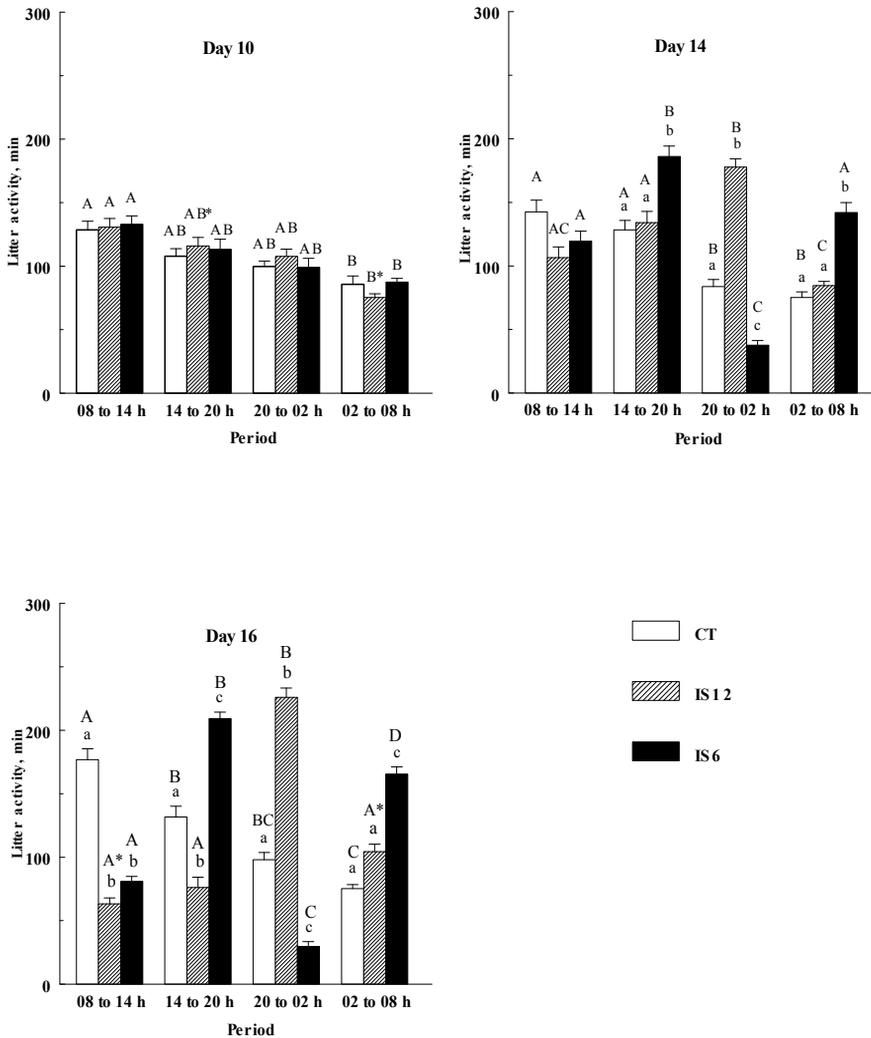
Day <sup>2</sup>	Treatment		
	CT n = 17	IS12 n = 14	IS6 n = 14
10	411.8 <sup>xy</sup> $\pm$ 16.7	429.6 <sup>x</sup> $\pm$ 17.0	434.7 $\pm$ 16.5
14	423.7 <sup>axy</sup> $\pm$ 13.5	502.9 <sup>by</sup> $\pm$ 15.5	483.1 <sup>ab</sup> $\pm$ 20.2
16	474.0 <sup>x</sup> $\pm$ 14.5	469.7 <sup>xy</sup> $\pm$ 10.7	483.1 $\pm$ 12.0
21	397.4 <sup>y</sup> $\pm$ 16.9	428.1 <sup>x</sup> $\pm$ 13.9	447.7 $\pm$ 17.5
28	378.4 <sup>ay</sup> $\pm$ 21.1	466.6 <sup>b,xy</sup> $\pm$ 13.7	479.4 <sup>b</sup> $\pm$ 18.0

<sup>a,b</sup> Within a row, means with different superscript letters differ ( $P < 0.05$ ).

<sup>x,y</sup> Within a column, means with different superscript letters differ ( $P < 0.05$ ).

<sup>1</sup> Results obtained by continuous observations. CT = conventional treatment; IS12 = intermittent suckling with 12-h intervals; IS6 = intermittent suckling with 6-h intervals. Both intermittent suckling regimens began on d14, and intermittent suckling litters were weaned between d 41 to d 45. CT litters were weaned on d 21.

<sup>2</sup> Day of the experiment; d 0 designated as the day on which most of the litters were born.



**Figure 3.1. Litter activity (min; mean  $\pm$  SE) per period on day 10, 14, and 16 of lactation.**

CT = conventional treatment (d 21); IS12 = intermittent suckling regimen with 12-h separation intervals; IS6 = intermittent suckling regime with 6-h separation intervals. After the onset of intermittent suckling (d 14), IS12 sows were present between 2000 and 0800 and IS6 sows were present between 1400 and 2000 and between 0200 and 0800. Different lowercase letters on bars of one period indicate differences between treatments ( $P < 0.05$ ). Different uppercase letters on bars of one treatment indicate differences in time ( $P < 0.05$ ). \* Indicates a tendency for a difference ( $0.10 > P > 0.05$ ).

Litter activity was greatest in the morning and decreased gradually in all treatments during the afternoon, evening, and night, resulting in a lower activity at night ( $P < 0.04$ ) compared with the morning in all 3 groups (Figure 3.1). This pattern was maintained throughout lactation in litters of the CT. During the first day of the IS regimen (d 14), total litter activity in IS12 litters was greater ( $P = 0.04$ ) compared with the CT piglets; activity in IS6 litters was intermediate (Table 3.2). Furthermore, the onset of IS resulted in a marked change in the activity pattern over the 24-h cycle in the IS litters (Figure 3.1). After 2 d of IS (d 16), litter activity in IS12 litters was lower ( $P < 0.001$ ) during the absence of the sow (morning and afternoon) and greater ( $P < 0.001$ ) during the first 6 h after return of the sow (evening) compared with the CT litters (Figure 3.1). Similarly, IS6 activity was also lower ( $P < 0.001$ ) during absence of the sow (morning and evening) and greater ( $P < 0.001$ ) during the presence of the sow (afternoon and night) compared with the CT litters (Figure 3.1). The activity pattern observed on d 16 was maintained on d 21 and d 28 for all treatments (data not shown).

Total litter activity was similar between treatments on d 16 and 21, but not on d 28, when litters in both IS treatments had greater activity ( $P < 0.02$ ) compared with the weaned litters of the CT (Table 3.2). The CT litters had lower ( $P < 0.001$ ) activity after weaning at d 21 and 28 compared with preweaning values at d 16. Although total litter activity was similar in both IS groups between d 14 and 28, the proportion of litter activity observed during absence of the sow was greater in IS12 litters compared with IS6 litters (Figure 3.2).

### **Effect of IS on Nursing Behavior**

Total nursing time and frequency on d 10, before the beginning of IS, was similar in all 3 treatments (Table 3.3). On d 14 and 16, total nursing frequency was lower ( $P < 0.001$ ) in IS litters compared with CT litters, but no differences existed between both IS treatments (Table 3.3). On d 14 and 16, total nursing time of IS12 litters was also lower ( $P < 0.001$ ) compared with CT litters; IS6 was intermediate. However, if total nursing time of litters was expressed as nursing time per hour of time together (24 h for CT and 12 h for IS litters), IS6 litters spent more time (14.2 min/h;  $P < 0.001$ ) nursing on d 16 compared with CT litters (8.5 min/h); IS12 litters were intermediate (11.0 min/h). At d 21 and 28, IS6 litters had a greater ( $P < 0.02$ ) nursing frequency compared with IS12 litters. Total nursing time, however, was not different between the IS treatments on both days.

**Table 3.3.** Nursing behaviors (means  $\pm$  SE) of continuously suckled pigs (CT) or intermittently suckled pigs with short (IS6) or long (IS12) separation intervals<sup>1</sup>

Nursing Behavior	Treatment	Day of experiment <sup>2</sup>					
		10	14	16	21	28	
Total nursing time, min/d	CT	217.1 $\pm$ 11.6	204.1 <sup>a</sup> $\pm$ 8.6	202.4 <sup>a</sup> $\pm$ 11.2	--	--	
	IS12	208.7 <sup>x</sup> $\pm$ 12.1	133.0 <sup>y,ab</sup> $\pm$ 7.4	132.0 <sup>y,b</sup> $\pm$ 9.8	148.2 <sup>y</sup> $\pm$ 6.5	160.3 <sup>y</sup> $\pm$ 7.8	
	IS6	206.2 <sup>x</sup> $\pm$ 11.8	163.4 <sup>y,ab</sup> $\pm$ 9.6	169.9 <sup>y,ab</sup> $\pm$ 6.6	165.3 <sup>y</sup> $\pm$ 8.6	169.4 <sup>y</sup> $\pm$ 12.7	
Total nursing frequency, n/d	CT	36.7 $\pm$ 1.3	35.4 <sup>a</sup> $\pm$ 1.4	34.9 <sup>a</sup> $\pm$ 1.2	--	--	
	IS12	37.2 <sup>x</sup> $\pm$ 0.9	24.1 <sup>y,ab</sup> $\pm$ 1.0	24.5 <sup>y,b</sup> $\pm$ 1.1	20.9 <sup>z,a</sup> $\pm$ 0.8	21.5 <sup>z,a</sup> $\pm$ 0.7	
	IS6	34.8 <sup>x</sup> $\pm$ 1.3	25.4 <sup>y,b</sup> $\pm$ 1.1	25.8 <sup>y,b</sup> $\pm$ 1.0	25.8 <sup>y,b</sup> $\pm$ 1.1	24.3 <sup>y,b</sup> $\pm$ 0.8	
Successful nursings, %	CT	93.6 $\pm$ 1.6	92.7 <sup>a</sup> $\pm$ 2.0	93.4 <sup>a</sup> $\pm$ 1.5	--	--	
	IS12	90.6 <sup>x</sup> $\pm$ 1.9	72.7 <sup>y,ab</sup> $\pm$ 2.7	77.0 <sup>z,b</sup> $\pm$ 3.7	84.6 <sup>z,a</sup> $\pm$ 3.2	86.4 <sup>x,a</sup> $\pm$ 3.2	
	IS6	91.8 <sup>x</sup> $\pm$ 1.8	75.0 <sup>y,b</sup> $\pm$ 3.0	76.4 <sup>y,b</sup> $\pm$ 2.3	72.9 <sup>y,b</sup> $\pm$ 3.5	74.4 <sup>y,b</sup> $\pm$ 2.4	
Successful nursing frequency, n/d	CT	34.1 $\pm$ 0.8	32.5 <sup>a</sup> $\pm$ 0.8	32.4 <sup>a</sup> $\pm$ 0.8	--	--	
	IS12	33.6 <sup>x</sup> $\pm$ 0.6	17.3 <sup>y,b</sup> $\pm$ 0.6	18.4 <sup>y,b</sup> $\pm$ 0.5	17.4 <sup>y</sup> $\pm$ 0.3	18.3 <sup>y</sup> $\pm$ 0.4	
	IS6	31.7 <sup>x</sup> $\pm$ 0.7	18.7 <sup>y,b</sup> $\pm$ 0.3	19.5 <sup>y,b</sup> $\pm$ 0.4	18.4 <sup>y</sup> $\pm$ 0.4	17.9 <sup>y</sup> $\pm$ 0.4	

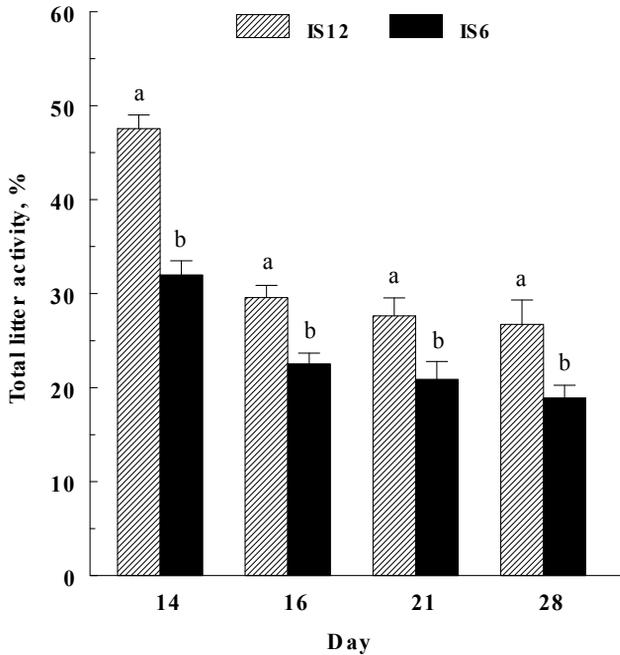
	CT	0	3.8 ± 2.4	1.8 <sup>a</sup> ± 1.3	--	--
Standing nursings, %	IS12	1.2 <sup>x</sup> ± 1.2	5.3 <sup>xy</sup> ± 2.0	5.6 <sup>y<sup>b</sup></sup> ± 2.1	3.8 <sup>xy</sup> ± 2.1	5.5 <sup>y</sup> ± 1.6
	IS6	4.5 ± 4.5	4.7 ± 1.3	6.0 <sup>b</sup> ± 3.5	4.7 ± 1.8	3.8 ± 1.8
Sow-ended nursings, %	CT	36.1 ± 4.8	37.6 ± 3.8	38.9 <sup>ab</sup> ± 5.2	--	--
	IS12	39.1 ± 6.8	41.7 ± 6.2	48.1 <sup>a</sup> ± 5.8	42.1 <sup>a</sup> ± 4.4	42.8 ± 6.0
	IS6	31.9 ± 3.3	30.6 ± 3.9	29.2 <sup>b</sup> ± 2.7	27.5 <sup>b</sup> ± 4.4	34.1 ± 4.2

<sup>a,b</sup> Differences between treatments per day are indicated with different superscripts ( $P < 0.05$ ).

<sup>x-z</sup> Differences between days within treatments are indicated with different subscripts ( $P < 0.05$ ).

<sup>1</sup> Results obtained by continuous observations. CT = conventional treatment; IS12 = intermittent suckling with 12-h intervals; IS6 = intermittent suckling with 6-h intervals. Both intermittent suckling regimens started on d14 and intermittent suckling litters were weaned between d 41 to d 45. The CT litters were weaned on d 21.

<sup>2</sup> Day of the experiment; d 0 was designated as the day on which most litters were born



**Figure 3.2. Percentage of total litter activity observed during absence of the sow in both intermittent suckling treatments (%; mean  $\pm$  SE).** Intermittent suckling regimens began on d 14. IS12 = intermittent suckling regime with 12-h separation intervals; IS6 = intermittent suckling regime with 6-h separation intervals. After the onset of intermittent suckling (d 14), IS12 sows were present between 2000 and 0800 and IS6 sows were present between 1400 and 2000 and between 0200 and 0800. Different letters on bars indicate differences between treatments ( $P < 0.05$ ).

Intermittent suckling reduced the percentage of successful nursings in both IS treatments compared with with the CT ( $P < 0.002$ ; Table 3.3). In contrast to the IS6 treatment, the percentage of successful nursings increased gradually from d 14 to 28 in the IS12 treatment. This resulted in a greater ( $P < 0.002$ ) percentage of successful nursings in the IS12 treatment on d 21 and 28 (Table 3.3) compared with the IS6 treatment. However, no differences in frequency of successful nursings existed between the 2 IS treatments (Table 3.3).

Within each treatment, the percentage of sow-ended nursings was unaltered over time (Table 3.3). However, on d 16 and 21, a lower percentage ( $P < 0.04$ ) of sow-ended nursings was observed in IS6 litters compared with IS12 litters.

**Table 3.4.** Piglet behavior (proportion of total observations; %) of CT and IS litters on d 17, 24 and 37 of the experiment<sup>1</sup>.

Behavior, % of total observations	CT			IS12			IS6		
	d <sup>2</sup> 17	d 24	d 37	d 17	d 24	d 37	d 17	d 24	d 37
Inactive	65.8 ± 1.8 <sup>xy,a</sup>	73.5 ± 1.5 <sup>x,b</sup>	62.2 ± 1.4 <sup>x,c</sup>	69.8 ± 1.5 <sup>x,a</sup>	64.9 ± 2.5 <sup>y,b</sup>	61.6 ± 2.0 <sup>x,b</sup>	63.7 ± 0.7 <sup>y,a</sup>	65.6 ± 1.4 <sup>y,ab</sup>	68.8 ± 1.6 <sup>y,b</sup>
Eating behavior*	0.1 ± 0.1 <sup>x,a</sup>	5.0 ± 0.7 <sup>x,b</sup>	7.1 ± 0.7 <sup>x,c</sup>	0.8 ± 0.3 <sup>y,a</sup>	1.5 ± 0.6 <sup>y,b</sup>	4.3 ± 0.6 <sup>y,c</sup>	0.4 ± 0.2 <sup>x,a</sup>	0.5 ± 0.1 <sup>z,a</sup>	1.8 ± 0.2 <sup>z,b</sup>
Exploratory behavior	5.1 ± 0.4 <sup>a</sup>	11.7 ± 1.5 <sup>x,b</sup>	15.0 ± 0.9 <sup>x,c</sup>	4.9 ± 0.4 <sup>a</sup>	9.3 ± 0.9 <sup>xy,b</sup>	10.9 ± 1.0 <sup>y,b</sup>	6.5 ± 0.6	7.6 ± 0.6 <sup>y</sup>	8.2 ± 0.9 <sup>y</sup>
Social behavior	1.7 ± 0.1 <sup>xy,a</sup>	2.1 ± 0.2 <sup>xy,a</sup>	3.8 ± 0.3 <sup>x,b</sup>	1.3 ± 0.1 <sup>x,a</sup>	2.5 ± 0.3 <sup>x,b</sup>	2.4 ± 0.2 <sup>y,b</sup>	2.0 ± 0.2 <sup>y</sup>	1.5 ± 0.2 <sup>y</sup>	1.5 ± 0.2 <sup>z</sup>
Aggressive behavior*	0.5 ± 0.1 <sup>a</sup>	0.5 ± 0.1 <sup>a</sup>	1.0 ± 0.2 <sup>x,b</sup>	0.6 ± 0.1	0.8 ± 0.1	0.5 ± 0.1 <sup>y</sup>	0.6 ± 0.1	0.6 ± 0.2	0.3 ± 0.1 <sup>y</sup>
Manipulative behavior*	0.3 ± 0.0 <sup>a</sup>	0.8 ± 0.4 <sup>a</sup>	2.4 ± 0.4 <sup>x,b</sup>	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.1 <sup>y</sup>	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1 <sup>y</sup>
Play behavior	0.5 ± 0.1 <sup>x,a</sup>	0.4 ± 0.1 <sup>x,a</sup>	0.7 ± 0.1 <sup>x,b</sup>	0.5 ± 0.1 <sup>x</sup>	0.6 ± 0.1 <sup>xy</sup>	0.5 ± 0.1 <sup>x</sup>	0.9 ± 0.1 <sup>y,a</sup>	0.7 ± 0.1 <sup>y,a</sup>	0.2 ± 0.1 <sup>y,b</sup>
Nursing behavior	18.0 ± 1.2 <sup>x</sup>	-----	-----	14.2 ± 0.7 <sup>y</sup>	13.7 ± 0.7 <sup>x</sup>	12.9 ± 0.7	17.8 ± 1.1 <sup>x,a</sup>	17.3 ± 1.0 <sup>y,a</sup>	12.9 ± 1.2 <sup>b</sup>
Sow-directed behavior	2.1 ± 0.3	-----	-----	1.3 ± 0.2	1.5 ± 0.2	1.2 ± 0.1	1.7 ± 0.2	1.9 ± 0.2	1.6 ± 0.3

<sup>a-c</sup> For each treatment, means with different superscripts are different ( $P < 0.05$ ), indicating differences between days within each treatment.

<sup>x-z</sup> For each day, means with different superscripts are different ( $P < 0.05$ ), indicating differences between treatments per day.

<sup>1</sup> Results were obtained scan sampling observations. CT = conventional treatment; IS12 = intermittent suckling with 12-h intervals; IS6 = intermittent suckling with 6-h intervals. Both intermittent suckling regimens started on d14, and intermittent suckling litters were weaned between d 41 to d 45. CT litters were weaned on d 21.

<sup>2</sup> Day of the experiment; d 0 was designated as the day on which most of the litters were born.

\* Differences of behavior marked with an asterisk were tested nonparametrically

### **Effect of IS on Piglet Behavior**

The proportion of total observations ( $n = 180$ ) spent per behavioral category is presented in Table 3.4. Three days after the onset of IS (d 17), the proportion of total observations spent inactive in IS12 litters was greater compared with IS6 litters ( $P = 0.04$ ), but inactivity in both IS treatments was similar to the CT. Inactivity of IS12 litters decreased over time ( $P < 0.001$ ). In contrast, inactivity of IS6 litters increased over time ( $P < 0.001$ ; d 37) resulting in a greater inactivity compared with IS12 litters on d 37 ( $P = 0.01$ ). Weaning of CT litters (at d 21) resulted in a transient increase in inactivity (d 24;  $P < 0.001$ ).

Shortly after the onset of IS (d 17), eating behavior in both IS treatments was greater compared with the CT ( $P < 0.001$  and  $P = 0.06$ , for IS12 and IS6, respectively). Although eating behavior increased in both IS treatments with time, it was greater ( $P < 0.02$ ) from d 17 onward in the IS12 treatment compared with the IS6 treatment.

On d 17 (3 d after start IS), the proportion of total observations spent on exploratory behavior was similar for all treatments. In both the IS12 and conventional treatments, exploratory behavior increased over time ( $P < 0.001$ ), whereas it was relatively stable over time in IS6 litters.

On d 17, time spent on aggressive or manipulative behavior was similar in all treatments. The proportion of total observations spent on manipulative behavior was relatively unaltered over time for both IS treatments but increased over time in CT litters ( $P < 0.001$ ). Both on d 24 and 37, a large proportion of the manipulative behavior in the weaned CT litters consisted of belly nosing ( $41.5 \pm 6.9\%$  and  $57.5 \pm 4.6\%$  of manipulative behavior, respectively). Belly nosing was not, or only rarely, observed in the unweaned IS litters (0% and 0.1% of total observations for the IS12 and IS6 litters, respectively, on d 37). The incidence of aggressive behavior was quite low and, similarly to manipulative behavior, remained relatively unaltered over time in both IS treatments. Aggressive behavior was greater in CT litters 2 wk after weaning (d 37), compared with preweaning values (d 17;  $P = 0.01$ ).

A considerable proportion of total observations was spent on nursing behavior in all treatments on d 17. Nursing behavior was lower ( $P < 0.04$ ) in the IS12 litters compared with both the CT and IS6 litters. Interestingly, IS did not reduce the proportion of total observations spent on nursing behavior in IS6 litters compared with CT litters. The difference in nursing behavior between both IS treatments

lasted until d 24 ( $P = 0.006$ ), but disappeared on d 37 because of a sharp decrease in nursing behavior in IS6 litters ( $P < 0.001$  compared with d 17).

### Relation Between Behavior and Feed Intake or Performance

Feed intake of litters was correlated with eating behavior of the sow's 6 selected piglets in all treatments on d 17 and 24 (Table 3.5). On d 37, irrespective of treatment, no correlation between eating behavior and feed intake of litters was observed.

**Table 3.5.** Relationship between eating behavior and ADFI (g/piglet daily) of continuously suckled pigs (CT) or intermittently suckled pigs with short (IS6) or long (IS12) separation intervals<sup>1</sup>

Feed intake period <sup>2</sup>	Treatment	Day of scan sampling	Correlation coefficient	<i>P</i> -value
d 16 to d 20	CT	d 17	0.57	0.016
	IS12	d 17	0.92	< 0.001
	IS6	d 17	0.94	< 0.001
d 23 to 27	CT	d 24	0.64	0.006
	IS12	d 24	0.95	< 0.001
	IS6	d 24	0.64	0.015
d 34 to 41	CT	d 37	-0.04	0.89
	IS12	d 37	0.13	0.66
	IS6	d 37	0.36	0.23

<sup>1</sup> CT = conventional treatment; IS12 = intermittent suckling with 12-h intervals; IS6 = intermittent suckling with 6-h intervals. Both intermittent suckling regimens started on d14, and intermittent suckling litters were weaned between d 41 to d 45. CT litters were weaned on d 21.

<sup>2</sup> ADFI was calculated per experimental period; d 0 was designated as the day on which most litters were born.

In the CT and IS6 treatments, total litter activity was correlated with feed intake only on d 16, when a tendency was found in the IS6 treatment ( $r = 0.49$ ,  $P = 0.09$ ). However, at d 21 and 28, total litter activity and feed intake were positively

correlated in the IS12 treatment (Table 3.6). This correlation was even greater when only activity during the sow's absence was used for the calculations on d 21 and 28 and was not found when only the activity during the sow's presence was used.

Total nursing time and frequency were correlated with feed intake only on d 28, when total nursing time of IS12 litters showed a negative correlation with feed intake ( $r = -0.65$ ,  $P = 0.02$ ). Total nursing time was correlated with piglet growth of CT litters on d 14 ( $r = -0.50$ ,  $P = 0.05$  for successful nursings) and for IS12 litters on d 16 ( $r = 0.63$ ,  $P = 0.02$  for all nursings and  $r = 0.70$ ,  $P = 0.005$  for successful nursings) and on d 28 ( $r = -0.61$ ,  $P = 0.03$  for all nursings). Frequency of nursings was only positively correlated with litter growth on d 10 in the IS6 treatment ( $r = 0.50$ ,  $P = 0.08$  for all nursings and  $r = 0.64$ ,  $P = 0.02$  for successful nursings).

**Table 3.6.** Relationship between piglet activity and ADFI (g/ piglet daily) on d 21 and 28 in intermittently suckled piglets with a long separation interval (IS12)<sup>1</sup>

Feed intake <sup>2</sup>	Activity	Correlation coefficient	P-value
ADFI d 20 to 23	Total activity d 21	0.67	0.009
ADFI d 20 to 23	Activity during sow's absence d 21	0.80	< 0.001
ADFI d 20 to 23	Activity during sow's presence d 21	0.15	0.60
ADFI d 27 to 29	Total activity d 28	0.66	0.01
ADFI d 27 to 29	Activity during sow's absence d 28	0.89	< 0.001
ADFI d 27 to 29	Activity during sow's presence d 28	-0.41	0.16

<sup>1</sup> Intermittent suckling started on d 14, and intermittently suckled litters were weaned between d 41 to d 45. IS12 = intermittent suckling with 12-h intervals (n = 14).

<sup>2</sup> ADFI was calculated per experimental period; d 0 was designated as the day on which most litters were born.

## DISCUSSION

The objectives of the current study were to determine how IS with an extended lactation period would affect litter activity, nursing behavior, and general behavioral patterns during lactation, and whether it would result in altered behavioral patterns associated with piglet distress. The behavioral observations

described in this paper were part of a larger experiment, which was focussed on the effect of weaning regimen, IS in combination with extended lactation vs. conventional, on post-weaning piglet performance and feed intake (Berkeveld et al., 2007). The main objective of that study was to investigate whether IS during extended lactation was a suitable management strategy to improve postweaning piglet performance. As a result, the 2 IS regimens were compared with a CT, and the experimental design lacked a weaning regimen with only an extended lactation (without IS). Because CT litters were weaned at d 21, only short-term effects of IS on piglet behavior, during the first week of the regimen, are compared with CT litters. Additionally, differences in piglet behavior throughout lactation between the 2 IS treatments are discussed. However, caution must be used in attributing changes in the behavioral pattern of IS litters entirely to the IS regimen. Age might also have had an influence on the observed piglet behavior during extended lactation.

Under (semi)natural conditions, piglets are weaned gradually, between 11 to 17 wk of age (Jensen, 1986; Stolba and Wood-Gush, 1989; Boe, 1991). During this period, they make a gradual transition from sow's milk to a nonmilk diet. During the weaning process, the nursing frequency and the number of sow-initiated nursings decrease, whereas the number of sow-ended nursings and the time spent away from the piglets increase (Jensen, 1988; Jensen and Recen, 1989). Similar to free-ranging sows, both the time spent in the lactation pen and the number of nursings decrease during lactation for sows in a sow-controlled housing system (Rantzer et al., 1995; Weary et al., 2002). As expected, the onset of IS in the current study resulted in a sharp decrease in nursing frequency in both IS treatments. Interestingly, the frequency of successful nursings in both IS treatments after the onset of IS corresponded closely to the decreased nursing frequency found in a sow-controlled housing system during a 12-h presence of the sow (Pajor et al., 2002). In contrast to free-ranging sows, and irrespective of treatment, the percentage of sow-ended nursings was similar over time. Moreover, it was lower (on d 14) compared with 55 % on d 13 postpartum reported previously (Valros et al., 2002). The difference in proportion of sow-ended nursings can probably be attributed to the housing conditions of sows during lactation (with vs. without a farrowing crate). Maternal behavior has a great influence on observed nursing behaviours (Pitts et al., 2002). The small sample size in the current study may have been inadequate to overcome individual differences and reveal differences between treatments in nursing behavior. As a result, the lack of treatment or time effects

should be interpreted with care, especially when variation within treatments is high.

The greater nursing frequency in IS6 litters compared with IS12 litters might indicate that IS6 litters maintained a stronger motivation for nursing behavior compared with the IS12 litters, which is supported by the lower proportion of total observations spent on ingestive behavior. The difference in nursing frequency and the greater percentage of unsuccessful nursings were probably caused by a high nursing frequency directly after reunion with their dams, which occurred twice daily in IS6 litters and only once daily in IS12 litters. On the other hand, the IS12 sows may have been less compliant to nurse their litter than IS6 sows, because the percentage of sow-ended nursings was greater in IS12 litters. Although the number of successful nursings was comparable between both IS treatments, the IS6 litters had a greater growth in the first week following IS (Berkeveld et al., 2007) while creep feed intake was still relatively low. Considering this, it might be questioned whether the defined 'unsuccessful' nursings (nursings followed by a consecutive nursing within 20 min) were indeed without any milk yield for the piglets. However, the findings are similar to data indicating that a shorter interval between nursings leads to a greater proportion of unsuccessful nursings, but also results in a greater milk yield (27%) and greater growth (44%) of litters compared with litters with longer nursing intervals (Špinka et al., 1997). Furthermore, a greater nursing frequency results in a greater mammary gland mass (Auldust et al., 2000). Interestingly, nonnutritive nursings increase plasma prolactin concentration, which increases proliferation of mammary glands and promotes lactational performance (Rushen et al., 1993; Špinka et al., 1999). Plasma prolactin concentrations of sows decrease rapidly within the first 2 h after removal of piglets, but are restored rapidly to normal lactation levels after reunion with their litters (Bever et al., 1978). After weaning, a reduction of plasma prolactin concentrations to a constant level of 1 to 2 ng/ml takes place within 5 to 6 h (Bever et al., 1978; Rojkittikhun et al., 1991). To our knowledge, no studies are available describing a diurnal rhythm of prolactin secretion in lactating sows, eliminating a possible effect of differences in light regimen. Therefore, we hypothesize that in the current study, sows in the IS6 treatment might have maintained greater prolactin levels because of shorter separation intervals compared with sows in the IS12 treatment, possibly resulting in greater milk production and subsequently greater litter growth.

The greater total litter activity in IS treatments on d 14 may have been the result of restless activity associated with a sudden, previously unexperienced separation

from the sow (Fraser et al., 1998), because this difference completely disappeared on d 16. After the onset of the IS regimens, litter activity coincided closely with presence of their dam, resulting in dramatic changes in 24-h activity patterns compared with CT litters. Nevertheless, the influence of light and dark on the activity of litters should also be recognized. The IS12 litters were separated from their sows during the day, but one separation period of the IS6 litters was during the evening, when the lights were dimmed. As observed in the CT litters, piglets are normally less active during the dark period compared with the light period. Therefore, some of the observed differences in litter activity pattern can be attributed to the light regimen. However, as shown in Figure 3.1 (d 16), the contact frequency with their dams had a stronger influence on the activity pattern of IS litters than the light regimen. In addition, Kirkwood and Smith (1983) demonstrated that piglets separated from their sows overnight consumed similar amounts of feed compared with piglets with daytime separations (12 h). The greater contact frequency of IS6 litters with their dams might account for the attenuated stimulation of eating behavior, and consequently lower feed intake compared with IS12 litters. Interestingly, the observed differences in both eating and nursing behavior between IS treatments agree with a greater estimated dependency on milk for their growth in IS6 litters compared with IS12 litters (Berkeveld et al., 2007). In conclusion, we postulate that the observed differences in piglet behavior of IS litters were mainly attributable to the separation regimen, and were slightly influenced by light conditions.

In line with previous findings, weaning of CT litters resulted in an increased inactivity, which might be considered a symptom of stress (Colson et al., 2006). However, we suspect that the increased inactivity of IS6 litters in late lactation is most probably explained by the decrease in nursing behavior and was an indication of the piglets' comfort in their environment, rather than a sign of distress. Several behavioral patterns are known to be associated with postseparation piglet distress (Dybkjaer, 1992), including belly nosing, manipulating pen mates, and aggression. Belly nosing is most probably associated with the need to suckle (Metz and Gonyou, 1990; Gonyou et al., 1998). The absence of belly nosing in IS litters, maternally deprived from d 14 onward, might indicate that complete weaning is necessary to induce development of belly-nosing behavior, because weaning of CT litters at d 21 induced belly nosing. Similarly, the weaned CT litters demonstrated a greater proportion of total observations spent on manipulative behavior and aggression, whereas these behavioral patterns were absent in IS litters. Results of

the current study indicate that IS during an extended lactation is not associated with development of behavioral patterns indicative of postseparation piglet distress, suggesting that repeated maternal separation does not induce the behavioral patterns associated with complete separation of sow and piglets after weaning. However, no conclusions can be drawn for the behavioral development after weaning, because no behavioral observations were made in IS litters after complete weaning.

In conclusion, IS reduced the nursing frequency, and litter activity largely coincided with the presence of the sow during an extended lactation period. Separation of the sow and piglets during 12 consecutive hours in IS12 litters reduced the time spent on nursing behavior and, similarly to weaned CT litters, increased the time spent on exploratory and ingestive behavior during lactation, possibly improving adaptation to the postweaning state. Distribution of total separation time (12h/d) over 2 periods synchronized litter activity even more to the presence of the sow, because they spent more time on nursing and less on eating behavior. Finally, both IS regimens were not associated with any behavioral patterns indicative of piglet distress. As such, an intermittent suckling regimen may provide a gradual adaptation to the postweaning situation without causing obvious behavioral distress.

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# CHAPTER 4

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## **FEED INTAKE AND GROWTH OF PIGLETS SUBMITTED TO INTERMITTENT SUCKLING REGIMENS DIFFERING IN TIMING AND DURATION**

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Submitted

## ABSTRACT

The aim of this study was to determine how age at the start of an intermittent suckling regime (IS) and the duration of the regimen affect pre- and postweaning piglet feed intake and performance. Sows were continuously present until the start of the IS regime, and separated from their litter for 12 h each day during IS until final weaning. Intermittent suckling started at d 14 (IS14) or d 21 (IS21) of lactation, and litters were either weaned after ~ 1 wk of IS (IS14-S and IS21-S; n = 17 and n = 14, respectively) or ~ 4 wk of IS (IS14-L and IS21-L; n = 17 and n = 16, respectively). Piglets had ad libitum access to creep feed from 1 wk before the onset of IS onward. One wk of IS (d 14 to 21) increased creep feed intake of IS14 litters compared to the IS21 litters that were still suckling continuously in that period ( $P < 0.001$ ). Postponing the onset of IS from 2 to 3 wk of age increased feed intake during the first wk of IS considerably ( $P < 0.005$ ). Moreover, the reduction in growth after weaning of IS-S litters was of shorter duration for IS21-S than for IS14-S litters. In IS21-L litters, feed intake during the first and second wk of IS was similar to feed intake in IS14-L litters of the same age (in their second and third wk of IS). Weaning of IS-L litters increased their feed intake markedly and resulted in a postweaning ADFI similar to IS-S litters that were weaned earlier. Postweaning ADG was only slightly decreased in IS14-L, and even increased in IS21-L litters compared to their preweaning ADG. At the end of the experiment (d 49 to 56), there were no differences in BW, growth or feed intake between the 4 IS treatments. In conclusion, onset of IS at an older age (d 21) markedly improved feed intake stimulation, and shortened the period of postweaning growth reduction when piglets were weaned after 1 wk of IS. Combining IS with an extended lactation period apparently resulted in a more gradual adaptation to weaning, irrespective of age at onset, in terms of piglet growth and feed intake.

## INTRODUCTION

Weaning of piglets is associated with a reduced nutrient intake (van Beers-Schreurs and Bruininx, 2002) and reduced piglet growth (Okai et al., 1976; Colson et al., 2006). A greater creep feed intake during the preceding suckling period results in greater postweaning feed intake and growth (Bruininx et al., 2002). However, creep feed intake during the suckling period is usually low and highly variable between and within litters (Pajor et al., 1991).

Intermittent suckling (IS), separation of sow and piglets during a fixed period of the day, stimulated creep feed intake during the suckling period (Kuller et al., 2004; Kuller et al., 2007). As a result, IS litters (onset at d 14, weaned at d 26) had an improved feed intake and growth in the first wk postweaning compared with conventionally weaned litters (Kuller et al., 2004). Moreover, combining IS with an extended lactation resulted in a gradual adaptation to weaning, i.e. markedly reduced the postweaning growth check compared to conventional weaning (Berkeveld et al., 2007b; Chapter 2). However, feed intake during the first wk of IS, when IS started at d 14 of lactation, was rather low and quite variable between litters (Kuller et al., 2004; Berkeveld et al., 2007b; Chapter 2). In a conventional 4-wk suckling period, a large proportion of total feed intake is consumed during the last wk before weaning (Pajor et al., 1991; Bruininx et al., 2004). The question therefore arises, whether it is sensible to subject piglets to an IS regime at a time normally associated with low voluntary feed intake. In this light, it is postulated that piglets have a higher feed intake when first submitted to intermittent suckling at a later age (e.g. 21 d). If so, the time needed to reach a level of preweaning feed intake that ensures a continued substantial nutrient intake postweaning, ultimately preventing a postweaning growth check, might be shorter.

The aim of the current study, therefore, was to determine the effect of piglet age at onset of IS and duration of IS on pre- and postweaning piglet feed intake and growth. Intermittent suckling began at d 14 or d 21 of lactation and was applied for a short ( $\approx 1$  wk) or prolonged period ( $\approx 4$  wk).

## MATERIALS AND METHODS

### **Animals, Housing and Diet**

The Ethics Committee for animal experiments of Wageningen University and Research Centre approved the following experimental design.

The experiment was conducted between March 2006 and March 2007, at De Haar Research Farm (Wageningen University and Research Centre, the Netherlands). A total of 64 multiparous sows (Topigs40-line, Topigs, Vught, the Netherlands) with an average parity of  $5.0 \pm 0.2$  were randomly selected from commercial breeding farms, and used in 5 replicates. Piglets were either Topigs40 x TEMPO crossbreed (replicate 2 and 3) or Topigs40 x Pietrain crossbreed (replicate 1, 4, and 5).

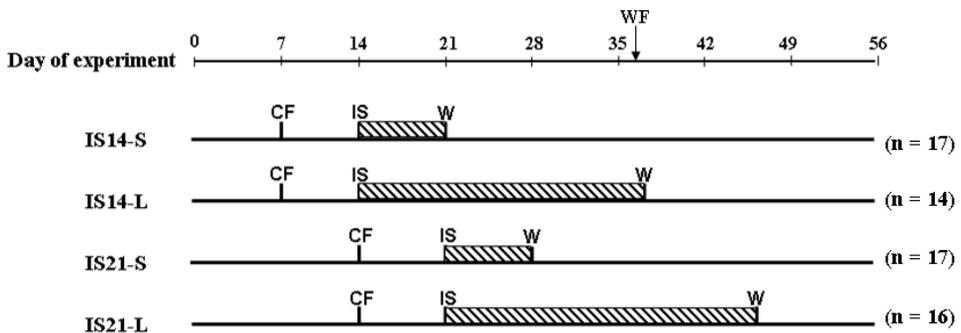
One week before farrowing, pregnant sows were individually housed in farrowing pens with a farrowing crate to prevent crushing of piglets. The farrowing pens (2.15 x 2.25 m) consisted of 4.39 m<sup>2</sup> of concrete floor and 0.45 m<sup>2</sup> of slatted floor. Each morning, the pen was cleaned and wood shavings were provided as bedding. A heated area was provided for the piglets by an infrared light during the first 10 d of life, and by floor heating. Artificial lighting was provided between 0800 and 2000 and was dimmed during the night.

Litter size was standardized within 2 d after farrowing by cross-fostering, resulting in an average litter size of  $10.9 \pm 0.3$  piglets. One day after farrowing piglets were weighed and received an ear tattoo for individual identification. Within 3 d of farrowing, piglets received an i.m. iron injection (Prevan 200, Eurovet Animal Health, Bladel, The Netherlands). Within each replicate, the beginning of the experimental procedure (d 0) was designated as the day at which most of the litters were born. Litters were born from 2 d before to 2 d after d 0.

To ensure an equal level of habituation to creep feed between treatments, litters were offered creep feed ad libitum from 1 wk before start of IS (Figure 4.1). Creep feed was offered in a piglet feeder with 2 feeding places (first week: 7 cm/feeding place; from the second week onward: 15 cm/feeding place). Until d 35 of the experiment, a milk-based commercial creep feed was offered (11.44 MJ NE/kg; 19% milk products; CP 17.8%; 1.25% lysine; Rijnvallei, Wageningen, The Netherlands). From d 37 until the end of the experiment (d 56), a commercial creep feed for weaner pigs (9.94 MJ NE/kg; 5% milk products; CP 17.9%; 1.16% lysine; Rijnvallei, Wageningen, The Netherlands) was offered to the piglets of all treatments. During a 2-d transition period (d 35 to d 37), the 2 diets were mixed (1:1) to ensure a gradual transition to the new diet. During the entire experiment drinking water was continuously available with 1 drinking nipple per pen. Sows were fed an increasing amount of feed (9.15 MJ/kg NE, 140 g/kg CP, 7.7 g/kg lysine) after farrowing until they attained the maximal allowance of 1% of BW plus 0.5 kg per piglet on d 11.

## Treatments

Within each replicate, litters were allocated to one of 2 groups differing in timing of IS, according to sow parity and sow BW. Litters were subjected to IS from d 14 (IS14;  $n = 34$  litters) or d 21 (IS21;  $n = 30$  litters) of lactation onward. Litters of both IS groups were housed in separate, but identical, farrowing rooms to prevent possible disturbing effects of nursing IS21 litters in the same room, when IS14 litters were separated from their sow. During IS, sows were separated from their litter for 12 h/d (from 0745 until 1945) and housed individually in a different room to prevent any visual or auditory contact with their piglets. This separation from their litters was expected to induce ovulation in the sows within a wk after onset of IS; the reproductive aspects were part of a separate study (Gerritsen, 2007).



**Figure 4.1.** Schematic presentation of the interventions within each treatment.

CF = start of creep feed; WF = start of weaner diet; IS = onset of intermittent suckling; W = weaning. Shaded bars indicate period of IS. IS14-S = IS started at d 14 and litters were weaned at  $d 21.8 \pm 0.4$ ; IS14-L = IS started at d 14 and litters were weaned at  $d 39.9 \pm 0.3$ ; IS21-S = IS started at d 21 and litters were weaned at  $d 27.7 \pm 0.2$ ; IS21-L = IS started at d 21 and litters were weaned at  $d 46.9 \pm 0.4$ .

According to the time of ovulation of the sow, as determined by transrectal ultrasonography, litters of both IS groups were allocated to either a short (weaning at ovulation; IS-S) or a long intermittent suckling period (weaning 20 d after ovulation; IS-L). This resulted in 4 different IS treatments (see Figure 4.1), with litters subjected to: 1) IS from d 14 and weaned between d 18.5 and 25.5 ( $d 21.8 \pm 0.4$ ;  $n = 17$ ; IS14-S treatment) 2) IS from d 14 and weaned between d 36 and 41 ( $d$

39.9 ± 0.3; n = 17; IS14-L treatment) 3) IS from d 21 and weaned between d 26.5 and 29.5 (d 27.7 ± 0.2; n = 14; IS21-S treatment), or 4) IS from d 21 and weaned between d 42 and 49 (d 46.9 ± 0.4; n=16; IS21-L treatment). At weaning, each litter was moved from its farrowing pen to a nursery pen. The nursery pen consisted of 2.63 m<sup>2</sup> of slatted floor and 2.45 m<sup>2</sup> concrete floor. Litters remained in their nursery pen until the end of the experiment (d 56).

### **Measurements**

Piglets were weighed individually on the first day after farrowing, and at weekly intervals (experimental day 14, 21, 28, 35, 42, 49 and 56). Creep feed residuals (g per litter) were determined simultaneously with weighings of the piglets. Around weaning, piglets were weighed on the day of weaning (W), and at 3 d (W3) and at 7 d (W7) after weaning instead of the standard weekly intervals.

### **Calculations**

Cumulative feed intake per litter at each weighing day was calculated by summing the total feed intake up to that weighing day, and expressed as average per piglet.

To be able to compare possible effects of IS regimens on piglet growth, relative piglet growth was calculated. Relative piglet growth was defined as the body weight piglets gained between two subsequent weighings expressed as percentage of the weight at the first weighing, and calculated as:  $[(BW_{\text{weighing2}} - BW_{\text{weighing1}})/BW_{\text{weighing1}}] * 100\%$ .

The IS litters subjected to a short IS period were weaned either at d 21.8 ± 0.4 (IS14-S litters) or at d 27.7 ± 0.2 (IS21-S litters). To compare performance and feed intake of all treatments on d 21 and 28, piglet BW and feed intake of IS14-S litters that were weaned before d 21 (i.e. between d 18.5 and 20; n = 4) and of IS21-S litters that were weaned before d 28 (i.e. d 25.5 and 27; n = 6), were estimated by linear extrapolation, based on their preweaning growth and feed intake (between d 14 and weaning or d 21 and weaning for IS14-S and IS21-S, respectively).

### **Statistics**

Data are presented as means ± SE. All statistical analyses were performed with a PROC MIXED model using SAS (SAS Inst. Inc., Cary, NC). Correlations were calculated using Pearson correlation coefficients. Although litter was used as the experimental unit, all data are expressed as average per piglet. Effects were

considered significant if  $P < 0.05$ , and a tendency if  $0.05 \leq P < 0.10$ ; in post-hoc testing the Bonferroni correction was applied.

Data on piglet BW and growth were analyzed using PROC MIXED with treatment and replicate as fixed factors, sow as random factor and birth weight and age at d 0 as co-variable. Since litter was considered to be the experimental unit, average piglet BW and ADG per litter were used in the above mentioned model. Interactions between treatment and replicate were never observed, and therefore the interaction was excluded from the analyses.

Data on feed intake were analyzed using PROC MIXED with treatment and replicate as fixed factors, sow as random factor, and age at d 0, BW at d 14, and litter size at d 14 as co-variable. Since interactions between treatment and replicate were never observed, the interaction was excluded from the analyses.

In an attempt to investigate possible influences of breed in more detail, replicate was replaced by breed as a fixed factor in the above mentioned models. Although there were significant effects of breed on BW, ADG and ADFI on some of the experimental days (greater values for Pietrain than for TEMPO crossbreed in general), there was no interaction between treatment and breed during statistical analysis. Therefore, effect of breed is not further discussed in the current paper.

## RESULTS

The percentage of piglets treated for illness from d 14 up to d 56 was similar in all treatments ( $3.5 \pm 0.5\%$ ), and mainly consisted of joint inflammation in the legs. From d 14 until the end of the experiment (d 56), piglet loss (1.8%) was similar in all treatments.

### Piglet Feed Intake

Feed intake of IS14 litters 1 wk before onset of IS (d 7 to 14) was negligible. Between d 14 and 21 feed intake was increased by IS in IS14 litters compared to that of continuously suckled IS21 litters (Table 4.1;  $P < 0.001$  overall for IS14 versus IS21 litters). Postponing the onset of IS from d 14 to d 21 resulted in a higher feed intake during the first wk of IS and shifted the distribution of cumulative feed intake during lactation to a higher level ( $218 \pm 38$  g/piglet and  $612 \pm 152$  g/piglet on average for IS14-S and IS21-S litters; Figure 4.2). The difference in feed intake level related to the age at onset of IS was sustained throughout the 3 consecutive weeks of IS when comparing feed intake levels of IS14-L and IS21-L

( $P < 0.005$ ; Figure 4.3). The greater feed intake stimulation by IS in IS21 litters resulted in a similar feed intake level during their first wk of IS compared to similar aged IS14-L litters in their second wk of IS, although values were numerically lower (Table 4.1).

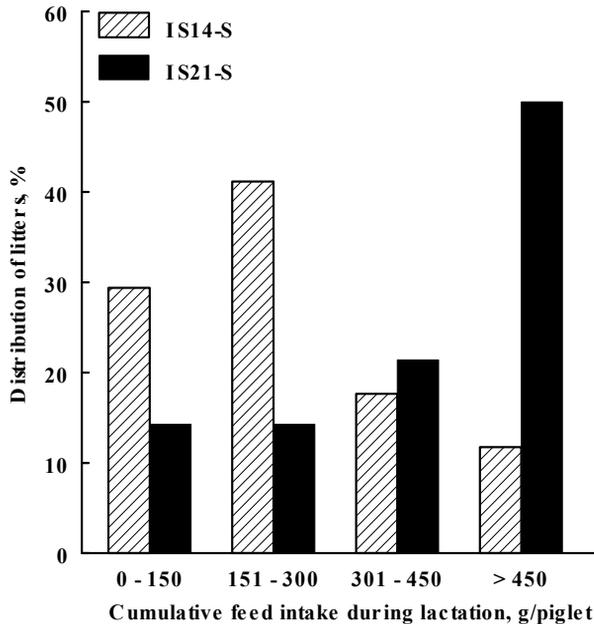
**Table 4.1.** Average daily feed intake ( $\text{g} \cdot \text{piglet}^{-1} \cdot \text{d}^{-1}$ ; means  $\pm$  SE) of piglets per treatment

Day	Treatment <sup>1</sup>			
	IS14-S	IS14-L	IS21-S	IS21-L
14 to 21	27 $\pm$ 6 <sup>ab</sup>	37 $\pm$ 7 <sup>a</sup>	9 $\pm$ 5 <sup>bc</sup>	10 $\pm$ 3 <sup>c</sup>
21 to 28	214 $\pm$ 16 <sup>a</sup>	126 $\pm$ 17 <sup>b</sup>	78 $\pm$ 15 <sup>b</sup>	93 $\pm$ 15 <sup>b</sup>
28 to 35	444 $\pm$ 28 <sup>a</sup>	307 $\pm$ 22 <sup>bc</sup>	372 $\pm$ 18 <sup>b</sup>	264 $\pm$ 21 <sup>c</sup>
35 to 42	655 $\pm$ 26 <sup>a</sup>	506 $\pm$ 33 <sup>b</sup>	665 $\pm$ 33 <sup>a</sup>	391 $\pm$ 29 <sup>c</sup>
42 to 49	827 $\pm$ 35 <sup>a</sup>	799 $\pm$ 36 <sup>a</sup>	821 $\pm$ 34 <sup>a</sup>	618 $\pm$ 35 <sup>b</sup>
49 to 56	1093 $\pm$ 34 <sup>a</sup>	1102 $\pm$ 42 <sup>a</sup>	1068 $\pm$ 37 <sup>a</sup>	1094 $\pm$ 46 <sup>a</sup>

<sup>a-c</sup> Within a row, means with different superscript letters differ ( $P < 0.05$ ). The shaded cells indicate the first period postweaning.<sup>1</sup>IS14-S = intermittent suckling starting at d 14 during a short lactation (n = 17); IS14-L = intermittent suckling at d 14 during an extended lactation (n = 17); IS21-S = intermittent suckling starting at d 21 during a short lactation (n = 14); IS21-L = intermittent suckling starting at d 21 during an extended lactation (n = 16).

In their first wk postweaning (d 21 to 28), feed intake of IS14-S litters was markedly increased compared with the similar aged, unweaned IS14-L and IS21 litters ( $P < 0.001$ ; Table 4.1). Likewise, feed intake of IS21-S litters was increased by weaning compared to the unweaned IS21-L litters (d 28 to 35), and from the second wk postweaning (d 35) onward it was similar to the IS14-S litters that had been weaned earlier (Table 4.1). Like in IS-S litters, weaning of IS-L litters increased their feed intake rapidly. In the last wk of the experiment (d 49 to 56) feed intake was similar in all treatments.

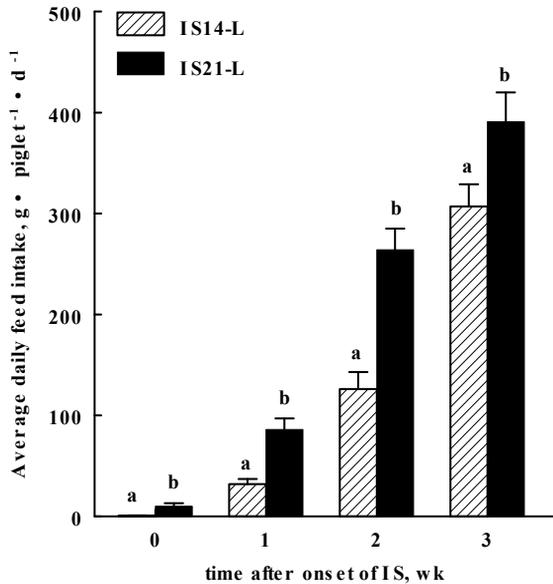
Feed intake in the wk before and after weaning were not correlated in IS-S litters, and only tended to be correlated in IS-L litters ( $r = 0.42$  and  $P = 0.09$ ,  $r = 0.52$  and  $P = 0.06$  for IS14-L and IS21-L litters, respectively).



**Figure 4.2.** Distribution of litters over 4 classes of cumulative feed intake until weaning. IS14-S = IS from d 14 and weaned at d  $21.8 \pm 0.4$ ; IS21-S = IS from d 21 and weaned at d  $27.7 \pm 0.3$ .

### Piglet BW and Growth

Piglet BW and ADG were similar in all treatments during the first 2 wk of the experiment. Between d 14 and 21 IS14 litters showed a reduced ADG compared with IS21 litters that were continuously suckled ( $P < 0.001$  overall for IS14 versus IS21 litters; Table 4.2), and consequently, a lower BW at d 21 ( $P = 0.002$  overall for IS14 versus IS21 litters). Initiating IS at a later age (d 21 vs d 14) also resulted in a reduced ADG between d 21 and 28 for IS21 litters compared with their ADG in the wk before onset of IS (d 14 to 21; Table 4.2). Relative growth during the first wk of IS was similar for IS21 litters ( $30.3 \pm 3.1\%$ ) and IS14 litters ( $33.2 \pm 1.6\%$ ).



**Figure 4.3.** Average daily feed intake of piglets ( $\text{kg} \cdot \text{piglet}^{-1} \cdot \text{d}^{-1}$ ; means  $\pm$  SE) in the week before IS and during 3 consecutive weeks of IS. <sup>a,b</sup> Within each experimental period, differences between the 2 treatments (IS14-L, IS21-L) are indicated with different superscripts. Onset of IS was on d 14 for IS14-L litters (wk 0 = d 7 to 14) and on d 21 for IS21-L litters (wk 0 = d 14 to 21). Both IS14-L and IS21-L litters were weaned during their fourth wk of IS.

Weaning of IS14-S and IS21-S litters resulted in a reduced ADG ( $P < 0.004$ ) compared to litters that were still suckling (IS14-L and IS21-L, respectively; Table 4.2). In IS14-S litters, the reduced ADG lasted for at least 2 wk (Table 4.2). In IS21-S litters, however, the reduction in ADG lasted for only 1 wk (d 28 to 35). Although weaned IS14-S and IS21-S litters showed a reduced ADG in the first wk postweaning compared to intermittently suckled counterparts (IS14-L and IS21-L, respectively), their ADG in the first 3 d after weaning ( $235 \pm 25$  g/piglet/day and  $312 \pm 31$  g/piglet/day, respectively) was greater than their preweaning ADG ( $159 \pm 15$  g/piglet/day from d 14 to weaning and  $212 \pm 21$  g/piglet/day from d 21 to weaning, respectively). Weaned IS14-L litters had a slightly lower ADG ( $430 \pm 26$  g/piglet/day) and IS21-L litters had a greater ADG ( $603 \pm 51$  g/piglet/day) in the first 3 d postweaning compared to preweaning values ( $457 \pm 36$  g/piglet/day and  $585 \pm 34$  g/piglet/day, respectively). From d 35 onward, piglet growth was similar

in all treatments (Table 4.2), and at the end of the experiment (d 56) no differences in piglet BW were observed (Table 4.3).

**Table 4.2.** Average daily gain of piglets ( $\text{g} \cdot \text{piglet}^{-1} \cdot \text{day}^{-1}$ ; means  $\pm$  SE) per treatment

Period	Treatment <sup>1</sup>			
	IS14-S	IS14-L	IS21-S	IS21-L
0 to 14	178 $\pm$ 10	205 $\pm$ 13	181 $\pm$ 10	196 $\pm$ 6
14 to 21	173 $\pm$ 13 <sup>a</sup>	201 $\pm$ 14 <sup>ab</sup>	242 $\pm$ 12 <sup>bc</sup>	260 $\pm$ 7 <sup>c</sup>
21 to 28	221 $\pm$ 23 <sup>a</sup>	311 $\pm$ 24 <sup>b</sup>	210 $\pm$ 21 <sup>a</sup>	252 $\pm$ 23 <sup>ab</sup>
28 to 35	364 $\pm$ 25 <sup>a</sup>	448 $\pm$ 17 <sup>b</sup>	331 $\pm$ 27 <sup>a</sup>	441 $\pm$ 13 <sup>b</sup>
35 to 42	473 $\pm$ 20	439 $\pm$ 29	438 $\pm$ 20	452 $\pm$ 25
42 to 49	543 $\pm$ 32	518 $\pm$ 31	585 $\pm$ 55	558 $\pm$ 25
49 to 56	658 $\pm$ 30	722 $\pm$ 44	609 $\pm$ 54	695 $\pm$ 32

<sup>a,b</sup> Within a row, means with a different superscript letter differ ( $P < 0.05$ ). The shaded cells indicate the first period postweaning.<sup>1</sup> The IS regimes started on d 14 or 21, respectively, for IS14 and IS21 regimes and lasted for 1 (IS-S) or 4 wk (IS-L). IS14-S = intermittent suckling starting at d 14 during a short lactation ( $n = 17$ ); IS14-L = intermittent suckling at d 14 during an extended lactation ( $n = 17$ ); IS21-S = intermittent suckling starting at d 21 during a short lactation ( $n = 14$ ); IS21-L = intermittent suckling starting at d 21 during an extended lactation ( $n = 16$ ).

### Relation Between Piglet Feed intake and Growth

Feed intake and ADG in the first wk of IS were correlated in IS14 litters ( $r = 0.61$ ,  $P < 0.001$ ), but no such correlation was observed in the still continuously suckled IS21 (but creep feed exposed) litters in that period (d 14 to 21). Similarly, feed intake and ADG in the first wk of IS were correlated in IS21 litters ( $r = 0.82$ ,  $P < 0.001$ ; d 21 to 28). Irrespective of onset of IS, distinct positive correlations were found between feed intake and ADG in both IS-L groups during the 3 consecutive weeks of IS ( $r > 0.86$  and  $P < 0.001$  for IS14-L and  $r > 0.74$  and  $P \leq 0.001$  for IS21-L). Feed intake and ADG in the first wk after weaning were correlated in IS-S

litters ( $r = 0.81$ ,  $P < 0.001$  and  $r = 0.89$ ,  $P < 0.001$  for IS14-S and IS21-S), but not in IS-L litters ( $r < 0.4$ ,  $P > 0.10$ ). Cumulative feed intake during lactation was correlated to ADG in the first 3d after weaning for IS-S litters ( $r = 0.54$ ,  $P = 0.02$  and  $r = 0.58$ ,  $P = 0.02$  for IS14-S and IS21-S), but not for IS-L litters ( $r < 0.2$ ,  $P > 0.50$ ).

**Table 4.3.** Body weight of piglets (kg / piglet; means  $\pm$  SE) per treatment

Day	Treatment <sup>1</sup>			
	IS14-S	IS14-L	IS21-S	IS21-L
0	1.50 $\pm$ 0.03	1.41 $\pm$ 0.04	1.46 $\pm$ 0.04	1.40 $\pm$ 0.04
14	4.02 $\pm$ 0.14	4.21 $\pm$ 0.15	4.09 $\pm$ 0.16	4.20 $\pm$ 0.12
21	5.21 $\pm$ 0.16 <sup>a</sup>	5.68 $\pm$ 0.23 <sup>ab</sup>	5.79 $\pm$ 0.20 <sup>ab</sup>	6.02 $\pm$ 0.16 <sup>b</sup>
28	6.85 $\pm$ 0.24 <sup>a</sup>	7.87 $\pm$ 0.36 <sup>ab</sup>	7.28 $\pm$ 0.21 <sup>ab</sup>	7.80 $\pm$ 0.20 <sup>b</sup>
35	9.37 $\pm$ 0.33 <sup>a</sup>	11.03 $\pm$ 0.46 <sup>b</sup>	9.59 $\pm$ 0.24 <sup>a</sup>	10.92 $\pm$ 0.26 <sup>b</sup>
42	12.68 $\pm$ 0.38 <sup>a</sup>	14.10 $\pm$ 0.61 <sup>b</sup>	12.66 $\pm$ 0.32 <sup>a</sup>	14.08 $\pm$ 0.32 <sup>b</sup>
49	16.53 $\pm$ 0.54 <sup>a</sup>	17.75 $\pm$ 0.72 <sup>ab</sup>	16.75 $\pm$ 0.45 <sup>ab</sup>	17.98 $\pm$ 0.36 <sup>b</sup>
56	21.25 $\pm$ 0.60	22.75 $\pm$ 0.89	21.02 $\pm$ 0.57	22.91 $\pm$ 0.54

<sup>a,b</sup> Within a row, means with a different superscript letter differ ( $P < 0.05$ ). The shaded cells indicate the first weighing postweaning.<sup>11</sup> IS14-S = IS from d 14, weaned at  $21.8 \pm 0.4$  ( $n = 17$ ); IS14-L = IS from d 14, weaned at  $39.9 \pm 0.3$  ( $n = 17$ ); IS21-S = IS from d 21, weaned at  $27.7 \pm 0.2$  ( $n = 14$ ); IS21-L = IS from d 21, weaned at  $46.9 \pm 0.4$  during an extended lactation ( $n = 16$ ).

## DISCUSSION

The objective of the current study was to determine the effect of piglet age at onset of IS and duration of IS on pre- and postweaning piglet growth and feed intake. Postponing the onset of IS from 2 to 3 wk of age increased feed intake during the first wk of IS considerably. Moreover, the period of postweaning growth reduction of IS-S litters, in comparison to their intermittently suckled counterparts (IS-L) lasted shorter for IS21-S than for IS14-S litters. Since feed intake levels of IS14-L and IS21-L litters were not significantly different between d 21 and 35, subjecting

piglets to IS as early as d 14 instead of d 21 of lactation, does not seem to have an added value with respect to feed intake stimulation. Although ADG and BW were greater in intermittently suckled litters (IS-L), when compared to litters that were already weaned at that stage, we found no differences in BW, growth or feed intake between treatments at the end of the experiment (d 49 to 56).

In line with previous results of our group (Kuller et al., 2004; Berkeveld et al., 2007b; Chapter 2), 1 wk of IS between d 14 and 21 of lactation resulted in a greater feed intake compared to litters that were suckled continuously. In conventional pig husbandry, creep feed is often supplied during lactation to familiarize piglets with dry feed. Creep feed intake of piglets is normally rather low in the first 3 wk of lactation, but increases rapidly thereafter (Pajor et al., 1991; Bruininx et al., 2004). Bruininx et al. (2004) demonstrated that during a 4-wk lactation period, 65% of total creep feed intake is established in the last wk before weaning. A similar increase of feed intake by IS litters (onset IS at d 14) has been observed between d 21 and 25 of lactation (Kuller et al., 2004). As anticipated, postponing the onset of IS to d 21 in the current study resulted in a greater increase in feed intake during the first wk of IS (mean ADFI of  $32 \pm 5$  g/piglet/d versus  $86 \pm 11$  g/piglet/d for IS14 and IS21 litters, respectively). In both treatments, piglets were exposed to creep feed from 1 wk before onset of IS onward, ruling out any effect of habituation to creep feed. In general, nursing frequency of conventionally housed litters reaches a maximum at the beginning of the second wk of lactation and decreases gradually thereafter (Puppe and Tuchscherer, 2000). This decrease in milk production of the sow during the suckling period is associated with a concomitantly increased creep feed intake (Puppe and Tuchscherer, 2000). Therefore, the older age at onset of IS might have stimulated piglets in an increasing degree to search for alternative nutrient sources, due to the gradually decreased milk production. Alternatively, the greater level of feed intake of IS21 litters might indicate that older piglets are able to adapt better to dry feed intake.

Intermittent suckling (12 h/d) has been shown to reduce both total daily nursing time and nursing frequency (Berkeveld et al., 2007a; Chapter 3). Although feed intake in the first wk of IS was increased when the onset of IS was postponed with 1 wk, a reduction in growth associated with the onset of IS was still present in the current study. So, this indicates that the increase in feed intake was insufficient to compensate for the lower milk intake. Alternatively, one might question whether the functional state of the gastrointestinal tract, which is still under development at this young age (Pluske et al., 2003), is adequate for processing of the ingested dry

feed. Yet, this assumption is not compatible with the observation (van Beers-Schreurs et al., 1998) that weaned 28-d old piglets, given an amount of ME in the form of sow milk, equal to that of commercial dry feed ingested voluntarily by controls in the same study (increasing from ~ 230 to 760 KJ/BW<sup>0.75</sup> within the first wk postweaning), had a similar BW at 1 wk postweaning as these controls (weaning at d 28).

The time spent on eating during the first 2 d after weaning increases when piglets are older at weaning (Worobec et al., 1999). Likewise, the older age at weaning of IS21-S litters in the current study (d 28) improved feed intake during the first wk postweaning (372 vs. 214 g/piglet for IS21-S and IS14-S, respectively). Moreover, the reduction in growth of IS-S litters after weaning, compared to litters that were still suckling (IS-L), was of shorter duration for IS21-S litters than for IS14-S litters. This is in line with previous findings in conventionally weaned pigs, that the duration of the postweaning growth check is shortened (from 7 to 4 d), when weaning age is increased from 21 to 28 d (Colson et al., 2006). Altogether, these findings point to a more rapid adaptation to the postweaning situation when piglets are older.

Extending lactation length in combination with intermittent suckling (IS starting at d 14, weaning at 6 wk) has been shown to increase preweaning feed intake and markedly reduced the postweaning growth check, compared to conventional weaning at d 21 (Berkeveld et al., 2007b; Chapter 2). In the latter study, we attributed these beneficial effects on postweaning performance to the intermittent suckling regimens applied, as well as to the age at weaning. It might be questioned, considering the increase in ADG in the first 3 d after weaning in IS-S litters of the current study (compared to their preweaning ADG), whether a prolonged period of IS (as applied in the IS-L litters) is essential for a gradual adaptation to the postweaning situation. However, the postweaning ADG of IS-S litters was reduced compared to that of their still intermittently suckled counterparts. This suggests that a longer period of IS before weaning after all is necessary to prevent a negative effect of weaning on piglet growth.

We estimated the energy requirements of young piglets (ME, MJ/piglet/day; based on CVB report 1995; Everts et al., 1995) and the percentage of feed-derived and milk-derived energy intake. The relative contribution of dry feed to energy intake increased progressively throughout the suckling period of IS-L litters and reached some 56% and 61% for IS14-L and IS21-L litters at the time these litters were weaned. Interestingly, although the onset of IS in IS21-L litters was postponed for

1 wk, the relative contribution of dry feed to energy intake was similar to that for IS14-L litters between d 21 and 28 (27% and 32% for IS21-L and IS14-L, respectively), and between d 28 and 35 (47% and 56% for IS21-L and IS14-L litters, respectively). The piglets on the IS-L regime seemed to be acquainted sufficiently with dry feed to ensure an adequate level of feed intake once they were weaned definitively, because their level of feed intake after weaning was equal to that of the similar aged IS-S litters that were weaned 3 wk earlier. Furthermore, the ADG in the first 3 d after weaning was only slightly lower in IS14-L litters and even increased in IS21-L litters compared to their ADG in the wk before weaning. These findings indicate that, irrespective of onset, subjecting litters to a prolonged period of IS results in a more optimal adaptation to weaning. One may speculate, however, whether this prolonged period of IS could be shortened when it is started later, since the ADFI and the relative contribution of dry feed to energy intake between d 28 and 35 (wk before weaning IS14-L litters) was similar for IS14-L and IS21-L litters.

In conclusion, onset of IS at an older age (d 21 instead of d 14), markedly improved feed intake stimulation. Weaning of litters after 1 wk of IS (IS-S) resulted, irrespective of age at onset, in a postweaning growth reduction, when compared to the similar-aged intermittently suckled counterparts (IS-L). However, this period of postweaning growth reduction was shorter if piglets were submitted to IS at an older age. Irrespective of age at onset, IS combined with an extended lactation period resulted in a more gradual adaptation to weaning, as judged by postweaning growth and feed intake.

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# CHAPTER 5

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## **MEASURING INTESTINAL BLOOD FLOW IN GROUP- HOUSED WEANER PIGS USING PHYSIOGEAR™ I: A PILOT STUDY**

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## ABSTRACT

The objective of this study was to determine whether intestinal blood flow can be measured adequately in group-housed animals using the recently developed Physiogear<sup>TM</sup> I wireless flowmeter. We used the weaner pig as one of many possible animal models. Four 7-kg piglets were instrumented with a 3-mm flowprobe around the superior mesenteric artery (SMA) and SMA flow was measured pre- and post-weaning. During measurements, behaviour was recorded. The piglets did not show any abnormal behaviour and were not restrained by the flowmeter. Severe reductions (>75%) in SMA flow coincided with nursing (pre-weaning) and aggressive behaviour (post-weaning) and were only short-lived. Our results demonstrate that the Physiogear<sup>TM</sup> I flowmeter can be used to measure flow in group-housed animals without any human contact, providing the opportunity to relate flow measurements to undisturbed animal behavior.

## INTRODUCTION

A large part of the intestinal tract depends on the superior mesenteric artery (SMA) for blood supply. The SMA branches into several arteries leading to the submucosa from where they split into a web of parallel vessels. During intestinal hypoperfusion, i.e. when intestinal blood flow is impaired, the parallel vessels control the distribution of blood towards the different mucosal tissues preserving the blood flow towards the metabolic active and vulnerable mucosa and preventing ischemic cell damage. Consequently, partial experimental occlusion of the SMA for 1h in anaesthetised pigs did not result in an increased intramucosal pH or histologic lesions compared to control pigs unless SMA blood flow was reduced by at least 75% (Pargger et al., 1997). Intestinal hypoperfusion occurs as an adaptation to stress in order to maintain the perfusion of essential organs. Since weaning is associated with multiple stressors, it is relevant to investigate whether splanchnic hypoperfusion plays a part in intestinal dysfunction after weaning. Unfortunately, the current techniques to investigate the effect of splanchnic hypoperfusion on intestinal function require either complete anesthesia of animals (e.g. experimental occlusion) or human contact and individual housing (e.g. Doppler ultrasound). The recently developed Physiogear<sup>TM</sup> I flowmeter (Transonic Systems Inc., Ithaca, USA), however, is equipped with a telemetric device developed for blood flow measurements in conscious animals. The objective of this study was to determine whether intestinal blood flow can be measured adequately in group-housed weaner pigs without any human interference using the Physiogear<sup>TM</sup> I flowmeter.

## MATERIALS AND METHODS

Four female piglets (6.4 to 7.4 kg) from 2 litters were used for the experiment. Only female piglets were used, since the animal jacket (described below) was not designed to support urination of male piglets. Piglets were offered creep feed ad libitum from d 21 post partum onwards. During the entire experiment piglets had free access to drinking water. At d 26 post partum and after an overnight fast, 4 piglets were provided with a 3-mm transit time ultrasound flow probe (Transonic Systems Inc., Ithaca, USA) around the SMA. After pre-medication by an i.m. injection of ketamine (10mg/kg), midazolam (0.75 mg/kg) and glycopyrrolate (0.01 mg/kg), an ear vein was used for i.v. induction with propofol (depending on the piglet, 2-4 mg/kg was necessary). Subsequently, the piglets were intubated and

placed in right lateral recumbency on a warm water blanket. Anesthesia was maintained with isoflurane 1-2% administered in oxygen:air (1:1). The end-tidal pressure was kept between 4.5 and 5.5 kPa. A sufentanil bolus (2  $\mu\text{g}/\text{kg}$ ) was administered i.v. 2 minutes before incision and additional sufentanil boluses (1  $\mu\text{g}/\text{kg}$ ) if necessary. During anesthesia a Lactated Ringers solution (10 ml/kg/hour) was administered. Heart rate, blood oxygenation, inspiratory and expiratory gas analysis, respiration rate and rectal temperature were monitored during surgery. A small lateral incision ( $\pm 5$  cm) was made in the left flank, just 2 centimeters behind the last rib and without exposure of the abdominal cavity. Starting from the incision, the ventral side of the abdominal aorta was retroperitoneally explored manually to localize the origin of the SMA, just behind the adrenal gland. The flow probe was placed around the SMA after careful dissection from surrounding tissue. The flow probe cable was tunneled subcutaneously, exteriorized between the shoulder blades, and placed in a pouch on an animal jacket (Dog jacket Xsmall, Alice King Chatham Medical Arts, California, USA), which was wrapped up with Coflex (Andover, Salisbury, USA; Figure 5.1). After surgery, piglets received a single i.m. injection of buprenorphine (10  $\mu\text{g}/\text{kg}$ ) and an i.v. injection of meloxicam (0.4 mg/kg). After a short recovery period (2 to 3 h), piglets were returned to their litter. Additional i.m. injections of meloxicam (0.4 mg/kg) were administered once daily for 2 days after surgery. The piglets were given antibiotics by i.m. injection (20 mg/kg Ampicilline) from 2 d before until 3 d after surgery.

Blood flow was measured using the Physiogear<sup>TM</sup> I flowmeter (Transonic Systems Inc., Ithaca, USA). This pocket-size flowmeter (135 x 75 x 45 mm;  $\pm 200$  g) and a similar sized rechargeable battery pack were placed in 2 pouches on the animal jacket. The battery pack enabled flow measurements for a maximum of 8 consecutive hours. The Physiogear<sup>TM</sup> I flowmeter uses a Bluetooth<sup>TM</sup> communication link to send the flow signal (128 Hz) to a remote computer with software for data collection (Physioview<sup>TM</sup>; Transonic Systems Inc., Ithaca, USA). The Physioview<sup>TM</sup> software registers the real-time flow signal and displays both the real-time and the average blood flow ( $F_{\text{avg}}$ ), calculated by filtering the real-time flow signal using a  $1/10$  Hz second-order Butterworth filter. For every data file, the mean value for the  $F_{\text{avg}}$ , designated as total mean flow ( $F_{\text{total}}$ ), was calculated using a custom routine programmed in Microsoft<sup>®</sup> QBasic version 1.1. Furthermore, periods of severely reduced flow, defined by a  $F_{\text{avg}}$  which is over 75% reduced compared to the  $F_{\text{total}}$  in that specific data file, were registered.

From d 28 to d 30 post partum, SMA flow was measured during 2 to 3 consecutive hours per piglet. Piglets were weaned between d 31 to d 34 post partum. Instrumented piglets were weaned with both littermates and non-littermates and housed together in a fattening pen. On the day of weaning, SMA flow was measured from 2 h before until 8 h after weaning. From d 35 until d 39 post partum, SMA flow was measured during 2 to 3 consecutive hours per piglet. All flow measurements were accompanied by video-recordings to monitor piglet behavior. The video-recordings were analyzed and nursing, eating and aggressive behavior of the piglets were recorded continuously.

On d 40 post-partum, experimental piglets were sacrificed by intracardial injection of T61 (2ml, Intervet Nederland BV, The Netherlands) to inspect the flowprobe.



**Figure 5.1** Piglet instrumented with flowmeter.

## RESULTS

The Physiogear<sup>TM</sup> I flowmeter was able to send the flow signal to a remote computer in a separate observation room  $\pm$  10 m away. The Coflex protected the animal jacket sufficiently and neither the animal jackets nor the flowmeter were damaged. The animal jacket with or without flowmeter did not restrain the piglets, since piglets did not display any abnormal behavior, were equally active as littermates and participated in the nursings during lactation. During post-mortem examination, encasing of the flow probe with purulent exudate and connective tissue was observed in one piglet. In this piglet, the amplitude of the real-time flow decreased dramatically during the consecutive measurements. Therefore, this piglet was omitted from data analysis. In the other piglets, no excessive connective tissue, collateral vessels or other abnormalities were observed around the flow probe.

The  $F_{\text{total}}$  of the data files in the consecutive measurements after weaning, was higher in piglet 2 compared to piglet 1 and 3 (Table 5.1). During inactivity, the  $F_{\text{avg}}$  was constant and the real-time flow showed a constant pattern of flow fluctuations with heart rate. During activity, the  $F_{\text{avg}}$  showed more variation and the real-time flow showed higher amplitude. During social interactions, like fighting or nursing, the  $F_{\text{avg}}$  decreased dramatically (e.g. Figure 5.2). Periods of severely ( $>75\%$ ) reduced  $F_{\text{avg}}$  were observed in all piglets (Table 5.1), but were only of short duration and often associated with nursing before and aggressive behavior after weaning. Furthermore, severely ( $>75\%$ ) reduced  $F_{\text{avg}}$  was observed during catching and holding of the piglet to install or remove the flow meter.

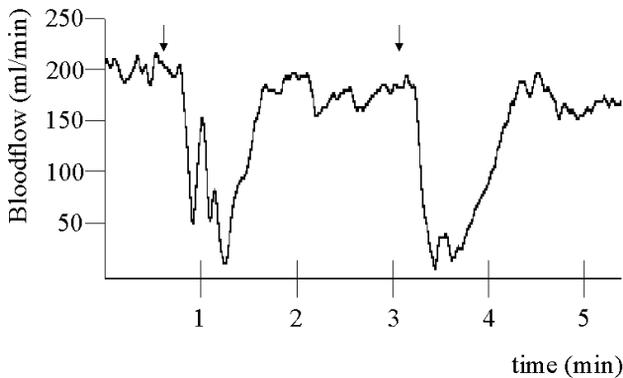
**Table 5.1.** Total mean flow ( $F_{\text{total}}$ ) and severe reductions ( $<75\%$ ) in average blood flow ( $F_{\text{avg}}$ ) per measurement period. Piglets were weaned on consecutive days, indicated by the shaded columns.

Parameter	Pig	Day post-partum										
		28	29	30	31	32	34	35	36	37	38	39
# reductions ( $<75\%$ ) in $F_{\text{avg}}$	1	3	2	-	-	-	24	7	5	2	1	0
	2	1	-	1	-	3	-	0	0	0	-	-
	3	-	7	15	14	-	-	0	1	2	0	0
Max. duration of reductions (s)	1	5	7	-	-	-	24	32	10	5	9	-
	2	2	-	4	-	4	-	-	-	-	-	-
	3	-	70	37	24	-	-	-	5	3	-	-
$F_{\text{total}}$ (ml/min)	1	120	107	-	-	-	212	173	140	165	191	217
	2	210	-	-	-	217	-	372	368	429	-	-
	3	-	121	116	123	-	-	146	127	138	132	125
Measurement time (min)	1	160	137	-	-	-	679	132	138	186	125	50
	2	71	-	164	-	660	-	161	180	162	-	-
	3	-	128	60	667	-	-	85	145	165	154	97

## DISCUSSION

In this study we determined whether the recently developed Physiogear<sup>TM</sup> I wireless flowmeter is a suitable system to measure blood flow in group-housed conscious pigs. In this study we used the weaner pig as one of many possible models. Our results demonstrate that the Physiogear<sup>TM</sup> flowmeter can be used to measure flow in group-housed animals. Furthermore, it enables to measure blood

flow in conscious animals without human interference, which provides the opportunity to relate flow measurements to undisturbed animal behaviour. For instance, in our study, the aggressive interactions due to mixing with non-littermates at weaning, resulted in severe, but short-lived decreases in intestinal flow. Given the fact that weaning coincides with multiple stressors, the Physiogear™ I flowmeter might be a useful tool to determine the role of hypoperfusion in intestinal dysfunction after weaning.



**Figure 5.2.** A representative example of severe reductions in intestinal blood flow due to aggressive interactions. Start of the interactions is indicated with arrows.

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# CHAPTER 6

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**CITRULLINE AND I-FABP:  
LONGITUDINAL MARKERS OF POSTWEANING  
SMALL INTESTINAL FUNCTION IN PIGS?**

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## ABSTRACT

The objective of the current study was to investigate whether plasma citrulline or intestinal fatty acid binding protein (I-FABP) concentrations might be used as longitudinal markers for small intestinal function in piglets after weaning. Plasma citrulline and I-FABP levels were measured longitudinally in weaned and unweaned piglets, and related to intestinal absorption values, i.e. plasma mannitol and D-xylose concentrations in a sugar absorption test. Within each litter ( $n = 10$ ), 2 piglets with a close-to-litter-average body weight were selected. At  $20.8 \pm 0.4$  d of age, the selected piglets per litter were either weaned conventionally (CW) or remained with the sow (UNW). One day before, and 0.5, 2, 4 and 7 d after weaning of the CW piglets, the selected piglets of both groups were subjected to a sugar absorption test. After a 2 h fast, an oral dose of 2mL/kg sugar solution was administered, containing 50 mg/kg mannitol and 100 mg/kg D-xylose. One hour after administration a blood sample was taken from the jugular vein for determination of plasma I-FABP, citrulline, mannitol and D-xylose concentrations. Plasma I-FABP concentration showed a great variation within treatments, and no difference in plasma I-FABP levels between the CW and UNW treatment ( $P = 0.63$ ) was observed. D-xylose absorption was not different between treatments ( $P = 0.83$ ). Mannitol absorption, however, was lower in the weaned CW piglets compared to the UNW piglets ( $P = 0.003$ ), with the lowest value on d 4 postweaning. Weaning also reduced plasma citrulline concentrations in the CW compared to the UNW treatment ( $P < 0.001$ ). On d 4 and 7 postweaning plasma citrulline levels of CW piglets were lower ( $P < 0.001$  and  $P = 0.0013$ ) compared to preweaning values. Furthermore, in the CW treatment plasma citrulline concentrations correlated to plasma mannitol levels at d 4 postweaning ( $r = 0.89$ ,  $P = 0.008$ ) and overall ( $r = 0.76$   $P = 0.001$ ). Based on the results of the current study, plasma citrulline concentration seems to be a possible marker for monitoring intestinal function in pigs after weaning.

## INTRODUCTION

Weaning of piglets at a young age (3 to 4 wk) is associated with villous atrophy (Hampson, 1986; Hedemann et al., 2003; Pluske et al., 1996b), reduced absorption (Hampson and Smith, 1986; Nabuurs et al., 1996) and increased permeability (Spreeuwenberg et al., 2001; Verdonk et al., 2007) in the small intestine. A recent review of Montagne et al. (2007), describing the main intestinal markers of gut architecture and function in piglets after weaning, demonstrates that most of these parameters are end-point measurements. A biological marker of intestinal function would enable longitudinal monitoring, and as a consequence, reduce the number of required animals. To our knowledge, such a marker has not been described for pigs.

Fatty acid binding proteins (FABPs) are small cytoplasmatic proteins involved in the intracellular buffering and transport of long chain fatty acids. When cell damage occurs, small proteins like FABPs diffuse through the interstitial space into the vascular space and become detectable in the plasma. Therefore, FABPs are considered to be potential plasma markers for the detection of tissue injury, such as cardiac injury, liver rejection, and inflammatory and ischemic bowel disease (Pelsers et al., 2005). Although some FABPs, like H(ear)-FABP and L(iver)-FABP, show a multi-tissue expression, the expression of I-FABP is restricted to the intestinal tract (Glatz and van der Vusse, 1996; Pelsers et al., 2005), with the highest tissue contents in the jejunum (Pelsers et al., 2003). Elevated levels of I-FABP are detected in human patients with small bowel obstruction (Cronk et al., 2006), necrotizing enterocolitis (Guthmann et al., 2002), and other intestinal diseases (Pelsers et al., 2003). Based on the acute increase (within 30 min) in plasma I-FABP concentrations after experimentally induced intestinal ischemia in pigs, it was suggested that plasma I-FABP concentration might be used as a sensitive marker of damage to the intestinal mucosa in pigs (Niewold et al., 2004). Citrulline, a nonprotein amino acid, is the nitrogen end product of glutamine metabolism and is produced exclusively by the enterocytes of the small bowel (Windmueller and Spaeth, 1981). In patients with short-bowel syndrome, the plasma citrulline concentration was found to be a simple and reliable marker of absorptive bowel length and absorptive function (Crenn et al., 2000; Jianfeng et al., 2005). In addition, plasma citrulline levels have been used to monitor cancer treatment-related gut damage (Lutgens et al., 2003; Lutgens et al., 2004; Lutgens et al., 2005), development of enteral tolerance in infants (Rhoads et al., 2005), and

enterocyte mass in villous atrophy associated small bowel diseases (Crenn et al., 2003). Based on these studies, it was hypothesized that plasma citrulline might be a promising marker for monitoring postweaning intestinal function in piglets.

In the current study, plasma citrulline and I-FABP levels were measured longitudinally in weaned and unweaned piglets, and related to intestinal absorption values, i.e. plasma mannitol and D-xylose concentrations in a sugar absorption test.

## MATERIALS AND METHODS

### Experimental Design

The Ethics Committee for animal experiments of the Faculty of Veterinary Medicine at Utrecht University approved the experimental design, including all procedures involving animals.

Ten litters of multiparous sows were used during 4 farrowing replicates (Tolakker Research Farm, Faculty of Veterinary Medicine, Utrecht University, the Netherlands). Selection of litters was based on litter size ( $\geq 10$  piglets) and selection of piglets on average piglet weight per litter (close to the overall average piglet weight of litters per replicate). Within each litter, 2 piglets with a close-to-litter-average body weight were selected, resulting in a total of 20 piglets. During the suckling period no solid feed was offered to the piglets. At  $20.8 \pm 0.4$  d of age, per litter one piglet was weaned conventionally (CW) and one piglet continued lactation (UNW). The UNW piglets ( $n = 10$ ) remained with their sow, whereas the CW piglets ( $n = 10$ ) were weaned and housed in weaner pens together with four unfamiliar age-matched piglets (Pig and Poultry Research Unit, Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, the Netherlands). From the day of weaning the CW piglets onward, piglets of both treatment groups had ad libitum access to solid feed (Table 6.1; Romelko Geel, Sondag Voeders BV, Veghel, The Netherlands). Piglets had ad libitum access to water throughout the entire experiment.

One day before weaning of the CW piglets, and at 0.5, 2, 4 and 7 d after weaning, piglets of both treatment groups were subjected to a sugar absorption test. Piglets were fasted for 2 hours by removal of solid feed and water in both groups and separation from the sow in the UNW group. Thereafter, an oral dose of 2mL/kg sugar solution, containing mannitol (50 mg/kg; Sigma, St.Louis, MO) and D-xylose (100 mg/kg; Sigma, St.Louis, MO) dissolved in water, was administered. One hour after administration, a blood sample was taken by venipuncture of a

jugular vein. Two-mL aliquots of each blood sample were transferred to a heparin-coated and an EDTA-coated tube, and after centrifugation (10 min, 3000 rpm at 4°C) plasma samples were stored at -80°C.

**Table 6.1.** Composition of postweaning piglet diet (as fed basis)

	<b>Content, %</b>
Wheat	11
Corn	15
Barley	37
Whey powder	7.0
Lactose	3.2
Soybeans FF toasted	6.0
Extracted soybeanmeal	3.0
Soy protein concentrate	4.0
Potato protein	2.1
Coconut oil	1.0
Fish meal	1.0
Soy bean oil	1.0
Di calcium phosphate	0.3
salt	0.2
Vitamin and mineral premix	8.2
<b>Chemical analysis</b>	
Dry matter, %	88.3
Crude protein, %	17.8
Crude fat, %	4.2
Crude fiber, %	2.9
Ash, %	5.6
Lysine, %	1.3
Fosfor, %	0.5
Zinc, mg/kg	135.0
Copper, mg/kg	160.0
Metabolisable energy, MJ ME/kg	14.0
Net energy, MJ NE/kg <sup>a</sup>	10.3

<sup>a</sup> Calculated with the use of the Dutch feed tables (CVB, 1997)

### **Determination of plasma Mannitol and D-xylose concentration**

The EDTA plasma samples obtained 1 hr after administration of the sugar solution were used to determine plasma mannitol and D-xylose concentrations as markers for intestinal absorption (Cox et al., 1999; Miller et al., 1984). Due to a limited volume, some of the plasma samples could not be analysed (see table 6.3 for exact sample number per sampling day). The standards and reagents were from Sigma-Aldrich (St. Louis, MO, USA), solvents were from Baker (Deventer, NL). Hibitane (50mg/L) was added to all solutions containing carbohydrates to prevent bacterial growth. An aliquot of 200  $\mu$ L internal standard solution of Trehalose (300  $\mu$ mol/L in 20:80 Methanol: Water, v/v) and 100  $\mu$ L salicine was added to 100  $\mu$ L of each EDTA plasma sample. The mixture was blown dry thoroughly under a stream of nitrogen at 40°C before adding a light-protected solution of 12.5 mg hydroxylamin-HCl in 500  $\mu$ L aniline and the mixture was incubated during 10 minutes at 60°C. After cooling to room temperature, 300  $\mu$ L BSTFA/1%TMCS were added, tubes were closed and samples were incubated during 10 minutes at 18-28°C (room temperature). After centrifugation at 3000 rpm, supernatants were transferred to autosampler vials. A volume of 2  $\mu$ L was injected (with 1  $\mu$ L air plug in front of the sample) into an 1177 split injector (containing a plug of glass wool) of a Varian CP-3900 type gas chromatograph equipped with an autosampler and a 50 m \* 0,25 mm i.d. CPSil-5CB column (Varian, Middelburg, the Netherlands; 0,12  $\mu$ m film thickness). The injector was operated at 280°C and the column gas (helium) flow rate was constantly 2 mL/min. The detector was operated at 300°C with a makeup gas (nitrogen) flow rate of 25 mL/min, flame gas (hydrogen) of 30 mL/min and flame gas (air) of 300 mL/min. Temperature profile started initially at 184°C during 2 min, followed by a rise of 30°C/min to 200°C (7 min hold), and subsequently followed by a rise of 30°C/min to 240 °C (17 min hold) and ended by a rise of 30°C/min to 290°C (1 min hold) to clean the column. Data handling was performed using Galaxy software (Varian) using areas and internal standard method.

Due to a detection limit, plasma concentrations of mannitol or D-xylose below 2  $\mu$ M remained undetected. In those cases, this detection limit was the value applied for the plasma concentrations of mannitol (n = 5) or D-xylose (n = 21) in the statistical analysis.

### **Determination of plasma I-FABP concentration**

The I-FABP concentration of EDTA-plasma samples were determined using a commercial ELISA test kit (HyCult biotechnology BV, Uden, The Netherlands). This ELISA test kit was developed to measure I-FABP concentration in human plasma, but has been demonstrated to be suitable for measuring I-FABP concentrations in pig plasma (Niewold et al., 2004). Samples were analyzed in duplicate and concentrations are expressed as equivalents of human I-FABP. Preliminary analyses revealed that there was a strong ‘sow-effect’ on the plasma I-FABP concentration ( $P < 0.001$ ). Therefore, plasma I-FABP concentrations of the sows (except for one slaughtered sow) were determined during the second half ( $\approx$  d 70) of their subsequent pregnancy.

### **Determination of plasma Citrulline concentration**

Plasma citrulline concentrations were analysed by automated ion-exchange chromatography performed on a Jeol Amino-Tac (JLC-500/V, Tokyo, Japan) with post column ninhydrin derivatization. The detection range was from 3 to 1000  $\mu$ M, with a maximal inaccuracy of 14%. The plasma citrulline concentrations of one piglet in the UNW treatment showed a distinctly different kinetic (higher values) compared to the other piglets. Data of this piglet on plasma citrulline concentration were considered to be outliers, and therefore omitted from data analysis.

### **Calculations and Statistics**

Postweaning growth check was defined as the reduction in average daily gain (ADG) at d 2 postweaning compared with the ADG in the last wk before weaning. The relative postweaning growth check (%) was calculated as:  $100 [(ADG_{\text{preweaning wk}} - ADG_{\text{d2 postweaning}}) / ADG_{\text{preweaning wk}}]$ . Although piglets of the UNW treatment were not weaned, the relative growth was calculated using the same time periods as for the CW treatment.

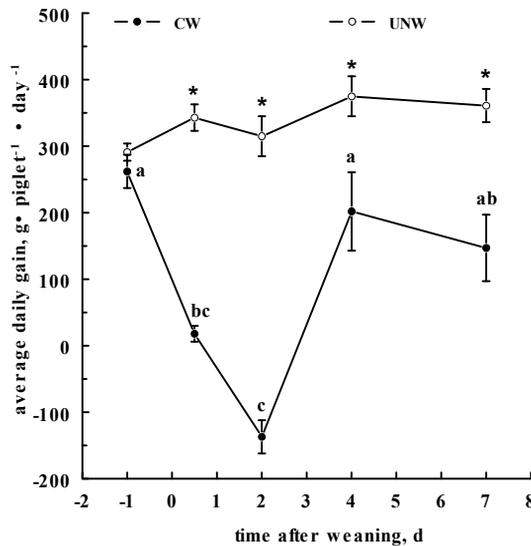
Longitudinal measurements per animal can not be considered as independent observations, and thus repeated measurements analysis of variance (Littell et al., 1998) using the MIXED procedure in SAS (SAS Inst. Inc., Cary, NC) was performed for piglet bodyweight, piglet growth and plasma variables. Treatment, day and their interaction were included in the model as fixed factors, replicate and sow were included as random factor, and piglet as repeated measurement, with an autoregressive covariance structure. For data on piglet body weight, birthweight was included as co-variable, and for data on piglet growth and plasma variables,

body weight at d 21 of age (day of weaning for CW piglets) was included as co-variable. Plasma I-FABP concentrations were log-transformed to obtain homogeneity of variances. All values are presented as means  $\pm$  SE. Effects were considered significant if  $P < 0.05$ , and a tendency if  $0.05 \leq P < 0.10$ ; for post-hoc testing the Bonferroni correction was applied.

## RESULTS

### Piglet Performance

During the experiment none of the piglets had to be treated for illness, and during post-mortem pathological examination of the CW piglets, none of the organs showed abnormalities. No differences in piglet body weight or growth were observed between treatments at 20 d of age, before beginning of the treatments (Figure 6.1 and Table 6.2).



**Figure 6.1.** Average daily gain (g/piglet) of weaned (●- CW; n=10) and unweaned (○ - UNW; n = 10) piglets (Means  $\pm$  SE). <sup>a-c</sup> Differences in piglet growth within a treatment ( $P < 0.05$ ) are indicated with different letters per sampling day. \* Differences in piglet growth between treatments ( $P < 0.05$ ) are indicated with an asterisk per sampling day.

Weaning of the CW piglets resulted in a markedly reduced growth compared to preweaning values, and resulted in a lower growth compared to UNW piglets (overall  $P < 0.001$ ; Figure 6.1). The relative postweaning growth check of CW piglets was  $155 \pm 9\%$ , whereas the UNW piglets gained weight and had a relative increase in growth of  $6 \pm 9\%$  in the same period. Consequently, CW piglets had a lower piglet body weight compared to UNW piglets from 2 d postweaning onward ( $P < 0.001$ ; Table 6.2).

**Table 6.2.** Bodyweight of piglets per treatment (Means  $\pm$  SE)

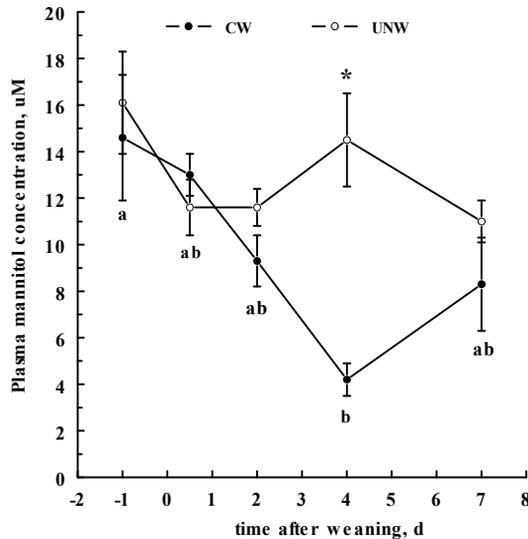
Day after weaning	Treatment <sup>1</sup>		P- value
	UNW	CW	
-1	$6.9 \pm 0.3^a$	$6.7 \pm 0.4^{ab}$	n.s.
0.5	$7.4 \pm 0.3^{ab}$	$6.7 \pm 0.4^{ab}$	0.077
2	$7.9 \pm 0.3^{bc}$	$6.5 \pm 0.3^a$	$< 0.001$
4	$8.7 \pm 0.4^c$	$6.9 \pm 0.4^{ab}$	$< 0.001$
7	$9.7 \pm 0.4^d$	$7.3 \pm 0.5^b$	$< 0.001$

<sup>1</sup> UNW = unweaned; CW = conventionally weaned; P-value indicates differences between treatments per sampling day. <sup>a-d</sup> Different letters indicate differences between means of sampling days within a treatment.

### Plasma Mannitol and D-xylose concentrations

Plasma mannitol concentrations were different between the CW and UNW treatment (overall  $P = 0.003$ ; Figure 6.2). Weaning of the CW treatment resulted in a transient decrease in plasma mannitol concentration at 1 h after oral application, with lower values observed at d 4 postweaning compared to preweaning values ( $P < 0.001$ ; Figure 6.2). In contrast, no differences in mannitol absorption between sample days were observed in the UNW treatment. The marked decrease at d 4 postweaning resulted in a lower mannitol concentration in the CW compared to the UNW treatment ( $P = 0.01$ ). Overall, plasma mannitol concentrations on d 4 postweaning were correlated to the relative postweaning growth check ( $r = -0.73$ ,  $P = 0.005$ ), i.e. piglets with a more reduced growth at d 2 postweaning had lower

mannitol absorption values at d 4 postweaning. No correlations were observed overall on other sampling days, or within each separate treatment.



**Figure 6.2.** Plasma mannitol ( $\mu\text{M}$ ) concentrations of weaned ( $\bullet$  - CW) and unweaned ( $\circ$  - UNW) piglets at 1 h after oral administration. All values are means  $\pm$  SE. <sup>a-c</sup> Differences in mannitol concentration within a treatment ( $P < 0.05$ ) are indicated with different letters per sampling day. \* Different ( $P < 0.05$ ) from the value of weaned piglets on that sampling day. Number of observations similar to those of plasma D-xylose concentrations (Table 6.3).

There was no difference in plasma D-xylose concentrations at 1 h after oral application between the CW and UNW treatments (overall  $P = 0.83$ ; Table 6.3). The plasma D-xylose concentration in the CW treatment was quite variable between sampling days, and higher on d 2 and 7 postweaning ( $P = 0.013$  and  $P = 0.05$ ) compared to the preweaning value. In the UNW treatment, D-xylose concentrations also varied considerably, but no differences between sampling days were found.

### Plasma I-FABP concentration

There was no difference in I-FABP plasma concentration between the CW and UNW treatments (overall  $P = 0.63$ ; Figure 6.3). Moreover, mean I-FABP plasma concentration were nearly similar at all sampling days in both treatments. Variation

in plasma I-FABP concentration was high, resulting in large standard errors (Figure 6.3). When examining the data in more detail, the high variation was suspected to be caused by a ‘sow-effect’; plasma I-FABP levels of the UNW and CW piglet from the same litter were almost similar (data not shown). Indeed, there was a strong correlation between the I-FABP concentration of sows and the average I-FABP concentration of their offspring on all treatment days ( $r > 0.87$ ,  $P < 0.003$ ).

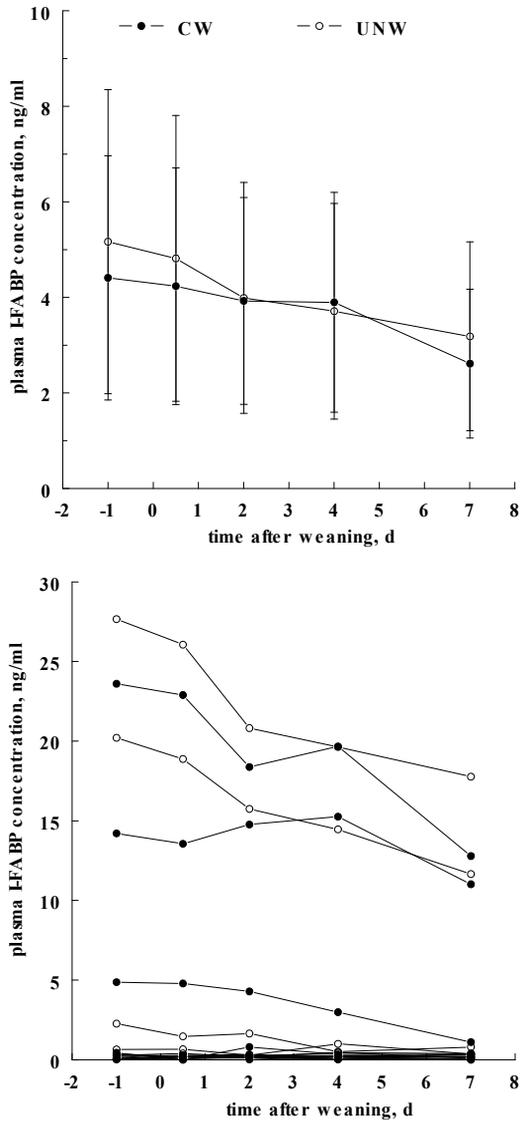
**Table 6.3.** Plasma D-xylose concentrations ( $\mu\text{M}$ ) 1 h after oral administration per treatment (Means  $\pm$  SE).

Day Postweaning	Treatment <sup>1</sup>			
	UNW		CW	
	Mean	SE (n)	Mean	SE (n)
-1	35.6	7.8 (10)	17.6 <sup>a</sup>	6.9 (10)
0.5	23.4	6.4 (9)	34.6 <sup>ab</sup>	5.3 (9)
2	40.6	8.2 (10)	53.5 <sup>b</sup>	4.2 (10)
4	37.9	5.6 (9)	23.0 <sup>ab</sup>	8.2 (8)
7	32.3	2.3 (9)	53.9 <sup>b</sup>	19.1 (7)

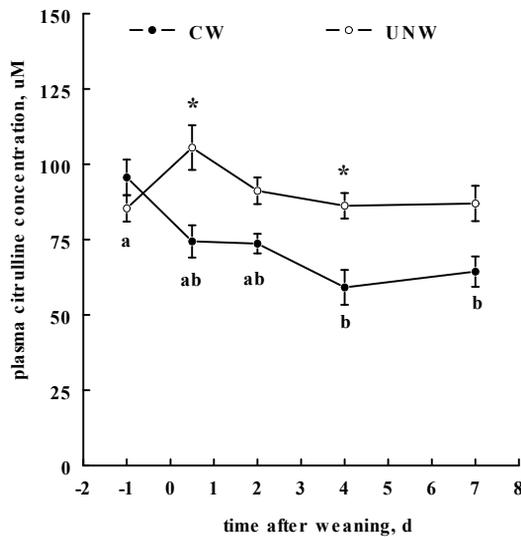
<sup>1</sup> UNW = unweaned; CW = conventionally weaned. <sup>a,b</sup> Different letters indicate differences between means of sampling days within a treatment.

### Plasma I-FABP concentration

There was no difference in I-FABP plasma concentration between the CW and UNW treatments (overall  $P = 0.63$ ; Figure 6.3). Moreover, mean I-FABP plasma concentration were nearly similar at all sampling days in both treatments. Variation in plasma I-FABP concentration was high, resulting in large standard errors (Figure 6.3). When examining the data in more detail, the high variation was suspected to be caused by a ‘sow-effect’; plasma I-FABP levels of the UNW and CW piglet from the same litter were almost similar (data not shown). Indeed, there was a strong correlation between the I-FABP concentration of sows and the average I-FABP concentration of their offspring on all treatment days ( $r > 0.87$ ,  $P < 0.003$ ).



**Figure 6.3.** Plasma I-FABP concentration (ng/mL) of weaned (● - CW; n = 10) and unweaned (○ - UNW; n = 10) piglets. The upper graph shows the means ± SE per treatment, the lower graph shows values per piglet. Note differences between scales.

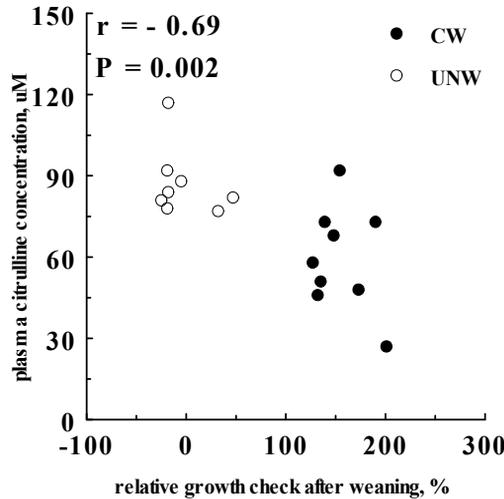


**Figure 6.4.** Plasma citrulline concentration of weaned (●- CW; n = 10) and unweaned (○ - UNW; n = 9) piglets. All values are means  $\pm$  SE. <sup>a-c</sup> Differences in citrulline concentration within a treatment ( $P < 0.05$ ) are indicated with different letters per sampling day. \* Different ( $P < 0.05$ ) from the value of weaned piglets on that sampling day.

### Plasma Citrulline concentration

Plasma citrulline concentrations differed between CW and UNW treatments (overall  $P = 0.0001$ ; Figure 6.4). Weaning of CW litters reduced citrulline levels and resulted in lower citrulline concentrations on d 4 and 7 after weaning ( $P < 0.001$  and  $P = 0.001$ ) compared to preweaning values. Overall, the relative postweaning growth check was correlated to plasma citrulline values on all postweaning sampling days ( $r < -0.50$ ,  $P < 0.04$ ), with the highest correlation on d 4 postweaning ( $r = -0.69$ ,  $P = 0.002$ ; Figure 6.5).

No correlation between relative postweaning growth check and citrulline concentration was observed within each separate treatment. Plasma citrulline concentrations correlated to plasma mannitol levels at d 4 postweaning in the CW treatment ( $r = 0.89$ ,  $P = 0.008$ ) and overall ( $r = 0.76$ ,  $P = 0.001$ ; Figure 6.6). On the other sampling days, no correlation was observed (overall or per separate treatment) between plasma citrulline and mannitol concentrations.

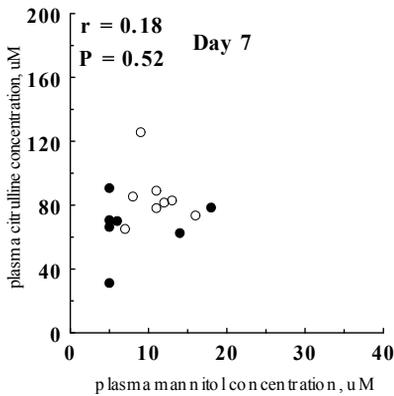
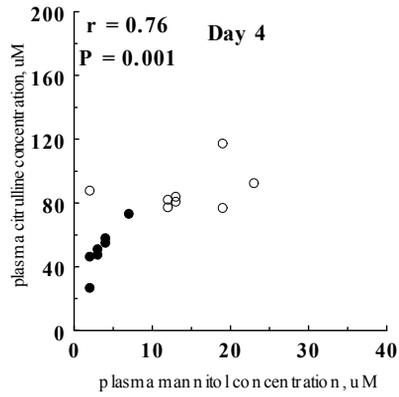
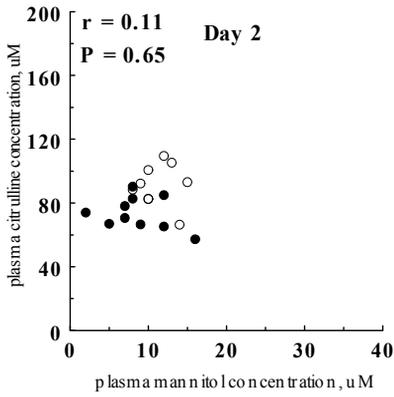
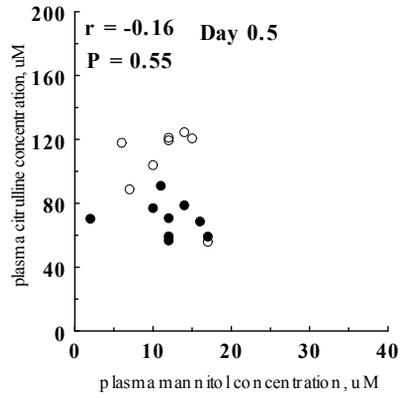
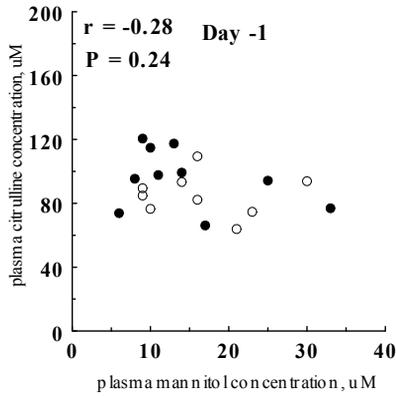


**Figure 6.5.** Relation between relative postweaning growth check (%) and plasma citrulline concentration at d 4 postweaning for weaned (● - CW) and unweaned (○ - UNW) piglets. Overall r- and P-value is given. Piglets with a more reduced growth have a higher relative postweaning growth check. A negative postweaning growth check indicates that some of the UNW piglets were gaining weight in the period after weaning the CW piglets.

## DISCUSSION

Commonly used parameters of intestinal function or enterocyte mass in pigs, such as morphology (Hampson, 1986; Pluske et al., 1996b; Hedemann et al., 2003), absorption (Hampson and Smith, 1986; Nabuurs et al., 1996) or permeability (Spreeuwenberg et al., 2001; Verdonk et al., 2007) are often labour-intensive and/or require sacrificing of the animals. The objective of the current study was to investigate whether plasma citrulline and I-FABP concentrations might be used as longitudinal markers for small intestinal function in piglets after weaning. Results of the current study indicate that plasma citrulline concentration, but not I-FABP concentration, seems to be a possible marker for monitoring intestinal function in postweaning pigs.

**Figure 6.6 (next page).** Plasma citrulline concentration (µM) in relation to plasma mannitol concentration (µM) per sampling day for weaned (● - CW) and unweaned (○ - UNW) piglets. Number of observations on plasma mannitol concentrations are similar to those of plasma D-xylose concentrations (Table 6.3). Overall r- and P-value are given per sampling day.



● CW  
○ UNW

Although psychological stressors are assumed to make a contribution to weaning associated intestinal dysfunction (Moeser et al., 2007), weaning associated anorexia plays the major part in postweaning shortening of villi (Pluske et al., 1996a; van Beers-Schreurs et al., 1998). The severe reduction in piglet growth, together with the decreased mannitol absorption at 4 d postweaning observed in the current study seems to be indirect evidence for occurrence of weaning-associated villous atrophy. However, in contrast with experimentally induced intestinal ischemia in weaned pigs of 20-25 kg (Niewold et al., 2004), no elevated levels of plasma I-FABP were observed after weaning of piglets in the current study. It should be noted, however, that the piglets used in the current experiment originate from only one farm, with no history of major weaning-associated problems. Under experimentally induced ischemic conditions, a 90-% flow reduction through the superior mesenteric artery (SMA), results in a rapid increase in plasma I-FABP levels within 30 min after onset of occlusion (Niewold et al., 2004). Villous atrophy, however, is greatest at 2 to 3 d postweaning (Spreeuwenberg et al., 2001; Hedemann et al., 2003) and therefore changes in I-FABP levels might have a different kinetic than morphological changes. The timing of blood samples may not have captured the change in I-FABP, if any. Another possible explanation for the lack of increased I-FABP levels might be the type of the induced tissue injury, because the mechanisms causing the intestinal tissue injury during severe ischemia and after weaning might be different. Unlike with ischemia, the weaning associated villous atrophy is believed to be primarily caused by a reduced cell division at the base of the villi, whereas the shedding at the extrusion zone proceeds, ultimately resulting in shortening of villi.

The observed I-FABP concentrations showed a great variation between piglets. Apart from a few extreme values, preweaning I-FABP levels of the current study correspond to previously reported values in pigs (Niewold et al., 2004). Niewold et al (2004) also showed a considerable variation in baseline levels of I-FABP and they suspected that this was caused by an unknown cross-reacting factor in the plasma, not interfering with changes in I-FABP levels in time. Interestingly, results of the present study suggest that the variation in I-FABP levels is a 'litter-effect', since the mean I-FABP concentration of weaned and unweaned littermates were highly correlated to the I-FABP level in the plasma of the mother during her subsequent pregnancy.

Plasma citrulline concentrations were affected by treatment in the current study, resulting in decreased citrulline levels after weaning. This seems to be in line with

the lower citrulline levels observed in patients with villous-atrophy associated small bowel diseases (Crenn et al., 2003). However, in the current study the reduction in citrulline levels appeared to be induced rather quickly, since plasma citrulline levels already tended to be decreased at 12 h postweaning compared with preweaning levels. The absence of nutrients in the intestinal lumen has been demonstrated to induce villous atrophy in pigs parenterally fed for 24 h (Niinikoski et al., 2004). A 12-h fast, however, was not associated with any detrimental effects on villous morphology in the small intestines of pigs (Hartke et al., 2005). Therefore, one might question whether the observed rapid decrease in the current study is really caused by villous atrophy and/or reduced intestinal function. Previous research on isolated pig enterocytes has demonstrated that the availability of precursors (glutamine) in the medium influences the production rate of citrulline by the enterocytes (Wu et al., 1994b). So, the fast decrease in citrulline levels observed 12 h postweaning in the current study might be a reflection of nutritional state rather than of intestinal function (or enterocyte mass).

However, plasma citrulline levels were even more lowered at d 4 and 7 postweaning, when piglet growth was restored to preweaning levels, indicating that the level of nutrient intake was higher than maintenance requirements. In addition, mannitol absorption was also decreased at d 4 postweaning and was correlated to the observed plasma citrulline levels at that day (with an overall  $r = 0.76$  and  $P = 0.001$ ). Previous studies on the correlation between sugar absorption and citrulline concentration are conflicting. In a study of Jianfeng et al. (2005) citrulline levels were correlated with the 5-h D-xylose recovery in urine of patients with short bowel syndrome. In contrast, no correlations were observed between plasma citrulline concentrations and several sugar absorption/permeability tests in patients with cancer treatment-induced gut toxicity (Lutgens et al., 2005). The lack of correlation in the latter study was supposedly due to a different time course of the markers, i.e. citrulline levels being more sensitive and specific for measuring small bowel epithelial cell loss. The proposed higher sensitivity of plasma citrulline for alterations in gut (dys)function might give an explanation for the lack of correlation between plasma citrulline and mannitol on the other sampling days in the current experiment.

An impressive amount of work has been done by Wu and co-authors to elucidate the intestinal amino acid metabolism in developing pigs. An interesting finding is the fact that enterocytes isolated from 23-d old weaned piglets (weaned at d 21) showed a 14-fold increase in citrulline production (from glutamine) compared to

enterocytes isolated from age-matched suckled piglets (Dugan et al., 1995). This increase seemed to be independent of diet, since it was also observed in 23-d old weaned piglets fasted for 2 d (Dugan et al., 1995), and might have been induced by elevated plasma cortisol concentrations associated with weaning (Flynn and Wu, 1997b; Flynn et al., 1999; Wu et al., 2000). The decreased plasma citrulline concentrations after weaning observed in the current study seem to be conflicting with these reports. However, it should be noted that the outcomes stem from different studies obtained under completely different experimental conditions. In the *in vitro* model, citrulline production is determined using a fixed amount of enterocytes, whereas the *in vivo* plasma citrulline concentrations observed in the current study were also determined by total enterocyte mass, which was assumed to decrease after weaning. Therefore, the citrulline levels measured postweaning in an *in vivo* model are a result of increased citrulline production potential per intestinal enterocyte on the one hand, and a reduced enterocyte mass on the other hand. In addition, in an *in vivo* model plasma citrulline levels are also influenced by the cellular uptake of the produced citrulline.

The plasma citrulline levels of the unweaned piglets in the current study correspond rather well to the levels of 29-d old suckling piglets ( $122 \pm 25 \mu\text{M}$ ;  $n = 7$ ; jugular vein sample) as reported previously by Flynn and Wu (1997). The somewhat lower plasma levels ( $87 \pm 6 \mu\text{M}$ ) of 28-d old unweaned piglets in the current study might be caused by the fact that these piglets were fasted for 3 h before the blood sample was obtained, which might also account for the lower variation in the current study compared to the study of Flynn and Wu (7% vs 21%). Previous research indicated that plasma citrulline concentration in the jejunal artery of 29-d old weaned piglets (weaned at d 21) decreased to  $43.0 \pm 4.8 \mu\text{M}$ , but was not significantly different from the level at weaning ( $50.6 \pm 6.9 \mu\text{M}$ ; Wu et al., 1994a). However, this was a cross-sectional study using cannulated piglets under complete anesthesia, complicating a comparison with the values of the current study. To our knowledge, the present study is the first that presents longitudinal data on plasma citrulline concentrations of piglets shortly after weaning.

Based on the results of the current study, plasma citrulline concentration seems to be a possible marker for the monitoring of intestinal function in pigs after weaning. It may be applied by biomedical researchers using pig models for the study of the processes involved in various human intestinal diseases (Burrin et al., 2003; Manzano et al., 2007; Pereira-Fantini et al., 2008). Moreover, it might give the opportunity to investigate intestinal function in pigs under practical conditions,

which was the rationale for the current study. In view of a possible practical use in the future, blood samples were obtained from the jugular vein in the current study. However, more insight is still needed in the relation between plasma citrulline concentration in jugular vein blood and a sampling location that is closer to the site of citrulline production, e.g. the portal vein. Further investigations should also focus on the relation between plasma citrulline concentration and macroscopic and morphologic small intestinal characteristics in pigs postweaning, which was beyond the scope of the current experiment.

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# CHAPTER 7

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**IMPROVING ADAPTATION TO WEANING:  
EFFECT OF INTERMITTENT SUCKLING REGIMENS ON  
PIGLET FEED INTAKE, GROWTH AND GUT  
CHARACTERISTICS**

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## ABSTRACT

Daily separation of sows and piglets during lactation, intermittent suckling (IS), improves feed intake and postweaning adaptation in piglets. The aim of the current study was to determine how, in piglets that have been subjected to IS, age at weaning and the duration of the preceding IS period contribute to postweaning adaptation through effects on feed intake, growth, and gut characteristics. All piglets had ad libitum access to creep feed from d 7 onward. Litters were subjected to conventional weaning (CW) or to 1 of 3 IS regimens. In CW, litters ( $n = 29$ ) had continuous access to the sow until weaning (d 26, d 0 = farrowing). During IS, litters had access to the sow between 1600 and 0600. Litters in the IS treatments were subjected to IS 1) from d 19 onward and weaned at d 26 (IS19-7D,  $n = 33$ ), or 2) from d 19 onward and weaned at d 33 (IS19-14D,  $n = 28$ ), or 3) from d 26 onward and weaned at d 33 (IS26-7D,  $n = 33$ ). One wk of IS before weaning on d 26 (IS19-7D) resulted in a similar weaning-associated relative growth check within the first 2 d after weaning compared to CW litters ( $72 \pm 13\%$  and  $90 \pm 7\%$ , respectively), but in a greater piglet growth and feed intake between d 2 and 7 postweaning ( $P = 0.014$  and  $P = 0.001$ ). Moreover, it prevented the weaning-associated villous atrophy observed at d 2 postweaning in CW litters. In IS piglets weaned after an extended lactation (d 33), a markedly smaller weaning-associated relative growth check was observed shortly postweaning ( $11 \pm 18\%$  and  $32 \pm 19\%$  for IS19-14D and IS26-7D litters, respectively). In these litters, feed intake and growth within the first 2 d after weaning were slightly greater when piglets experienced 2 wk of IS (IS19-14D) rather than 1 wk (IS26-7D;  $P = 0.03$  and  $P = 0.04$  for feed intake and growth, respectively). Irrespective of duration of IS, weaning after an extended lactation (d 33) with IS was not associated with postweaning villous atrophy. Irrespective of treatment, plasma citrulline concentrations were reduced at d 2 and d 8 postweaning compared to the values at weaning ( $P \leq 0.01$ ). No correlations were observed between postweaning plasma citrulline concentrations and postweaning small intestinal villous height. This study indicates that 1 wk of IS before weaning at d 26 of lactation improves feed intake and growth between d 2 and d 7 postweaning and prevents the weaning-associated villous atrophy observed in conventionally weaned piglets, although it did not prevent a profound growth check shortly after weaning. However, combining 1 wk of IS with an extended lactation (weaning at d 33) improved postweaning adaptation markedly in terms of growth, feed intake and gut characteristics.

Increasing the duration of IS from 1 to 2 wk slightly improved growth and feed intake shortly after weaning, but the contribution to postweaning adaptation seemed to be relatively small compared to extending lactation.

## INTRODUCTION

Conventional weaning of piglets is associated with a reduced postweaning nutrient intake (van Beers-Schreurs and Bruininx, 2002), reduced growth (Okai et al., 1976; Colson et al., 2006) and changes in gut integrity and function that increase the susceptibility to diarrhoea (van Beers-Schreurs et al., 1992; Nabuurs, 1998). Intermittent suckling (IS), a management strategy in which sow and piglets are separated during a fixed period of the day, stimulates preweaning creep feed intake and, as a result, improves postweaning feed intake and growth (IS from d 14, weaned at d 25; Kuller et al., 2004; Kuller et al., 2007a). Moreover, combining IS with an extended lactation period improved adaptation to weaning, as judged by the markedly reduced postweaning growth check and the substantial feed intake shortly after weaning (Berkeveld et al., 2007; Chapter 2). Average feed intake during the first wk of IS is low when 14-d old piglets are subjected to IS ( $218 \pm 38$  g/piglet), but is markedly increased ( $612 \pm 152$  g/piglet) when piglets are 1 wk older at initiation of IS (Chapter 4). One may question whether postponing the onset of IS to an older age, together with the associated higher feed intake, facilitates the adaptation to weaning. However, in the latter study initiation of IS at a later age coincided with a later weaning age as well, making it hard to evaluate the relative contribution of weaning age and age at the start of IS. Moreover, no comparison was made to a conventional weaning regimen.

Providing creep feed to piglets during the suckling period was found to improve their postweaning nutrient intake (Bruininx et al., 2002), and, as a result, to attenuate detrimental effects of weaning on intestinal structure and/or function (Kuller et al., 2007b). Moreover, in a study of Nabuurs et al. (1996) a 2-wk period of IS before weaning reduced the weaning-associated villus atrophy and increased postweaning small intestinal absorption, when creep feed was provided (Nabuurs et al., 1996). However, in the latter study no data on piglet feed intake or piglet growth was presented. More recent studies were mostly focussed on the effects of IS on piglet growth and feed intake (Kuller et al., 2004; Berkeveld et al., 2007; Kuller et al., 2007a; Millet et al., 2008). However, the effect of IS regimens on

postweaning small intestinal parameters, and the relation of these parameters with feed intake and growth in piglets subjected to IS remain to be elucidated.

The principal aim of the current study is to determine how age at weaning of piglets that have been subjected to IS, and the duration of the preceding IS period, each contribute to postweaning adaptation through effects on feed intake, growth and gut characteristics. Besides macroscopic and morphologic parameters of the small intestine, the plasma citrulline concentration may be a possible biological marker of intestinal function. Citrulline is the nitrogen end product of glutamine metabolism and is produced exclusively by the enterocytes of the small intestines (Windmueller and Spaeth, 1981). It is assumed to depend both on the metabolic activity of the enterocytes, as well as on the total enterocyte mass of the small intestines. Conventional weaning of piglets resulted in reduced plasma citrulline concentrations in piglets (Berkeveld et al. in press; Chapter 6). We anticipate that the villous atrophy often associated with weaning might play a role in these reduced plasma citrulline values. Therefore, another aim of the current study was to investigate the possible relation between postweaning small intestinal morphology and plasma citrulline concentrations.

## **MATERIALS AND METHODS**

### **Animals, Housing and Diet**

The Ethics Committee for animal experiments of Wageningen University and Research Centre approved the experimental design, including all procedures involving animals.

The experiment was conducted between April and November 2007 at the Sterksel research farm (Animal Sciences Group, Sterksel, the Netherlands). A total of 124 sows and their litters (TOPIGS20 (Landrace x York), TOPIGS, the Netherlands) with an average parity of  $3.5 \pm 0.1$  (ranging from 1 to 9) was used in 4 replicates. Piglets were TOPIGS20 x TEMPO (TOPIGS, the Netherlands) crossbreed. One week before farrowing, pregnant sows were individually housed in a farrowing pen (2.4 x 1.8 m) with farrowing crate. An infrared heated area was provided for the piglets from birth until d 14. Artificial lighting was provided between 0600 and 1800 and was dimmed during the night. At weaning, each litter was moved from the farrowing pen to its own nursery pen (1.77 x 2.65 m). Litters remained in the nursery pen until the end of the experiment (d 61).

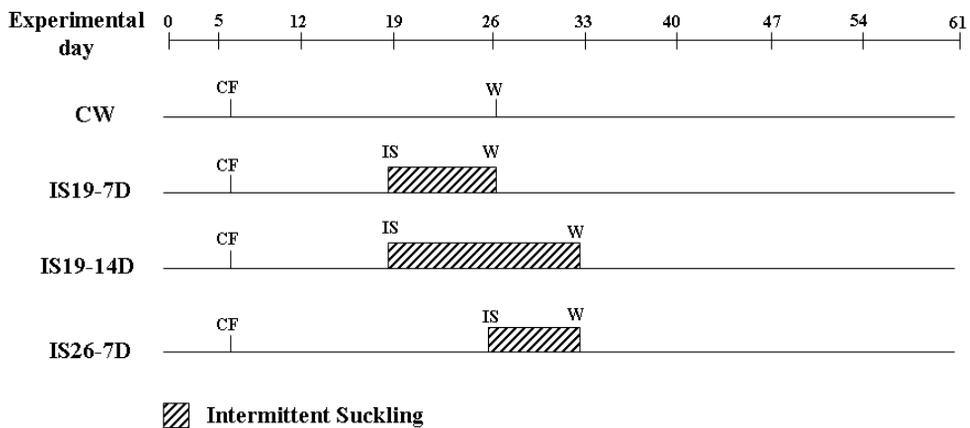
Litter size was standardized within 2 d after farrowing by cross fostering, resulting in an average litter size of  $12.4 \pm 0.1$  piglets. One day after farrowing piglets were weighed and received an eartag for individual identification. Within 1 wk after farrowing, piglets received an i.m. iron injection (Prevan 200, Eurovet Animal Health, Bladel, the Netherlands). Within each replicate, the beginning of the experimental procedure (d 0) was designated as the day at which most of the litters were born. Litters were born from 3 d before to 3 d after d 0, and piglet age at d 0 was  $0.4 \pm 0.2$  on average.

All litters were offered creep feed ad libitum from d 7 onward. From d 7 to 12, a 1:1 mix of two commercial creep feed diets was offered in a feeding bowl (diet 1, Havens Graanhandel NV, Maashees, the Netherlands; diet 2, 16.1% CP, 1.2% lysine, Almido Big, Havens Graanhandel NV, Maashees, the Netherlands). From d 12 until d 40, diet 2 was offered in a piglet feeder with 2 feeding places (15 cm/feeding place). From d 42 until the end of the experiment (d 61), a commercial creep feed for weaner pigs (diet 3; 17.1% CP, 1.15% lysine; Havo Opfok Sprint, Havens Graanhandel NV, Maashees, the Netherlands, The Netherlands) was offered. During a 2-d transition period (d 40 to d 42), diet 2 en 3 were mixed (1:1) for a gradual transition. During the entire experiment, drinking water was continuously available, provided by 1 drinking nipple per pen. Sows were fed an increasing amount of feed (Euro Airline Lactokorrel, 15% CP, 5.1% lysine, Cehave Landbouwbelang Voeders, Veghel, the Netherlands) after farrowing until the maximum allowance of 7.5 kg was reached at d 13 of lactation.

### **Treatments**

Within each replicate, sows were allocated to treatments based on sow parity and BW 1 wk before farrowing. The sow and her litter were subjected to conventional weaning (CW) or to 1 of 3 IS regimens (Figure 7.1). Litters receiving different treatments were housed in separate similar farrowing rooms to prevent possible disturbing effects of nursing litters in the same room, while other litters were separated from their sow during IS. In CW litters ( $n = 29$ ), piglets had continuous access to the sow until weaning at d 26. In the IS treatments, litters had continuous access to the sow until subjected to IS. Litters in the IS treatments were 1) subjected to IS from d 19 and weaned at d 26 (IS19-7D,  $n = 33$ ), or 2) subjected to IS from d 19 and weaned at d 33 (IS19-14D,  $n = 28$ ), or 3) subjected to IS from d 26 and weaned at d 33 (IS26-7D,  $n = 33$ ). During IS, sows were separated from their litter for 10 h/d (from 0600 until 1600) and housed individually in a different

room to prevent visual and auditory contact. During absence of the sow, an infrared light provided heating for the piglets. Litters in the IS19-7D and CW treatments were weaned at d 26, and those in the IS19-14D and IS26-7D treatments were weaned after lactation was extended for 1 wk (d 33). Piglets were weighed at 1 d after farrowing, and at d 19, 21, 26, 28, 33, 40 and 61. Litters weaned at d 33 (IS19-14D and IS26-7D) had an additional weighing at d 35. Creep feed residuals per litter were determined simultaneously with the weighing of the piglets.



**Figure 7.1.** Schematic presentation of the interventions within each treatment.

CF = start of creep feed (d 7); IS = onset of intermittent suckling; W = weaning. Shaded bars indicate period of IS. CW = Conventional Weaning at d 26; IS19-7D = IS from d 19, weaning at d 26; IS19-14D = IS from d 19, weaning at d 33; IS26-7D = IS from d 26, weaning at d 33.

### Collection of Samples

A total of 10 litters per treatment of the first 2 replicates were selected on d 25 based on litter feed intake (closest to treatment average). Within each of these litters, 3 piglets were selected based on their BW (closest to litter average), resulting in a total of 30 piglets per treatment (n = 120 piglets in total). The 3 piglets per litter were euthanized at weaning, or at d 2 or 8 postweaning, respectively, by intracardial injection of Euthesate® (0.75 to 1 ml/kg BW; Ceva Santé Animale, Naaldwijk, The Netherlands) after obtaining a 5-ml cardiac blood sample. The blood sample was transferred to a heparin-coated tube, and after centrifugation (10 min, 3000 rpm at 4°C) plasma samples were stored at -80°C.

The abdominal cavity was opened and gut segments (3 cm) for microscopy were obtained at ~10, ~50 and ~90% of the small intestinal length (duodenal, jejunal and ileal section). The gut segments were opened lengthwise and pinned on a piece of dental wax with the serosal side to the wax. Subsequently, the samples were fixed in 4% formalin solution with the mucosal side downwards to fix the villi vertically. The entire small intestine was dissected from the remaining mesentery, and the length and weight were determined. In the first replicate, small intestinal weight was only determined with its content. In the second replicate, the weight of the emptied small intestine was also determined ( $n = 5$  per treatment). In both replicates, the large intestine was isolated, and the weight was determined, with and without its content.

### **Histological procedure**

Two transverse tissue samples were cut from each gut segment using a stereo microscope. These tissue samples were dehydrated, embedded together in paraffin wax, and sectioned at 4 (or 5)  $\mu\text{m}$ . Hence, each section contained two transverse tissue samples of a gut segment. One section was transferred to a slide and stained with haematoxylin and eosin. In each slide, villous height and crypt depth were determined for at least 10 villi and crypts ( $17.4 \pm 0.2$  observations per slide on average) using an image analysis system with a monitor (Image Tool version 3.0, UTHSCSA Dental Diagnostic Science, Texas, USA). Villi and crypts were only measured when there was a complete longitudinal section of a villous and an associated crypt. The average villous height and crypt depth per slide was used as experimental observation.

### **Plasma citrulline concentration**

Plasma citrulline concentrations in plasma obtained before euthanization of the piglets were analysed by automated ion-exchange chromatography performed on a Jeol Amino-Tac (JLC-500/V, Tokyo, Japan) with post column ninhydrin derivatization (Berkeveld et al. in press; Chapter 6). The detection range was from 3 to 1000  $\mu\text{M}$ , with a maximal inaccuracy of 14%.

### **Calculations**

Cumulative feed intake at each time point was calculated by summing the total feed intake at previous time points.

A relative growth check was defined as the reduction in ADG in the first 2 d after weaning, from d 26 to 28 for IS19-7D and CW litters, and from d 33 to 35 for IS19-14D and IS26-7D litters, compared to the ADG in the last 5 d before weaning, and expressed as a percentage. The relative growth check (%) was calculated as:  $100 \cdot (\text{ADG}_{d21-26} - \text{ADG}_{d26-28})/\text{ADG}_{d21-26}$  for litters weaned at d 26 and as:  $100 \cdot (\text{ADG}_{d28-33} - \text{ADG}_{d33-35})/\text{ADG}_{d28-33}$  for litters weaned at d 33.

## Statistics

Data are presented as means  $\pm$  SE. Normally distributed data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Although litter was the experimental unit for ADFI, ADG and BW, and used as such in statistical analyses, all data were expressed per piglet. Effects were considered significant when  $P < 0.05$ ; in post-hoc testing the Bonferroni correction was applied. Correlations were calculated using Pearson correlation coefficients of SAS.

Because feed intake data of the first 3 wk (until d 21) were not normally distributed, they were analyzed using PROC NPAR1WAY, a non-parametric Kruskal-Wallis test. If this test detected an overall treatment effect, data of treatments were tested pair wise. From d 26 onwards, feed intake data were normally distributed and analyzed using the following model:

$$Y_{ij} = \mu + T_i + R_j + L_k + \text{age}_{ijk} + \text{bw19}_{ijk} + \text{ls19}_{ijk} + \text{par}_{ijk} + e_{ijk},$$

where  $Y_{ijk}$  = ADFI per piglet in a weighing interval,  $T_i$  = treatment,  $R_j$  = replicate as random effect,  $L_k$  = litter as random effect,  $\text{age}_{ijk}$  = litter age on d 0,  $\text{bw19}_{ijk}$  = mean litter BW (kg/piglet) at d 19,  $\text{ls19}$  = litter size on d 19, and  $\text{par}_{ijk}$  = parity of the sow. Data on piglet BW and ADG were analyzed as litter characteristics using the following model:

$$Y_{ijkl} = \mu + T_i + R_j + L_k + \text{age}_{ijk} + \text{bw19}_{ijk} + \text{par}_{ijk} + e_{ijk},$$

where  $Y_{ijkl}$  = mean litter ADG or BW (g/piglet),  $T_i$  = treatment,  $R_j$  = replicate as random effect,  $L_k$  = litter as random effect,  $\text{age}_{ijk}$  = litter age on d 0,  $\text{bw19}_{ijk}$  = mean litter BW (kg/piglet) at d 19, and  $\text{par}_{ijk}$  = parity of the sow. The co-variable  $\text{bw19}_{ijk}$  was omitted from the model for analysis on piglet birth weight and replaced by birth weight for analysis on mean litter ADG and BW at d 19.

Data on intestinal macroscopic parameters were analyzed with piglet as experimental unit, using the following model:

$$Y_{ijklm} = \mu + T_i + D_j + T_i * D_j + G_k + R_l + L_m + \text{age}_{ijklm} + \text{bw19}_{ijklm} + e_{ijklm},$$

where  $Y_{ijk}$  = intestinal parameter,  $T_i$  = treatment,  $D_j$  = day,  $T_i*D_j$  = interaction between treatment and day,  $G_k$  = gender,  $R_l$  = replicate as random effect,  $L_m$  = litter as random effect,  $age_{ijklm}$  = age on d 0,  $bw19_{ijklm}$  = BW at d 19. Data on plasma citrulline concentration were analyzed in the same model, but since the interaction between treatment and day was not significant ( $P > 0.05$ ) it was omitted from the model.

Data on intestinal morphology (villous height and crypt depth) were analyzed using the following model:

$$Y_{ijklmn} = \mu + T_i + D_j + T_i*D_j + G_k + LC_l + L_m + R_n + age_{ijklmn} + bw19_{ijklmn} + e_{ijklmn},$$

where  $Y_{ijk}$  = intestinal parameter,  $T_i$  = treatment,  $D_j$  = day,  $T_i*D_j$  = interaction between treatment and day,  $G_k$  = gender,  $LC_l$  = sample location,  $L_m$  = litter as random effect,  $R_n$  = replicate as random effect,  $age_{ijklm}$  = age on d 0,  $bw19_{ijklm}$  = BW at d 19. Since no interactions were observed between sample location, treatment and/or day, these interactions were omitted from the model. The measurements on villus height and crypt depth at the 3 sampling locations (duodenum, jejunum and ileum section) were averaged per piglet. These average values were used to present data on villous height and crypt depth per treatment, and to relate intestinal morphology to other parameters obtained in the study (such as piglet growth and plasma citrulline concentration).

## RESULTS

### Feed Intake

Creep feed intake before d 19 was negligible for all treatments. Between d 19 and 26, IS19-7D and IS19-14D litters were subjected to IS, whereas CW and IS26-7D litters were still continuously suckled. Feed intake of litters subjected to IS in this period (d 19 to 26) was still low, however, and did not differ from that of the continuously suckled CW and IS26-7D litters (Table 7.1). At d 26 both CW and IS19-7D litters were weaned, whereas the 2 other treatments (IS19-14D and IS26-7D) were weaned 1 wk later at d 33. Weaning (d 26) markedly increased feed intake in both CW and IS19-7D litters compared with the unweaned IS19-14D and IS26-7D litters, both during the first 2 d after weaning and from d 28 to 33. In the first 2 d after weaning, feed intake did not differ between litters weaned at d 26, but feed intake between d 28 and 33 was greater in IS19-7D litters compared to CW litters ( $P = 0.001$ ).

**Table 7.1.** Daily (ADFI) and cumulative (CUMFI) feed intake of piglets per treatment during lactation and after weaning

		Treatment <sup>1</sup>			
Day		CW	IS19-7D	IS19-14D	IS26-7D
ADFI (g/piglet/d)	19 to 21	7 ± 2	5 ± 1	5 ± 1	7 ± 1
	21 to 26	14 ± 3	19 ± 3	20 ± 4	20 ± 3
	26 to 28	103 ± 7 <sup>a</sup>	112 ± 9 <sup>a</sup>	37 ± 5 <sup>b</sup>	34 ± 6 <sup>b</sup>
	28 to 33	164 ± 9 <sup>a</sup>	203 ± 9 <sup>b</sup>	75 ± 10 <sup>c</sup>	55 ± 6 <sup>c</sup>
	33 to 35	--	--	244 ± 17 <sup>a</sup>	201 ± 14 <sup>b</sup>
	35 to 40 <sup>2</sup>	362 ± 13 <sup>a</sup>	375 ± 15 <sup>a</sup>	325 ± 12 <sup>b</sup>	322 ± 16 <sup>b</sup>
	40 to 61	808 ± 21 <sup>ab</sup>	829 ± 21 <sup>b</sup>	775 ± 19 <sup>ac</sup>	752 ± 24 <sup>c</sup>
CUMFI	12 to weaning, g/piglet	103 ± 2 <sup>a</sup>	126 ± 2 <sup>ab</sup>	587 ± 8 <sup>c</sup>	484 ± 6 <sup>bc</sup>
	12 to 61, kg/piglet	20.6 ± 0.5 <sup>a</sup>	19.6 ± 0.7 <sup>ab</sup>	18.7 ± 0.5 <sup>b</sup>	18.7 ± 0.6 <sup>b</sup>

<sup>a-c</sup> Within a row, means without a common superscript letter differ ( $P < 0.05$ ). The shaded cells indicate the first experimental period after weaning.

<sup>1</sup>CW = Conventional Weaning at d 26 ( $n = 29$ ); IS19-7D = IS from d 19, weaning at d 26 ( $n = 33$ ); IS19-14D = IS from d 19, weaning at d 33 ( $n = 28$ ); IS26-7D = IS from d 26, weaning at d 33 ( $n = 33$ ). ADFI = Average daily feed intake per piglet; CUMFI = Average cumulative feed intake per piglet. <sup>2</sup>Since feed residuals were not determined in the CW and IS19-7D litters on d 35, the ADFI was calculated between d 33 and 40.

In litters with extended lactation (weaning at d 33) no differences in feed intake were observed between IS19-14D and IS26-7D treatments during the suckling period. However, in the first 2 d after weaning feed intake was greater for litters subjected to 14 d of IS as compared to litters subjected to 7 d of IS ( $P = 0.03$ ; Table 7.1). Thereafter, feed intake of both treatments was similar. Between d 35 and 40, feed intake of litters weaned at d 33 was lower compared with that of litters weaned 1 wk earlier ( $P < 0.012$ ; Table 7.1). During the last 3 wk of the experiment (d 40 to 61), feed intake was still greater for IS19-7D litters than for IS19-14D and IS26-7D litters ( $P < 0.007$ ), and also greater in CW compared with IS26-7D litters ( $P = 0.003$ ; Table 7.1).

In all treatments, cumulative feed intake during lactation (Table 7.1) was correlated to feed intake in the first 2 d postweaning ( $r > 0.57$ ;  $P < 0.002$ ). At the end of the experiment, cumulative feed intake of IS litters weaned after extended lactation was lower than for CW litters ( $P = 0.014$  and  $P = 0.005$  for IS19-14D and IS26-7D litters, respectively) and tended to be lower than for IS19-7D litters ( $P = 0.087$  and  $P = 0.05$  for IS19-14D and IS26-7D litters; Table 7.1).

### Piglet Performance

Piglet mortality up to d 19, before onset of treatments was 9%. Piglet loss from d 19 until the end of the experiment (d 61) was similar in all treatments, and  $3.4 \pm 0.2\%$  on average.

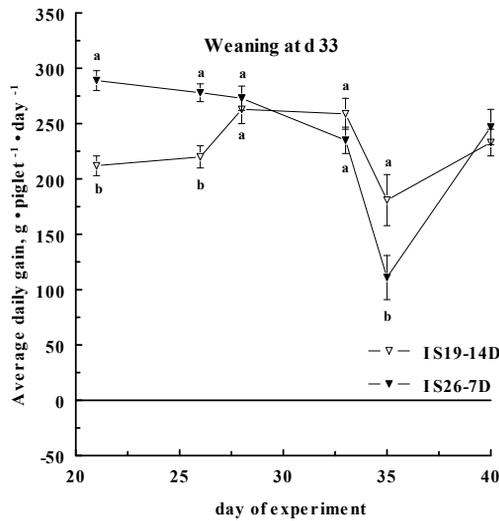
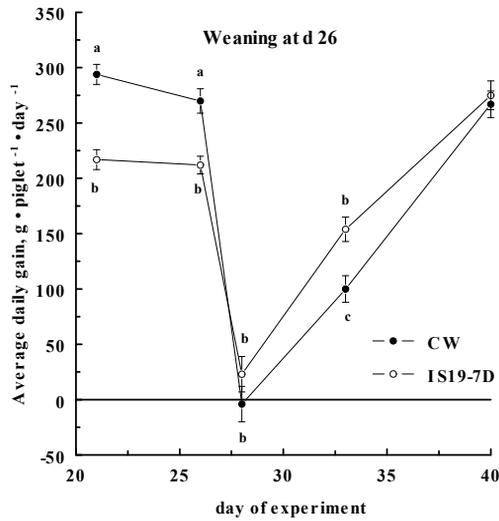
Piglet BW at the start of treatments (d 19) was slightly greater in IS26-7D litters compared with IS19-7D litters ( $P < 0.03$ ); CW and IS19-14D litters were intermediate (Table 7.2).

**Table 7.2.** Body weight of piglets (kg) during lactation and after weaning

Day	Treatment <sup>1</sup>			
	CW	IS19-7D	IS19-14D	IS26-7D
0	1.41 ± 0.04	1.41 ± 0.03	1.38 ± 0.04	1.42 ± 0.04
19	6.22 ± 0.14 <sup>ab</sup>	6.00 ± 0.14 <sup>a</sup>	6.16 ± 0.17 <sup>ab</sup>	6.35 ± 0.13 <sup>b</sup>
21	6.80 ± 0.15 <sup>a</sup>	6.44 ± 0.14 <sup>b</sup>	6.58 ± 0.18 <sup>b</sup>	6.80 ± 0.14 <sup>a</sup>
26	8.16 ± 0.19 <sup>a</sup>	7.50 ± 0.16 <sup>b</sup>	7.68 ± 0.20 <sup>b</sup>	8.12 ± 0.15 <sup>a</sup>
28	8.15 ± 0.18 <sup>a</sup>	7.54 ± 0.16 <sup>b</sup>	8.21 ± 0.21 <sup>a</sup>	8.66 ± 0.16 <sup>c</sup>
33	8.68 ± 0.19 <sup>a</sup>	8.33 ± 0.19 <sup>a</sup>	9.50 ± 0.24 <sup>b</sup>	9.84 ± 0.19 <sup>b</sup>
35	--	--	9.86 ± 0.26	10.06 ± 0.21
40	10.54 ± 0.25 <sup>a</sup>	10.23 ± 0.25 <sup>a</sup>	11.03 ± 0.27 <sup>b</sup>	11.30 ± 0.24 <sup>b</sup>
61	21.28 ± 0.47	21.48 ± 0.53	21.52 ± 0.47	21.72 ± 0.47

<sup>a-c</sup> Within a row, means without a common superscript letter differ ( $P < 0.05$ ). The shaded cells indicate the first weighing after weaning.

<sup>1</sup>CW = Conventional Weaning at d 26 (n = 29); IS19-7D = IS from d 19, weaning at d 26 (n = 33); IS19-14D = IS from d 19, weaning at d 33 (n = 28); IS26-7D = IS from d 26, weaning at d 33 (n = 33).



**Figure 7.2.** Average daily gain of piglets (g/piglet/d) during lactation and after weaning at d 26 (upper graph) or at d 33 (lower graph). <sup>a-c</sup> Data on piglet growth is presented in two graphs according to weaning age (d 26 or d 33). However, the model used for statistical analysis of piglet growth included all 4 treatments. Thus, at each experimental day, differences between the 4 treatments ( $P < 0.05$ ) are indicated with different superscript letters. CW = Conventional Weaning at d 26 (●;  $n = 29$ ); IS19-7D = IS from d 19, weaning at d 26 (○;  $n = 33$ ); IS19-14D = IS from d 19, weaning at d 33 (∇;  $n = 28$ ); IS26-7D = IS from d 26, weaning at d 33 (▼;  $n = 33$ ).

Therefore, treatment effects on BW and ADG were always corrected for BW at d 19 (see Statistics section). IS (starting at d 19) resulted in a reduced growth in IS19-7D and IS19-14D litters between d 19 and 21, and between d 21 and 26 of lactation when compared with that of the continuously suckled CW and IS26-7D litters in these periods ( $P < 0.001$ ; Figure 7.2). This reduced growth after onset of IS resulted in a lower BW for IS19-7D and IS19-14D litters at d 21 and 26 compared with CW and IS26-7D litters ( $P < 0.0001$ ; Table 7.2). Weaning (d 26) resulted in a dramatic decrease in piglet growth in both IS19-7D and CW litters (Figure 7.2), with a relative growth check of  $72 \pm 13\%$  and  $90 \pm 7\%$ , respectively ( $P > 0.05$ ). However, piglet growth between d 2 and 7 postweaning was greater for IS19-7D litters than for CW litters ( $P = 0.014$ ; Figure 7.2; d 28 to 33). The reduced growth after weaning resulted in a lower BW for IS19-7D and CW litters at d 33 compared to the intermittently suckled IS19-14D and IS26-7D litters ( $P < 0.0001$ ). Weaning after extended lactation (d 33) also reduced piglet growth, more so for litters subjected to 1 wk of IS (IS26-7D) than for litters with 2 wk of IS before weaning (IS19-14D;  $P = 0.037$ ; Figure 7.2). However, the relative postweaning growth check was markedly reduced after an extended lactation compared to the earlier weaned IS19-7D and CW litters ( $P < 0.05$ ):  $11 \pm 18\%$  in IS19-14D and  $32 \pm 19\%$  in IS26-7D litters. During the last 3 wk of the experiment (d 40 to 61), piglet growth was greater for IS19-7D than for IS26-7D litters ( $536 \pm 16$  and  $481 \pm 18$  g/piglet/d, respectively;  $P = 0.009$ ); CW and IS19-14D litters were intermediate ( $512 \pm 16$  g/piglet/d and  $499 \pm 12$  g/piglet/d, respectively). However, no differences in piglet BW were observed at the end of the experiment (d 61; Table 7.2).

After an extended lactation, piglet growth in the first 2 d postweaning was correlated to the cumulative feed intake during lactation ( $r = 0.44$  and  $P = 0.02$  for IS19-14D, and  $r = 0.52$  and  $P = 0.003$  for IS26-7D litters). This correlation was not observed in litters weaned at d 26 ( $r = 0.31$  and  $P = 0.10$  for CW, and  $r = 0.32$  and  $P = 0.07$  for IS19-7D litters).

### **Postweaning Intestinal Macroscopy**

Irrespective of treatment, small intestinal (SI) length numerically decreased in the first 2 d after weaning ( $P > 0.05$ ), but increased thereafter, resulting in a longer SI at d 8 postweaning compared to d 2 postweaning (overall  $P < 0.001$ ; Table 7.3). However, the relative decrease in small intestinal length within the first d 2 postweaning seems to be smaller for piglets weaned at d 33 ( $\sim 3\%$  for IS19-14D

and ~1% for IS26-7D) than for piglets weaned at d 26 (~ 9% for CW and IS19-7D). Moreover, the relative increase in small intestinal length between d 2 and d 8 postweaning seems to be greater for piglets weaned at d 33 (~23% for IS19-14D and ~12% for IS26-7D) than for piglets weaned at d 26 (~ 6% for CW and ~ 7% for IS19-7D). Overall, CW piglets had a smaller SI length compared with IS piglets, irrespective of IS regime (overall  $P < 0.02$ ). SI length did not differ overall between the IS treatments.

Small intestinal empty weight was similar in all treatments at weaning. Interestingly, weaning at d 26 reduced the SI empty weight to a numerically lower value at d 2 postweaning, whereas weaning at d 33 resulted in numerically higher values. At d 2 postweaning, SI empty weight was greater for IS26-7D piglets than for CW piglets ( $P = 0.04$ ); IS19-7D and IS19-14D were intermediate. At d 8 postweaning, piglets weaned after extended lactation (IS19-14D and IS26-7D treatment) had a greater SI empty weight compared to piglets weaned at d 26 (IS19-7D and CW treatment;  $P < 0.02$ ).

The empty weight of the large intestine (LI) increased after weaning in all treatments, resulting in higher values at d 2 compared to the values at weaning (overall  $P = 0.017$ ), and in higher values at d 8 postweaning compared to the preceding values (overall  $P < 0.001$ ). The empty weight of the LI was greater for IS19-14D and IS26-7D piglets than for the earlier weaned IS19-7D and CW piglets ( $P < 0.001$ ).

Cumulative feed intake during lactation was not correlated to the SI and LI empty weight in piglets weaned at either d 26 or d 33. Moreover, empty weight of the SI and LI at d 2 and 8 postweaning correlated to the feed intake in the first 2 d postweaning (overall  $r > 0.74$ ,  $P < 0.001$ ) and between d 2 and 7 postweaning (overall  $r > 0.70$ ,  $P < 0.001$ ), respectively. Only length of the SI at d 8 postweaning was correlated to feed intake (between d 2 and 7 postweaning; overall  $r = 0.50$ ,  $P = 0.001$ ).

### **Postweaning Intestinal Morphology**

Villous height and crypt depth were different between sample locations, with highest values at the proximal intestine, intermediate at the mid small intestine, and lowest at the distal end of the small intestine (overall  $P < 0.001$ ; data not shown).

Weaning reduced villous height in piglets of the CW treatment, resulting in shorter villi at d 2 postweaning compared to the values at weaning ( $P = 0.009$ ); villous height at d 8 was intermediate (Figure 7.3).

**Table 7.3.** Gut parameters<sup>1</sup> at weaning and at d 2 and 8 postweaning in each treatment<sup>2</sup>.

Treatment	CW			IS19-7D			IS19-14D			IS26-7D		
	0	2	8	0	2	8	0	2	8	0	2	8
Day postweaning												
SI length *	656 ± 25	596 ± 27	631 ± 24	720 ± 41	653 ± 39	696 ± 40	666 ± 34	649 ± 48	800 ± 30	696 ± 42	689 ± 34	771 ± 41
SI full weight	283 ± 16 <sup>a</sup>	291 ± 18 <sup>a</sup>	549 ± 19 <sup>b</sup>	316 ± 24 <sup>a</sup>	309 ± 21 <sup>a</sup>	568 ± 50 <sup>b</sup>	394 ± 24 <sup>a</sup>	486 ± 38 <sup>a</sup>	814 ± 30 <sup>b</sup>	377 ± 21 <sup>a</sup>	477 ± 33 <sup>a</sup>	863 ± 32 <sup>b</sup>
SI empty weight <sup>3</sup>	276 ± 17 <sup>ab</sup>	225 ± 13 <sup>a</sup>	368 ± 19 <sup>b</sup>	262 ± 29 <sup>ab</sup>	226 ± 25 <sup>a</sup>	307 ± 32 <sup>b</sup>	317 ± 35 <sup>a</sup>	348 ± 41 <sup>a</sup>	514 ± 34 <sup>b</sup>	321 ± 28 <sup>a</sup>	356 ± 39 <sup>a</sup>	593 ± 33 <sup>b</sup>
LJ full weight*	158 ± 10	230 ± 23	387 ± 18	159 ± 13	211 ± 15	380 ± 31	266 ± 23	347 ± 34	514 ± 25	280 ± 29	353 ± 34	576 ± 28
LJ empty weight*	89 ± 6	96 ± 7	165 ± 8	89 ± 8	94 ± 6	159 ± 11	129 ± 6	155 ± 11	212 ± 7	132 ± 9	152 ± 9	228 ± 8

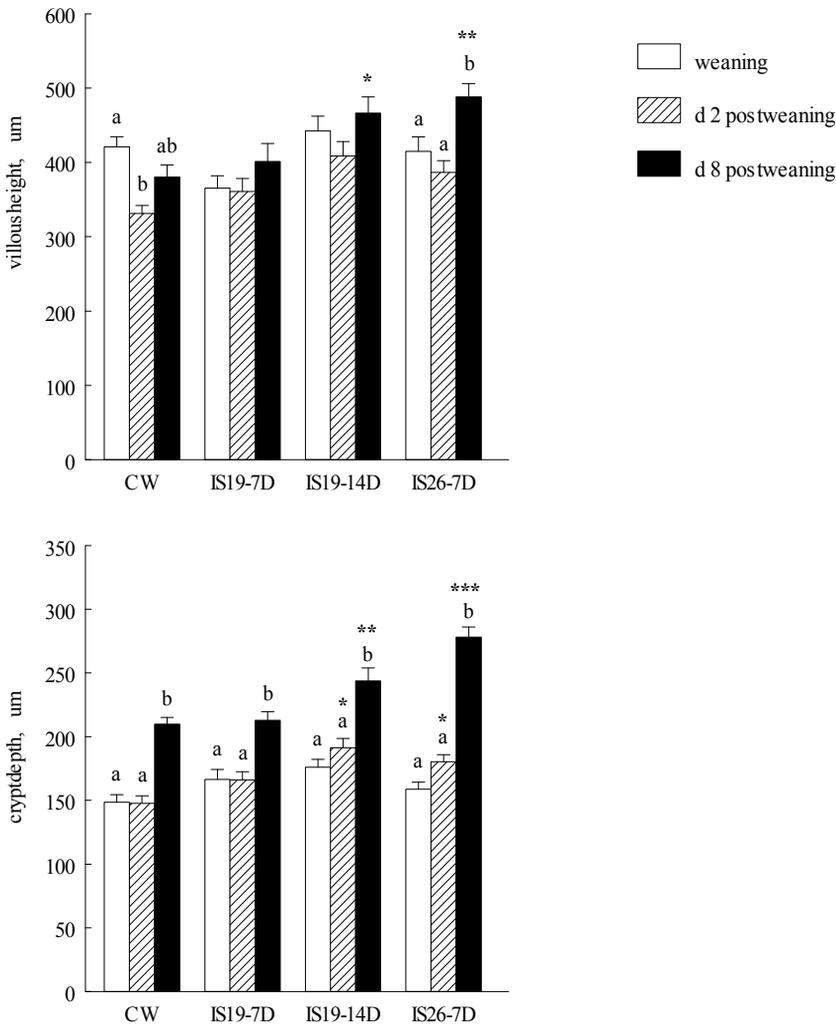
<sup>1</sup> All parameters are expressed in g, except for SI length, which was expressed in cm.

<sup>2</sup> CW = Conventional Weaning at d 26; IS19-7D = IS from d 19, weaning at d 26; IS19-14D = IS from d 19, weaning at d 33; IS26-7D = IS from d 26, weaning at d 33.

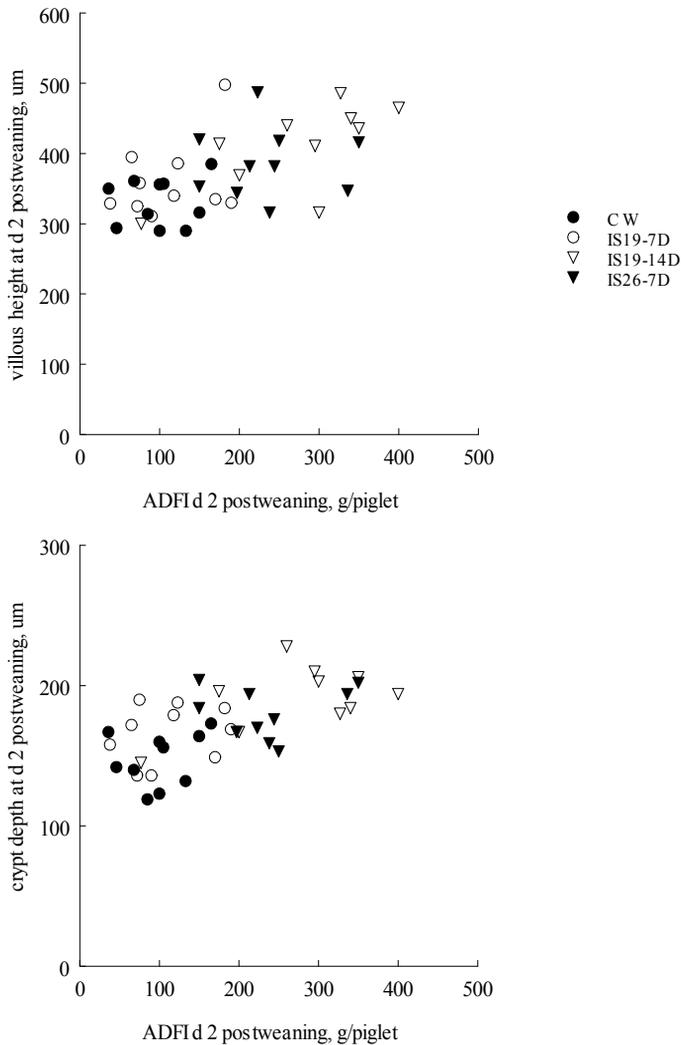
<sup>3</sup> The means ± SE displayed for the SI empty weight were based on observations of replicate 2 (n = 5).

<sup>a,b</sup> Within a row, means within each treatment with a different superscript letter are significantly different (P < 0.05).

\* Interaction between treatment and day was not significant for this parameter, therefore only overall P-values are given; SI length at d 8 > SI length at d 2 (overall P < 0.001). SI length of IS litters (irrespective of regimen) > SI length of CW litters (overall P < 0.02), no differences in SI length between IS regimens. LJ empty and full weight increase over time with d 0 < d 2 < d 8 (overall P < 0.001). LJ empty and full weight of IS19-14D and IS26-7D litters > IS19-7D and CW litters (overall P < 0.001).



**Figure 7.3.** Villous height (µm; upper graph) and crypt depth (µm; lower graph) in the small intestine at weaning and at 2 and 8 d postweaning. Values are means ± SE, n = 10 piglets. The measurements on villous height and crypt depth of the 3 sampling locations in the small intestine were averaged per piglet. <sup>ab</sup> Within each treatment, differences between the values per day are indicated with different superscript letters above the bars. \* Different from the value of CW treatment at that specific day; \*\* different from the values in the CW and IS19-7D treatment at that specific day; \*\*\* different from the values in all other treatments at that specific day. CW = Conventional Weaning at d 26; IS19-7D = IS from d 19, weaning at d 26; IS19-14D = IS from d 19, weaning at d 33; IS26-7D = IS from d 26, weaning at d 33.



**Figure 7.4.** Average daily feed intake (g/piglet/d) in the first 2 d postweaning in relation to villous height ( $\mu\text{m}$ ; upper graph;  $r = 0.58$ ,  $P < 0.0001$ ) and crypt depth ( $\mu\text{m}$ ; lower graph;  $r = 0.62$ ,  $P < 0.0001$ ) at d 2 postweaning. CW = Conventional Weaning at d 26; IS19-7D = IS from d 19, weaning at d 26; IS19-14D = IS from d 19, weaning at d 33; IS26-7D = IS from d 26, weaning at d 33.

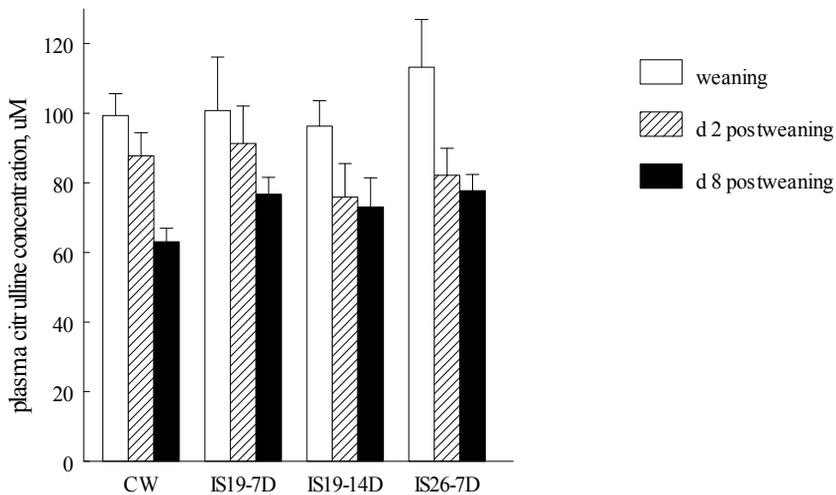
In contrast, villous height at d 2 postweaning was not different from the values observed at weaning in the IS treatments. Moreover, at d 8 postweaning, piglets weaned after an extended lactation (IS19-14D and IS26-7D treatment) had longer villi compared to conventionally weaned piglets (CW treatment;  $P = 0.0375$  and  $P < 0.001$ ; Figure 7.3). Irrespective of treatment, weaning resulted in deeper crypts at d 8 postweaning compared to values at weaning or 2 d after weaning (overall  $P < 0.001$ ; Figure 7.3). At d 2 postweaning, crypts were deeper in piglets of IS19-14D and IS26-7D treatments compared to piglets of the CW treatment ( $P < 0.001$  and  $P = 0.021$ ); values for IS19-7D were intermediate.

At d 8 postweaning, crypts were deeper in piglets of IS19-14D and IS26-7D treatments compared to piglets of the CW ( $P = 0.027$  and  $P < 0.001$ ) and IS19-7D treatment ( $P = 0.033$  and  $P < 0.001$ ).

Villous height and crypt depth at d 2 postweaning were both correlated to the ADFI in the first 2 d postweaning (overall  $r = 0.58$ ,  $P < 0.001$  and  $r = 0.62$ ,  $P < 0.001$ ; Figure 7.4). Moreover, villous height and crypt depth at d 2 postweaning were both correlated to the relative growth check in the first 2 d postweaning (overall  $r = -0.49$ ,  $P = 0.002$  and  $r = -0.60$ ,  $P < 0.001$ ). Villous height and crypt depth at day 8 postweaning were also correlated to the ADFI between d 2 and d 7 postweaning (overall  $r = 0.59$ ,  $P < 0.0001$  and  $r = 0.53$ ,  $P < 0.001$ , respectively). Only a weak correlation was found for villous height at d 8 postweaning and ADG between d 2 and d 7 postweaning (overall  $r = 0.38$ ,  $P = 0.04$ ), but not for crypt depth.

### **Postweaning Plasma Citrulline concentration**

Weaning reduced plasma citrulline concentrations resulting in lower levels on d 2 and d 8 postweaning compared to the values observed at weaning (overall  $P = 0.01$  and  $P < 0.001$ ; Figure 7.5). Postweaning plasma citrulline concentrations were not different between treatments. At weaning, plasma citrulline concentrations were negatively correlated to crypt depth (overall  $r = -0.36$ ,  $P = 0.022$ ). At d 2 postweaning, plasma citrulline concentrations were negatively correlated to crypt depth ( $r = -0.46$ ,  $P = 0.003$ ) and small intestinal empty weight ( $r = -0.62$ ,  $P = 0.004$ ). No correlations were observed between plasma citrulline concentrations and villous height. Moreover, no correlations were observed between plasma citrulline concentration at d 2 postweaning and the relative growth check, ADG or ADFI during the first 2 d postweaning. At d 8 postweaning, plasma citrulline was correlated to small intestinal length ( $r = 0.37$ ,  $P = 0.017$ ).



**Figure 7.5.** Plasma citrulline concentration ( $\mu\text{M}$ ) per treatment in jugular blood samples at weaning and at d 2 and 8 postweaning ( $n = 10$ ). CW = Conventional Weaning at d 26; IS19-7D = IS from d 19, weaning at d 26; IS19-14D = IS from d 19, weaning at d 33; IS26-7D = IS from d 26, weaning at d 33.

## DISCUSSION

The aim of the current study was to determine how age at weaning, after a period of IS, and the duration of IS, each contribute to the prevention of the detrimental effects on piglet performance and on gut characteristics associated with conventional weaning. In contrast to expectations, results of the current study demonstrate that the weaning-associated growth check the first 2 d after weaning was not prevented or reduced by 1 wk of IS before weaning (d 26). However, it did result in a greater feed intake and piglet growth from d 2 to 7 postweaning. Moreover, the postweaning villous atrophy at d 2 postweaning as observed in conventionally weaned piglets was prevented. The combination of 1 wk of IS with an extended lactation length (weaning at d 33) reduced the postweaning check considerably compared to piglets weaned at d 26, either with or without 1 wk of IS before weaning. This latter effect on the growth check was slightly more pronounced when IS was applied for 2 wk before weaning at d 33. Irrespective of

its duration, IS combined with extended lactation was not associated with postweaning villous atrophy.

Unexpectedly, subjecting litters to IS from d 19 to 26 of lactation did not stimulate their feed intake during this period as compared to that of continuously suckled litters in the current study. Previous experiments of our research group demonstrated a consistent stimulation of feed intake by IS during lactation, although the level of stimulation varied considerably, even between experiments under the same experimental conditions (Kuller et al., 2004; Berkeveld et al., 2007; Kuller et al., 2007a). In the current study, sow and piglets were, for practical reasons, separated for 10 h/d instead of the 12 h/d applied in the mentioned studies. A duration of daily separation of only 7 h was found not to be associated with any stimulatory effects of a 2-wk period of IS (d 14 to 28) on preweaning feed intake of piglets (Millet et al., 2008). Therefore, the 10-h separation period applied in the current experiment may have contributed to the lack of feed intake stimulation during lactation after 1 wk of IS. Even though preweaning feed intake was not stimulated by 1 wk of IS (from d 19 to 26), growth and feed intake between d 2 and 7 postweaning were greater for these IS litters than for conventionally weaned litters. Such an effect of IS has been observed previously in litters with a low preweaning feed intake level (Kuller et al., 2004). Apparently, IS not only influences preweaning feed intake, but may also have another influence causing a better postweaning performance (to be discussed in more detail below).

While 1 wk of IS before weaning at a conventional age (d 26) did not reduce the postweaning growth check, prolongation or postponing the period of IS for 1 wk before weaning did reduce the postweaning growth check markedly. This is in line with a previous study (Berkeveld et al., 2007), in which IS (d 14 onward) combined with an extended lactation period (weaning at  $43 \pm 1$  d) reduced the postweaning growth check to 14%. The shortened lactation length (from 43 to 33 d) and duration of IS (from 4 to 1 or 2 wk) of the current study seemed to have been equally effective in the prevention of a postweaning growth check. These positive effects on postweaning performance can not entirely be ascribed to the restriction in nursing time during the applied IS regimens. Age at weaning itself apparently has a crucial impact on how well piglets can cope with weaning, since a severe postweaning growth check was absent only when 1 wk of IS starting at 26 d of age, and not when starting at 19 d of age. Additional to increasing weaning age, subjecting piglets to an IS regimen does facilitate their adaptation, since feed intake

and growth shortly after weaning (at d 33) were improved by a longer period of IS (2 wk versus 1 wk) during lactation.

The observed values of villous height and crypt depth in the conventionally weaned piglets of the current study correspond to previously reported values (Nabuurs et al., 1993; van Beers-Schreurs et al., 1998). Villous height of conventionally weaned piglets was reduced by about 21% at d 2 postweaning compared to preweaning values. Similar reductions in villous height after weaning were shown to have a profound effect on intestinal absorption values (Nabuurs et al., 1996). Higher villi and deeper crypts were observed at d 8 after weaning in litters with an extended lactation combined with 1 or 2 wk of IS compared to conventionally weaned piglets (Figure 7.3), which is in line with previous findings of Nabuurs et al. (1996) that a 2-wk period of IS (8h/d) with supplemental feeding before weaning (at d 35) attenuates villous atrophy and improves intestinal absorption postweaning. Since in the current study no differences in intestinal morphology were observed between piglets weaned at a conventional age with or without 1 wk of IS in the current study, this appears to be an effect of age, rather than of the limited suckling time. Several authors (Pluske et al., 1996; van Beers-Schreurs et al., 1998), including data of the current study, have shown a positive correlation between villus height and feed intake, indicating the importance of feed intake for proper intestinal function. Therefore, the effect of age on gut morphology seemed to be mediated by the higher stimulation of feed intake when piglets were subjected to IS at an older age ( $339 \pm 18$  g/piglet versus  $104 \pm 18$  g/piglet in the first wk of IS, and  $2,013 \pm 94$  g/piglet versus  $1,239 \pm 56$  g/piglet in the first wk postweaning for IS26-7D and IS19-7D treatments, respectively; data not shown in results). Besides affecting intestinal morphology, macroscopic parameters of the intestine also seemed to be affected by age. The SI empty weight at d 2 after weaning is, for instance, numerically decreased compared to preweaning values in litters weaned at d 26, but not in those weaned at d 33. Moreover, SI empty weight at d 8 postweaning is greater in litters weaned at d 33 compared to those weaned at d 26. Surprisingly, 1 wk of IS before weaning at d 26 also prevented the post-weaning villous atrophy, despite the fact that feed intake before and within the first 2 d after weaning was not improved compared to conventionally weaned piglets. This suggests that beneficial effects of IS might also be mediated by factors other than an increased feed intake. One possible explanation could be the habituation of IS piglets to separation from their mother at the time they are weaned permanently. An attempt to eliminate nutritional stress at weaning by feeding piglets a high level

of sow milk attenuated, but did not completely prevent the weaning-associated villous atrophy (van Beers-Schreurs et al., 1998). Moreover, overnight maternal separation has been found to be associated with elevated basal cortisol levels in piglets (Klemcke and Pond, 1991). In addition, weaning of 19-d old piglets has been shown to activate stress signalling pathways, which mediate and contribute to the intestinal dysfunction, i.e. increased small intestinal permeability, associated with weaning (Moeser et al., 2007). Therefore, piglets habituated to repeated maternal separation in the IS regimen may have had a lower stress response after weaning, and this could have prevented or attenuated intestinal damage.

The plasma citrulline levels of the piglets at the day of weaning (d 26 or 33) correspond well to the levels of 29-d old suckling piglets reported previously ( $122 \pm 25 \mu\text{M}$ ;  $n = 7$ ; jugular vein sample; (Flynn and Wu, 1997). In line with previous findings of our group (Chapter 6), weaning reduced plasma citrulline concentrations. Plasma citrulline concentrations are lower in human patients with villous-atrophy associated with small bowel diseases compared to healthy subjects, and are considered to be a marker of enterocyte mass (Crenn et al., 2003). It was therefore anticipated that the postweaning reduction in plasma citrulline levels would be caused by the villous atrophy and/or impaired intestinal function associated with weaning. Unexpectedly, weaning of IS and CW litters in the current study resulted in a similar reduction of plasma citrulline concentrations, despite the fact that IS prevented a weaning-associated villous atrophy and resulted in a higher postweaning feed intake. Moreover, only a few and rather weak correlations were observed between plasma citrulline and crypt depth and none with villous height. Since the plasma citrulline concentration was determined in cardiac blood samples, it is the net resultant of both small intestinal citrulline production at the one hand and cellular uptake and/or metabolism at the other hand. Weaning is associated with numerous changes in the piglet environment, but one of the most important changes is the transition from a diet consisting mainly of milk to a nonmilk diet. Despite the fact that creep feed intake is already substantial in IS litters during extended lactation, the complete deprivation of sow's milk after weaning might possibly have altered the metabolism of citrulline produced in the small intestine after weaning, and thereby resulting in the lower plasma values, as observed after weaning in piglets of all treatments. So, to gain more insight in the relation between small intestinal citrulline production and morphological characteristics, a sampling location closer to the site of citrulline production, e.g. the portal vein (Wu et al., 1994), would be desirable.

In European conventional pig husbandry, piglets are weaned around 3 or 4 wk of age. As a result, weaning is associated with a reduced nutrient intake, growth and has a detrimental effect on intestinal structure and function. Results of the current study demonstrate that 1 wk of IS before weaning at the conventional age (d 26) does not prevent the weaning-associated growth check in the first 2 d after weaning, but does result in a greater feed intake and growth of piglets between d 2 and 7 after weaning. Moreover, it prevents the weaning-associated villous atrophy observed in conventionally weaned piglets. However, the fact that after 1 wk of IS before weaning at a 1-wk older age (d 33) a weaning-associated growth check was nearly absent indicates that age at weaning itself has a crucial impact on how well piglets can cope with weaning. Moreover, a longer period of IS before weaning (2 wk instead of 1 wk) at this older age improves the postweaning feed intake and growth of piglets shortly after weaning, suggesting an even more gradual adaptation to weaning. Like after 1 wk of IS before weaning at a conventional age (d 26) and irrespective of duration, villous atrophy did not occur when IS was applied before weaning at an older age (d 33). Despite the profound effects of IS (irrespective of regimen) on piglet performance in the short-term postweaning period, no differences in piglet BW were observed at the end of the experiment. It is postulated, however, that the beneficial effects of IS on postweaning performance might become even more pronounced (or long-lived) when piglets are weaned under suboptimal conditions, e.g. at farms with a history of postweaning diarrhoea. In conclusion, the results of the current study suggest that IS is a promising management strategy to improve the adaptation of piglets to weaning, by preventing postweaning detrimental effects on piglet nutrient intake, growth, and small intestinal morphology. Effects are most profound when IS is combined with an extended lactation.

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# CHAPTER 8

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## **GENERAL DISCUSSION**

Under (semi-)natural conditions, piglets are gradually weaned during a long lactation period with complete weaning occurring around 12 to 17 weeks of age (Jensen, 1986; Stolba and Wood-Gush, 1989; Bøe, 1991). Supervised by the sow, piglets become acquainted with nutrient sources other than sow milk and, with nursing frequency decreasing, they start consuming an increasing amount of solid feed. During this period, the young piglets make a gradual transition from a diet based on sow's milk to a nonmilk diet, ultimately achieving nutritional independence from the sow. This transition coincides with profound changes in intestinal function, enabling the digestion and absorption of nutrients from novel food sources (for review see Cranwell, 1995). Hence, under these (semi-) natural conditions, weaning is considered to be a long, gradual process rather than an event that takes place at a specific moment in time.

Over the last decades, the duration of the suckling period under farm conditions has been shortened dramatically. In conventional European pig husbandry piglets are weaned at a young age of 3 to 4 weeks. Conventional weaning is associated with an abrupt dietary change from sow's milk to dry feed, reallocation from the farrowing pen to a nursery pen, maternal deprivation, and often mixing with unfamiliar pen mates. Since the change to (nutritional) independency is a rather abrupt one, and takes place at a young age, piglets are physiologically not fully competent to deal with this situation. As a result, conventional weaning is often associated with a decreased nutrient intake (van Beers-Schreurs and Bruininx, 2002), a reduced piglet growth (Okai et al., 1976; Colson et al., 2006), display of piglet distress behavior (Dybkjaer, 1992), and changes in gut integrity and function after weaning that increase the susceptibility for diarrhoea (van Beers-Schreurs et al., 1992; Nabuurs, 1998). Considering the detrimental effects of conventional weaning on postweaning performance of piglets, it is desirable to improve their adaptation to weaning.

Considering the gradual weaning process under natural conditions, an obvious adjustment of husbandry conditions to resemble the natural weaning process would be to extend the suckling period. Under conventional housing conditions, an older age at weaning increased the postweaning feed intake (Leibbrandt et al., 1975), attenuated the postweaning growth reduction (Leibbrandt et al., 1975; Colson et al., 2006), reduced the development of stereotypic behavior (Metz and Gonyou, 1990), improved social skills later in life (Yuan et al., 2004), and attenuated the weaning-associated villous atrophy (Cera et al., 1988) and the detrimental effects on intestinal function (Moeser et al., 2007b). Improving piglet postweaning

performance by extending lactation length can only be profitable if reproductive performance of sows is not compromised. However, the inhibitory effect of suckling (Stevenson et al., 1981) and the metabolic constraints (for review see Prunier et al., 2003) on ovarian activity and ovulation during a prolonged continuous lactation will delay the establishment of the next pregnancy of the sow. During intermittent suckling (IS), sow and piglets are temporarily separated for a number of hours each day during the second part of lactation. This management strategy mimics the increasing amount of time spent away from the piglets during the lactation period, when sows themselves can control contact with their piglets (Bøe, 1991; Weary et al., 2002). Limiting the nursing time, when applied by IS, during the lactation period was found to induce ovulation during lactation (Matte et al., 1992; Gerritsen, 2007). Moreover, IS has been shown to improve piglet preweaning creep feed intake and to result in an improved postweaning piglet performance (Smith, 1960; Kirkwood et al., 1983; Nabuurs et al., 1996; Kuller, 2008), although some studies failed to show a beneficial effect of IS on postweaning performance (Hampson and Smith, 1986; Millet et al., 2008). The impact of IS seemed to vary between different studies, which presumably was related to the variation in age at onset, separation interval and duration of IS (piglet age at weaning) in those studies.

Hence, an extended lactation combined with intermittent suckling seems to be a promising strategy to encourage piglets to ingest solid feed during the suckling period, and to result in more mature piglets that are more capable of coping with the transition to weaning. Moreover, it is anticipated that sow reproductive performance will not be compromised by the extended lactation, since the onset of IS was shown to induce cyclicity of the sow, resulting in a lactational oestrus during the ongoing suckling period (described in Gerritsen, 2007).

In the current thesis we investigated how an extended lactation combined with IS regimens differing in separation interval (Chapter 2), age at onset, and duration (Chapter 4, 7) affected the adaptation to weaning, by measuring piglet growth and feed intake both during the suckling period and after weaning. An overview of the intermittent suckling regimens applied in the separate Chapters is given in Table 8.1. Since enforced maternal separation is associated with behavioral (and physiological) changes (Kuhn and Schanberg, 1998; Napolitano et al., 2003; Haley et al., 2005), one may question whether the repeated maternal separation of piglets subjected to IS also evokes such changes. Hence, we also investigated the effect of IS on piglet behavior (Chapter 3).

**Table 8.1** Applied intermittent suckling regimens, differing in separation interval, timing and duration.

Chapter	IS regimen	Age at onset	Duration	Daily separation time	Weaning age
2, 3	IS6	14 days	~ 4 weeks	2 x 6 hours	43 ± 1 days
2, 3	IS12	14 days	~ 4 weeks	12 hours	43 ± 1 days
4	IS14-S	14 days	~1 week	12 hours	21.8 ± 0.4 days
4	IS14-L	14 days	~ 4 weeks	12 hours	39.9 ± 0.3 days
4	IS21-S	21 days	~1 week	12 hours	27.8 ± 0.3 days
4	IS21-L	21 days	~ 4 weeks	12 hours	46.9 ± 0.4 days
7	IS19-7D	19 days	1 week	10 hours	26 days
7	IS19-14D	19 days	2 weeks	10 hours	33 days
7	IS26-7D	26 days	1 week	10 hours	33 days

At present, most parameters used to investigate the effects of weaning on piglet gut characteristics are end-point measurements, for which one needs to sacrifice the piglets (Montagne et al., 2007). In the present thesis we aimed to monitor postweaning intestinal function in the same piglets, whether or not subjected to a IS regimen, for a prolonged period of time. We have investigated selected parameters of interest, i.e.1) intestinal blood flow (Chapter 5), and 2) plasma citrulline and intestinal fatty acid binding protein (I-FABP) concentrations (Chapter 6), that might enable us to monitor postweaning intestinal function longitudinally.

In the penultimate chapter we investigated the effect of IS regimens differing in timing and duration on postweaning intestinal morphology, in addition to effects on piglet feed intake and growth (Chapter 7). Results of chapter 6 indicated that plasma citrulline was reduced by weaning and was positively correlated to sugar absorption values at day 4 postweaning. Based on these findings it was postulated that plasma citrulline concentration might be a possible marker of postweaning intestinal function (Chapter 6). Hence, in chapter 7 of this thesis the effect of IS on

postweaning plasma citrulline concentrations was determined and the relation to postweaning intestinal morphology was investigated (Chapter 7).

In the present chapter of this thesis, results of the previous chapters are integrated and discussed. The effect of separation interval on stimulation of preweaning feed intake is reflected in the first paragraph. Secondly, the effect of duration of IS, age at onset of IS and their interaction on piglet growth and feed intake is discussed. Next, we will consider the effect of extended lactation combined with IS on piglet behavior. After that, the effects of IS regimens on gut characteristics are discussed. Finally, some perspectives of intermittent suckling and suggestions for future research are presented.

### **EFFECTS OF SEPARATION INTERVAL ON PIGLET FEED INTAKE STIMULATION**

Previous studies indicated that the onset of IS at 14 days of age was associated with a reduction in growth during the suckling period, and concomitantly resulted in lower weaning weights of IS litters (weaned around 4 weeks of age) compared to continuously suckled litters (Kuller et al., 2004; Kuller et al., 2007a). In these studies, piglets and their dam were separated for 12 consecutive hours per day. We supposed that dividing total separation time over 2 intervals might (partly) prevent this reduction in growth, while still stimulating creep feed intake. Indeed, subjecting piglets to two 6-hours separation intervals attenuated, but did not prevent, the reduction of growth after onset of IS compared to a single separation interval of 12 hours (Chapter 2). However, we estimated that piglets maintained a greater dependency on milk for their growth in the two times 6 hours regimen compared to a single separation interval of 12 hours, which became evident from the lower feed intake stimulation, the higher nursing frequency, and the activity of the litter that was more synchronized to the presence of the sow (Chapters 2 and 3). Subjecting piglets to IS with a separation interval of 4 hours from 10 days of age until weaning (d 21) has been shown to result in an improved postweaning feed intake compared to continuously suckled piglets, but no difference was found if the latter were also offered creep feed during the suckling period (Hampson and Smith, 1986). Moreover, no difference in weaning weight was observed between intermittently or continuously suckled piglets with or without supplementary feed in the same study. Newton et al (1987a,b) demonstrated that separating piglets from their dam for 6 hours per day in the last week before weaning (at 3 to 4 week of age), without providing creep feed, did not affect litter weight at weaning. This

indicates that piglets subjected to IS are capable to (partly) compensate for the resulting milk intake deficit in the remaining hours with access to the sow. As a result, shorter separation intervals will most probably tone down the piglet's motivation in their search for nutrient sources other than milk. A recent study of Millet et al. (2008) indicated that separating sow and piglets for 7 hours per day during the last 2 weeks before weaning (day 28) did not improve creep feed intake of piglets during lactation at all, when compared to continuously suckled piglets (Millet et al., 2008). Furthermore, subjecting piglets to 1 week of IS before weaning did increase feed intake when a separation time of 12 hours was used (IS between day 14 and 21; Chapter 4), but not with a separation time of 10 hours (IS between day 19 and 26; Chapter 7), although piglets were 5 days older in the latter experiment.

In conclusion, these data indicate that the duration of a separation interval is of great importance for the stimulatory effect of IS on feed intake, and more so than the total separation time of sow and litter per day. Similar observations were made in a sow controlled housing system, where piglets of sows that spent more time away from their litter had a greater creep feed intake during the suckling period (Pajor et al., 2002).

## **EFFECT OF TIMING AND DURATION OF INTERMITTENT SUCKLING ON PIGLET PERFORMANCE**

### **Extending lactation length**

As described before, an older age at weaning was found to increase the postweaning feed intake (Leibbrandt et al., 1975), attenuated the postweaning growth reduction (Leibbrandt et al., 1975; Colson et al., 2006), reduced stereotype behavior (Metz and Gonyou, 1990) and weaning-associated villous atrophy (Cera et al., 1988) and attenuated the detrimental effects on intestinal function (Moeser et al., 2007b). On the other hand, a longer lactation period associated with an older weaning age postpones the next pregnancy of the sow. As mentioned at the beginning of this chapter, combining the extended lactation with IS was hypothesized to improve postweaning piglet performance, while maintaining a similar sow reproductive performance compared to conventional weaning management.

In previous studies on IS as an alternative lactation management strategy, it was mostly applied during a conventional lactation period of 3 to 4 weeks (Kirkwood et

al., 1983; Nabuurs et al., 1996; Kuller, 2008). Subjecting piglets to a short period of IS between 14 and 25 days of age resulted in a reduced piglet growth and concomitantly lower weaning weights compared to continuously suckled piglets (Kuller et al., 2004; Kuller et al., 2007a). In the same studies, IS piglets were found to have an improved feed intake and growth in the first week after weaning compared to conventionally weaned piglets, which compensated for their lower weaning weights (Kuller et al., 2004; Kuller et al., 2007a). Results of chapter 4 in the present thesis corroborated these results regarding the reduced growth of litters during IS (between d 14 and 21) and the lower weights at the time of weaning. In the same study, weaning of piglets after a short period of IS resulted in a reduced postweaning piglet growth when compared to still intermittently suckled piglets at that time. Hence, although a short period of IS was found to improve postweaning piglet growth compared to conventionally weaned piglets (Kuller et al., 2004; Kuller et al., 2007a), the IS piglets still experience a reduction of growth after weaning when compared to intermittently suckled piglets (Chapter 4). To conclude, a short period of IS commenced at 14 days of age, only partly prevented the growth reduction after weaning.

We hypothesized that when an extended lactation period is combined with IS, the older age at weaning together with a higher creep feed intake before weaning might be even more advantageous to prevent the detrimental effects of weaning on piglet performance. Indeed, extending the lactation length to 6 weeks in combination with IS from day 14 of age, markedly reduced the postweaning growth check compared to that of conventionally weaned piglets (Chapter 2). Moreover, growth of IS litters in the first week postweaning was only slightly lower compared to their growth during the week before weaning (Chapter 2 and 4). In addition, feed intake in the first week postweaning was only slightly lower compared to litters which had already been weaned 3 wk earlier (Chapter 2 and 4). Altogether, this suggests that piglets subjected to IS from 14 days of age during an extended 6-week lactation period are more capable to cope with the postweaning situation, as judged by their feed intake and growth, than piglets which were weaned after a short period of IS from day 14 of age. Results of chapter 2 suggested that the applied IS regimen, once daily for 12 hours (IS12) or separated twice daily for 6 hours (IS6), and thus limited suckling, contributed to the increased preweaning feed intake, considering the higher feed intake in IS12 litters. However, the beneficial effects of IS during an extended lactation on postweaning performance cannot be attributed entirely to

the limited time for suckling. The older age at weaning could also have contributed to the improved postweaning performance.

### **Piglet age at onset of intermittent suckling**

The initial contact of piglets with nutrient sources other than milk often is attained through exploration of the environment (Fraser et al., 1998). Exploratory behavior at d 17 of age, however, was found to constitute only 5% of the total observations in continuously suckled, conventionally housed piglets, whereas 18% consisted of suckling behavior (Chapter 3). The dependency on milk as predominant nutrient source at this young age becomes evident from the low feed intake levels of continuously suckled piglets observed in the first weeks of lactation (Chapter 2, 4 and 7; Pajor et al., 1991; Puppe and Tuchscherer, 2000). Although feed intake behavior was stimulated when piglets were subjected to IS at 14 days of age compared to continuously suckled piglets (Chapter 3), feed intake in the first week of IS was still rather low and quite variable between litters (Chapter 2 and 4; Kuller et al., 2004; Kuller et al., 2007a). Moreover, piglet growth was reduced after onset of IS at 14 days of age (Chapter 2 and 4), which is in line with previous findings (Kuller et al., 2004; Kuller et al., 2007a). These results indicate that piglets at this young age are not yet capable to compensate their reduced nutrient intake from milk (due to the imposed limited nursing time) with a sufficient level of dry feed intake. On the one hand, this might be caused by the fact that piglets are not yet acquainted sufficiently with dry feed. However, continuously suckled piglets were found to have 1 to 30 visits to the piglet feeder at day 13 of age (Kuller, 2008). Nevertheless, we and others suggested that this exploration of the piglet feeder was not nutrient driven, given the high number of nursings observed at this early stage of lactation (Chapter 3; Kuller, 2008; Puppe and Tuchscherer, 2000). On the other hand, the gastrointestinal tract, which is still under morphological and functional development at this young age (Pluske et al., 2003) and fully adapted to digestion of sow's milk, might not yet be capable to digest and absorb nutrients from the ingested dry feed.

Postponing the onset of IS with 1 week, to the age of 21 days, increased piglet feed intake about 3-fold in the first week of IS (Chapter 4). In contrast, in a different study of this thesis (Chapter 7) feed intake of litters subjected to IS between day 19 and 26 of age was not greater compared to that of continuously suckled litters in the same period. As mentioned previously, the shorter separation time (10 h/d) in

the study of chapter 7 might have played a part in this lack of feed intake stimulation.

Postponing the onset of IS to 21 days of age, irrespective whether or not it stimulated feed intake, was still associated with a reduced piglet growth (around 30% growth reduction), similar to that observed when IS was commenced at day 14 (Chapter 4 and 7). Postponing the onset of IS with another week (onset at day 26), however, appeared to attenuate the reduction in growth associated with the onset of IS (Chapter 7). Piglet growth in the latter treatment was only reduced with 5% in the first 2 days of IS and with 17% between day 2 and 7 after onset of IS (Chapter 7). Moreover, feed intake in the first week of IS was increased 3-fold, compared to litters in which IS began at a 1 week younger age (339 g versus 104 g per piglet, respectively, Chapter 7).

In conclusion, results of the present thesis demonstrated that an increased piglet age at onset of IS increases the extent to which feed intake is stimulated. Moreover, if piglets are subjected to IS at a young age, normally associated with low voluntary feed intake, the increased dry feed intake is not adequate to compensate for the deficit in milk intake due to the limited nursing time. However, it needs to be emphasized that even a short period of IS at a young age (11 days, from d 14 onward) can still be sufficient to improve piglet growth and feed intake in the first week after weaning, compared to conventionally weaned piglets (Kuller et al., 2004; Kuller et al., 2007a; Chapter 7).

### **Duration of intermittent suckling in relation to age at onset**

Extending the lactation to 6 weeks, with a 4-week period of IS from d 14 onward, resulted in a gradual adaptation to weaning, as judged by the markedly reduced postweaning growth check and a growth and feed intake (shortly after weaning) similar or slightly lower than that in piglets which already had been weaned 3 weeks earlier (Chapter 2). However, feed intake in the first week of IS (d 14 to 21) was very low and variable between litters (Chapter 2). As mentioned above, postponing the onset of IS to an older age resulted in greater stimulation of feed intake. Indeed, when the onset of a 4-week IS period was postponed to 21 days of age (IS21-L), feed intake during the first week of IS (and in the weeks thereafter) was similar to that of litters already experiencing two weeks of IS at that time (IS14-L; Chapter 4). Moreover, the estimated relative contribution of dry feed to energy intake was similar for both treatments (IS14-L and IS21-L) during the successive weeks of IS, suggesting that dependency on milk was similar for age-

matched piglets, despite differences in the experienced duration of IS. This indicates that the extent to which piglets subjected to IS are able to replace the deficit in milk intake (due to the limited nursing time) with ingestion of dry feed, is age-dependent rather than depending on the duration of IS already experienced in the preceding period. It is reasonable to assume that the age-related maturation of the gastrointestinal tract (Manners and Stevens, 1972; Kidder and Manners, 1980; Cera et al., 1988), and thereby the ability to process ingested dry feed, might in part be responsible for this effect.

Subjecting piglets to a shorter period of IS, during one week from 14 days of age, instead of the above mentioned 4 week period, still improved preweaning creep feed intake, but weaning of these piglets resulted in a period of reduced growth compared to piglets still intermittently suckled at that time (Chapter 4). Combining a short period of IS with an older age at onset of IS (21 days instead of 14 days of age) improved preweaning creep feed intake and shortened the period of reduced growth observed after weaning (Chapter 4). The concomitant older age at weaning increased the estimated relative contribution of dry feed to energy intake in the week before weaning from about 14% for piglets weaned at 3 weeks to 27% for piglets weaned at 4 weeks of age. In the study of chapter 7, one week of IS between 19 and 26 days of age did not attenuate the growth check observed in conventionally weaned piglets ( $72 \pm 13\%$  and  $90 \pm 7\%$ , respectively) nor did it improve piglet feed intake in the first 2 days after weaning. Hence, the piglets were probably still too dependent on milk for nutrient intake to prevent a postweaning growth check. However, the piglets subjected to IS did show an improved feed intake and growth later in the first week postweaning. These findings are in line with results of Kuller et al. (2004, 2007), who demonstrated that a short (11-day) period of IS before weaning at 26 days of age improves piglet growth and feed intake in the first week after weaning.

Postponing the onset of IS with another week, and thus extending lactation length with one week (IS from day 26 to 33), markedly reduced the growth check in the first 2 days postweaning (to  $32 \pm 19\%$ ), when compared with litters weaned one week earlier ( $72 \pm 13\%$ ; Chapter 7). This effect was ascribed to the older age at weaning and to the higher stimulation of feed intake associated with the older age at onset of IS. Piglet growth and feed intake in the first two days after weaning was improved even more when piglets had been subjected to a 2-week period of IS before weaning (day 33; Chapter 7). In these litters, growth check in the first two days was markedly reduced (to  $11 \pm 18\%$ ). In addition, results of the study of

chapter 4 suggested that the relative growth derived from feed in week 5 of lactation (with 2 weeks of IS from d 21) was estimated to be approximately 47%. Interestingly, these values seem to correspond well with the estimated growth from feed (45%) and with the growth check (14%) reported for litters weaned at 6 weeks of age, after being subjected to a 4-week period of IS (Chapter 2).

In conclusion, the results of the present thesis suggest that piglets submitted to IS and weaned at a conventional age (3 to 4 weeks) are not yet fully capable to cope with weaning, as judged by piglet growth and feed intake. However, when weaned at an older age (extended lactation) even a short (1-week) period of IS was found to result in a more gradual adaptation to weaning.

### **INTERMITTENT SUCKLING AND PIGLET BEHAVIOR**

Maternal deprivation can cause behavioral and physiological changes in piglets, such as an increased call rate or inhibition of GH secretion, which often contribute to the survival of the offspring during periods of maternal absence (Kuhn and Schanberg, 1998). Moreover, weaning of piglets was found to be associated with the development of behavioral patterns indicative for piglet distress, such as aggression, belly nosing, manipulation of pen mates and (in)activity (Dybkjaer, 1992). Since the submission of piglets to an IS regime imposes them to (repeated) periods of maternal separation, it was questioned whether IS is associated with similar behavioral changes.

Onset of IS resulted in a transient increase in activity of piglets on the first day compared to continuously suckled piglets, with a more pronounced effect in litters separated from their sow for 12 consecutive hours than for two times 6 hours (Chapter 3). We suggested that this increase in activity might have been the result of the restlessness commonly associated with a sudden (previously unexperienced) separation from the mother (Fraser et al., 1998). Besides a higher activity, piglets subjected to IS had vocalized more compared to continuously suckled piglets, but this was observed predominantly on the first day of IS. Later in lactation, an increased call rate did occur, but only when one entered the farrowing room shortly before the sows were returned to their litters (personal observations). The fact that the increased activity was transient and not observed anymore at 2 days after the onset of IS, suggests that the piglets quickly habituated to the periods of maternal absence. Our results corroborated data by Kuller (2008) who observed no differences in piglet non-feeding-related activity between IS and continuously

suckled piglets (both selected on a high feed intake level during lactation) on the third day after commencement of IS. Although total daily activity was not affected by IS (except for the first day), the activity pattern of the litters over the day was changed dramatically (Chapter 3). Activity of IS litters coincided closely with the presence of the dam, even more so when litters with two 6-hour separation intervals were compared to those with one 12-hour separation interval. This became evident from the higher percentage of activity during sow's absence in the latter litters. Again, this is in agreement with findings of Kuller (2008) who found lower activity levels of IS litters during separation from their dam compared to continuously suckled litters in that period of the day.

Nutritional, social and environmental stressors are suggested to play a part in the development of belly nosing behavior (Dybkjaer, 1992; Gardner et al., 2001; Li and Gonyou, 2002; Jarvis et al., 2008; Widowski et al., 2008), a stereotypic behavior, involving repeated rhythmic up-and-down massage movements with the snout at the flanks or belly of penmates, often observed after weaning of piglets. The repeated maternal deprivation during IS from 14 days of age did not result in development of belly nosing behavior during the 6-week suckling period (Chapter 3). In contrast, conventional weaning of piglets at day 21 under identical housing conditions did result in an increased expression of this behavior after weaning (Chapter 3). This suggests that complete weaning is necessary to induce this stereotypic behavior, since the alternately presence and absence of the sow during IS did not evoke such a behavioral response.

Separation of piglets from their dam at weaning, without mixing them with unfamiliar penmates, induces an increase in aggressive behavior (Fraser, 1978). The results of the study in chapter 3 demonstrated that subjecting piglets to IS during an extended lactation did not affect the proportion of aggressive or manipulative behavior during the suckling period. Similarly, the time spent on aggressive and manipulative behavior in litters submitted to IS between day 14 and 25 of age was similar to that of continuously suckled litters in that period (Kuller, 2008). Likewise, at 4 weeks of age no effect of sow controlled housing was observed on the percentage of aggressive behavior when compared to piglets in conventional housing (Rantzer et al., 1995). Although the sows are not forced to leave their litter in sow controlled housing systems, the total separation time of sow and piglets increased with increasing piglet age from 25% at 2 weeks of age to 75% at the time of these behavioral observations at 4 weeks of age (Rantzer et al., 1995). Interestingly, plasma cortisol concentrations of piglets in sow controlled

housing were found to be similar to conventionally housed piglets throughout the suckling period (Rantzer et al., 1995). However, only 1 blood sample was taken (at 9:00 in the morning 2 weeks before weaning) during the entire suckling period, and more frequent sampling is desirable to study possible effects of maternal deprivation on cortisol concentrations in more detail. In conclusion, the results of the present thesis (Chapter 3) and others suggest that IS (maternal deprivation) was not associated with lasting behavioral patterns indicative of piglet distress during the suckling period.

In the studies of the present thesis, an increased number of vocalizations in response to weaning was observed both in conventionally weaned as well as IS litters, although this response seemed to be somewhat delayed for the latter (personal observations). Hence, we questioned whether piglets submitted to IS are already accustomed to the separation from their dam at the time they are weaned. If so, repeated maternal separation during the lactation period might attenuate the weaning-associated behavioral (and physiological) changes observed in conventionally weaned piglets by us and others (Chapter 1 and 3). The present thesis does not provide any data on behavior of IS piglets after weaning and other studies on this topic are scarce. In an attempt to unveil possible effects, two studies investigating the effect of previously experienced maternal separation on postweaning behavior are discussed.

Time spent on exploratory, manipulative or aggressive behavior in the first 2 days postweaning was found to be similar in IS and conventionally weaned piglets (Kuller, 2008). Moreover, no differences in non-feeding related activity were observed between IS and conventional litters at their first day postweaning. However, as suggested by the author, these data should be interpreted with care since results were obtained using only a small number of litters. Similar to IS before weaning (Kuller, 2008), the periods of maternal absence in sow controlled housing did not affect the percentage of active, exploratory or aggressive behavior of piglets at day 3 postweaning compared to conventionally housed piglets (Rantzer et al., 1995). Moreover, no differences were observed in postweaning plasma cortisol concentrations (Rantzer et al., 1995). Results of these studies suggest that the postweaning behavior of piglets that experienced repeated periods of maternal separation during the lactation period is not different from that of piglets kept conventionally during lactation. We do want to emphasize that piglet behavior was only observed shortly after weaning in the latter studies and some behavioral patterns associated with piglet distress, like belly-nosing, are rarely seen

in the first days after weaning, but develop several days thereafter (Fraser, 1978; Worsaae and Schmidt, 1980). Since no data is currently available on the possible long-term effect of previously experienced periods of maternal separation (either by IS or sow-controlled housing) on postweaning behavioral or physiological traits, this remains to be elucidated.

## **EFFECT OF INTERMITTENT SUCKLING REGIMENS ON GUT CHARACTERISTICS**

### **Preweaning period**

Fasting has dramatic effects on small intestinal mucosal structure and function (Ferraris and Carey, 2000). Overnight nutritional depletion was found to result in an increased intestinal permeability and decreased villous height in humans (van der Hulst et al., 1998). The onset of IS (at 14 to 19 days of age) was associated with only a small, insignificant nutrient intake during the periods of sow absence, given the low feed intake levels of piglets during the first 2 days of IS (Chapter 2, 7). Hence, this indicates that piglets are imposed to repeated periods of (almost) fasting shortly after the onset of IS. One might hypothesize that these periods of underfeeding negatively affect small intestinal structure and/or function, as occurs with weaning. Yet, subjecting piglets to a single, short-term fasting of 6 or 12 hours was not associated with any detrimental effects on intestinal morphology (Hartke et al., 2005). But, IS piglets are subjected to repeated nonsuckling (and low nutrient intake) periods. Nabuurs et al. (1996) demonstrated that IS for 8 h/day without supplementary feeding between 3 and 5 weeks of age (hence repeated 8-h fasting periods), did not induce changes of intestinal function or net fluid absorption of the small intestine at weaning, when compared to continuously suckled piglets. Although piglets submitted to IS initially have a low feed intake and are limited in their nursing time, their body weight gain in the first two days after onset is only 26% lower than that of continuously suckled piglets (Chapter 7; recalculated from figure 1). Moreover, a normal mucosal proliferation and intestinal growth was found in 1-week old piglets when their enteral nutrient intake was reduced by 40% accomplished by parental feeding (Burrin et al., 2000). The results from the above studies seem to indicate that IS, although associated with a reduced nutrient intake at the onset, does not negatively affect intestinal structure or function per se.

Provision of creep feed during a continuous lactation was not associated with changes in small intestinal structure or absorption values at 21 days of age

(Hampson, 1986; Hampson and Kidder, 1986). It must be stated however, that in the latter study total feed intake of piglets during the lactation period was, as can be expected at this young age (Puppe and Tuchscherer, 2000), rather low with an average of 236 g per piglet (ranging from 0 to 363 g per piglet). In a study of Bruininx et al (2004) creep feed was supplemented with chromiumoxide (consumption of which colours the faeces green), which enabled the authors to designate individual piglets as good eaters, moderate eaters or non-eaters. In line with the above mentioned findings, the consumption of creep feed (eaters) did not affect morphology of the small intestine at 28 days of age when compared to non-eaters. Again, creep feed intake during the suckling period varied greatly between litters, with an average of 301 g per piglet (ranging between litters (containing both eaters and non-eaters) from 24 to 690 g per piglet). Although the supplementation of chromium-oxide allows one to distinguish between eating and non-eating piglets within litters, individual ingested creep feed could not be quantified in the study of Bruininx et al (2004). We suggest that preweaning feed intake might still have varied considerably between individual piglets designated as good or moderate eaters in the latter study, and this might have made it difficult to establish an evident effect of preweaning feed intake on intestinal morphology. Hence, more research is needed studying the effect of the creep feed quantity ingested by individual piglets and its effect on their intestinal development.

In accordance with the above mentioned studies using 3 or 4 week old suckling piglets (Hampson, 1986; Bruininx et al., 2004), villous height in the small intestine of piglets weaned around 32 days of age, was not affected by the supplementation of creep feed (Nabuurs et al., 1993a). Moreover, subjecting piglets to IS from 2 weeks before weaning, to stimulate food intake during the suckling period, did not affect villous height at weaning (Nabuurs et al., 1996). These findings seem to corroborate the absence of a significant difference in villous heights of piglets subjected to IS before weaning (day 26) compared to continuously suckled piglets observed in Chapter 7. It must be noted, however, that villous height in the small intestine of these IS piglets was reduced (though not significantly) at weaning compared to continuously suckled piglets. It was hypothesized that this might have been caused by the withdrawal of milk (and its trophic factors) during the periods of sow's absence and by the fact that the applied IS regimen in our study (Chapter 7) did not result in a greater feed intake during the suckling period compared to continuously suckled piglets. Similarly, subjecting piglets to a 2 week period of IS

without supplementary feed intake also resulted in a non-significant reduction of villous height at weaning (Nabuurs et al., 1996).

Providing supplementary creep feed to continuously suckled piglets did not affect crypt depth of the small intestine at 3 to 4 weeks of age (Hampson, 1986; Bruininx et al., 2004). However, Nabuurs et al. (1993) did find deeper crypts in supplemented continuously suckled piglets around 32 days of age. Moreover, subjecting piglets to IS with supplementary creep feed during the suckling period resulted in deeper crypts at the end of the suckling period compared to continuously suckled 32-day old piglets (Nabuurs et al., 1996). Similarly, in the study of chapter 7, a non-significant increase in crypt depth was observed in intermittently suckled piglets at the end of the suckling period.

So, based on the studies above, one might conclude that supplementation of creep feed itself already affected intestinal morphology (crypt depth) during a 5-week lactation period (but not during a 3-week lactation period). But, subjecting piglets to 2 weeks of IS before weaning at 5 weeks seemed to reinforce this effect, resulting in a greater crypt depth compared to similar-aged continuously suckled piglets.

### **Postweaning period**

One or two weeks of IS combined with an extended lactation (weaning at day 33) prevented the villous atrophy observed in piglets on the second day after conventional weaning and resulted in deeper crypts at day 2 and 8 postweaning as compared to piglets weaned at day 26 (Chapter 7). One might question whether this effect is solely due to the increased piglet age at weaning or whether the IS regimen also contributed to this effect. Conventional weaning in the study of chapter 7 was imposed only at day 26 of age, since an extended lactation length (without IS) would have compromised sow reproductive performance. As a result, the beneficial above described effects on postweaning intestinal morphology observed in the IS litters of this study could not be specifically ascribed to either the older age at weaning (33 days) or to the subjection to IS. Other investigators (Cera et al., 1988) reported that postponing weaning from 3 to 5 weeks of age attenuated, but did not prevent a weaning-associated villous atrophy (65% and 27% reduction in villous height at day 3 postweaning, respectively). Likewise, Nabuurs et al. (1996) reported a 10% reduction in villous height at day 4 after weaning of piglets at 32 days of age and this reduction in villous height was associated with lower net absorption values during a small intestinal segment perfusion (SISP) test.

A two week period of IS with supplementary feeding before weaning at day 32 prevented both the postweaning villus atrophy, with longer villi at day 4 postweaning compared to preweaning values, and the reduction in net absorption observed in continuously suckled piglets (Nabuurs et al., 1996). Furthermore, postweaning feed intake was positively correlated to villous height (Pluske et al., 1996; van Beers-Schreurs et al., 1998; Chapter 7) and net absorption (Kuller et al., 2007b), indicating the importance of feed intake for a proper intestinal function. Interestingly, subjecting piglets to two weeks of IS without supplementary feeding did not induce the above described beneficial effects on postweaning intestinal morphology and absorption (Nabuurs et al., 1996). Together, these results seem to indicate that the absence of postweaning villous atrophy observed in piglets weaned after an extended lactation combined with 1 or 2 weeks of IS (Chapter 7) was predominantly caused by an improved feed intake, stimulated by the limited nursing time before weaning.

Like with 1 or 2 weeks of IS before weaning at day 33, one week of IS before weaning at day 26 of age was found to prevent the weaning-associated villous atrophy observed in conventionally weaned piglets at 2 days after weaning (Chapter 7). This was quite a remarkable and unexpected result, given the fact that the preweaning feed intake and feed intake in the first 2 days postweaning were not increased, and that the growth check in the first two days after weaning was not reduced in these IS piglets (Chapter 7). At first sight, these findings do not seem to be compatible with the above mentioned observations of Nabuurs et al. (1996), which suggested that the beneficial effects of IS on postweaning intestinal structure were mediated by a higher preweaning (and consequently postweaning) feed intake. However, similarly to our findings, continuously suckled piglets in the study by Nabuurs et al. (1996) showed a significantly decreased villous height at day 4 postweaning compared to preweaning values, whereas the IS piglets without supplementary feed did not. This suggests that beneficial effects of IS might not solely be mediated by the increased feed intake. A possible explanation could be that IS piglets are habituated to separation from their mother at the time they are weaned definitively. An attempt to eliminate nutritional stress at weaning by feeding piglets a high level of sow's milk attenuated but did not completely prevent the weaning-associated villous atrophy (van Beers-Schreurs et al., 1998). Moreover, overnight maternal separation was found to be associated with elevated basal cortisol levels in piglets (Klemcke and Pond, 1991). In addition, weaning of 19-d old piglets appeared to activate stress signalling pathways, which mediate and

contribute to the intestinal dysfunction, i.e. increased small intestinal permeability, associated with weaning (Moeser et al., 2007a). Therefore, piglets habituated to repeated maternal separation in the IS regimen may have benefited by experiencing less of a stress response after weaning, preventing or attenuating intestinal damage.

## INTERMITTENT SUCKLING IN PERSPECTIVE

### **Getting closer to natural weaning**

As described before, nursing frequency of litters under conventional housing conditions remains high up to weaning. This is most probably due to the confinement of sows in farrowing crates, preventing them to get away from the demanding litter. The high nursing frequency during the lactation period coincides with a low voluntary creep feed intake at the time piglets are weaned (3 to 4 weeks of age). The abrupt dietary change at weaning, together with altered housing, mixing and sudden separation from the dam, often result in a reduced nutrient intake, reduced growth, impaired intestinal function and altered piglet behavior. Hence, it is desirable to alleviate the constraints of piglets in their adaptation to weaning.

In the past decade, organic pig farming has gained more interest. One of the regulations of organic pig farming according to the EU legislation is an extended lactation period of at least 40 days. Despite the extended lactation, weaning of piglets kept under organic farming conditions coincided with a stasis in growth and the occurrence of postweaning diarrhoea (van Krimpen et al., 2005; Binnendijk and van der Peet-Schwering, 2006). So, there is still plenty of improvement to be made. Results of the study described in chapter 2 of this thesis indicated that an extended 6-week lactation period combined with IS markedly reduced the postweaning growth check observed in conventionally weaned piglets. Hence, the application of IS on organic farms might be an effective way to improve postweaning piglet performance, ultimately leading to a gradual transition to weaning. Additionally, it might improve the reproductive performance of sows at organic farms, given the occurrence of lactational oestrus after commencement of IS (Gerritsen, 2007).

In current organic farming and in the IS studies described in this thesis, sow and piglets are confined in their farrowing pen during the entire lactation period. Alternative housing systems, however, providing piglets the opportunity to mingle with other piglets before weaning in a communal piglet area, reduced aggressive behavior after mixing (Weary et al., 1999). Moreover, it reduced the number of

nursings and increased postweaning feed intake (Weary et al., 1999). Combining a communal piglet area with sow controlled housing was found to increase preweaning creep feed intake and improved postweaning feed intake and growth of piglets (Weary et al., 2002). Piglets kept under the latter housing conditions during lactation, fought less when mixed at weaning (Weary et al., 2002). In addition to the beneficial effects on postweaning piglet performance, sows also benefited from the system. The nursing frequency decreased considerably during the 4-week lactation and sows consumed less food during lactation, but had similar bodyweight losses and weaning-to-oestrus intervals compared to conventionally housed sows (Weary et al., 2002). Moreover, it was stated that the welfare benefits to the sow were even more substantial (Pitts et al., 2002). Sows can leave their litter at will and reduce the udder stimulation, e.g. when their litter is large or when their condition is poor, and participate in the more complex social environment of the get away area (Pitts et al., 2002).

The disadvantage of a sow controlled housing or loose housing system, however, is the large variation by which the contact with the litter is reduced by the sow during the lactation period (Pajor et al., 2000; Pitts et al., 2002). Hence, a housing system that could regulate the time sows spent away from the piglets, would most probably reduce the variation between litters in terms of nursing frequency, preweaning creep feed intake and growth of piglets. A possible way to regulate this might be to impose the period of time spent away from the litter on the sows by subjecting them to IS. Results of our research group (Gerritsen, 2007; Kuller, 2008; present thesis) demonstrated that applying IS during the (extended) lactation period was associated with above mentioned benefits for piglets (increased preweaning feed intake and improved postweaning performance) and their dam (reduced number of nursings). Hence, the application of IS in “sow controlled” housing (i.e. group housing of sows during the separation interval, combined with a communal piglet area) could possibly improve the benefits of this lactational management even more. It would most probably also reduce the variation observed in sow controlled housing. In the present thesis, IS was applied using a standard separation interval (10 or 12 h) during the suckling period. Given the increasing time spent away from the litter during the lactation period when contact is controlled by the sow, one might ask to what extent a more gradual increase of the separation interval (h/day) might further improve the above proposed application of IS.

### **Intermittent suckling and gut health**

Piglets of herds with a history of postweaning diarrhoea generally have shorter villi and deeper crypts compared to piglets of a SPF herd, and these effects are even more marked in herds where diarrhoea is associated with mortality (Nabuurs et al., 1993a). Both rotaviruses and *Escherichia coli* were demonstrated to be important in the aetiology of diarrhoea in these piglets at weaning (Nabuurs et al., 1993b). Hence, in a subsequent study Nabuurs et al. (1996) studied the effect of the exposure of the small intestine to ETEC on small intestinal net absorption during a SISF test. The latter study demonstrated that exposure to ETEC reduces the net absorption of the small intestine at weaning and thereafter compared to the unexposed small intestine. Moreover, subjecting piglets to IS with supplementary feeding during the suckling period attenuated the reduction in small intestinal absorption after ETEC exposure. These results might indicate that IS could also have a beneficial effect on postweaning intestinal function of piglets in herds with a history of postweaning diarrhoea. The studies of the present thesis were conducted on experimental and commercial farms without a history of postweaning diarrhoea, and could therefore not be used and designed to substantiate this hypothesis.

In the last decades, sub-therapeutic levels of antibiotics have been included in the feed to improve piglet performance, especially in the postweaning period. However, it was demonstrated that the use of antimicrobial growth promoters could result in bacterial resistance. The ban on in feed antibiotics by the European Union (January 2006) asks for alternatives to these antibiotics. Otherwise, the disappearance of in feed antibiotics will probably have to be substituted with an increase in the use of therapeutic antibiotics. Possible alternatives are under investigation and include the improvement of the hygiene status of farms, or the addition of alternative substances to the piglet diet, such as prebiotics (Awati, 2005). Given the beneficial effects of IS on postweaning piglet performance observed in the current thesis, including the diminished effect of ETEC exposure after IS with supplementary feeding (Nabuurs et al., 1996), IS might also be considered as a strategy to reduce the use of antibiotics in pig husbandry.

### **Intermittent suckling and sow performance**

Results of the present thesis solely focussed on the consequences of intermittent suckling regimens during an extended lactation on piglet (postweaning) performance. As mentioned previously, improving piglet postweaning performance

by extending lactation length can only be profitable if reproductive performance of sows is not compromised. Hence, in addition and partly parallel to our studies, a considerable amount of research was performed investigating the effects of intermittent suckling regimens during an extended lactation on sow reproductive performance (Gerritsen, 2007). Subjecting sows to an IS regimen was found to induce lactational oestrus and ovulation in more than 80% of the sows, ultimately resulting in the establishment of the next pregnancy during the ongoing lactation period. In consecutive studies, the timing and duration of the applied IS regimens was varied. It was found that both an early onset of IS (at day 14) after parturition and a prolonged period of IS (up to 20 days after ovulation) tended to negatively affect embryo survival of the newly established pregnancy. Otherwise, the quality of lactational oestrus and of the subsequent pregnancy in sows submitted to an IS regimen was found to be rather comparable to that observed in conventionally weaned sows. Gerritsen (2007) described, besides timing and duration of IS, some other factors, such as breed, parity, method of separation and boar contact, that might affect the number of sows responding to IS with follicle growth and ovulation. In addition, since the effect of IS on embryo survival of the next pregnancy was only studied during early pregnancy, it was stated that more insight is needed in the effects of IS regimens on the performance of the litter conceived during the extended lactation period. Finally, it was concluded that further studies taken these factors into account are necessary before implementation of IS in pig husbandry.

#### FUTURE RESEARCH

In this paragraph we presented several recommendations for future studies. First, suggestions are made for further studies to unravel the mechanisms behind postweaning intestinal dysfunction in pigs. Thereafter, some suggestions are made for applied research questions are formulated, investigating the benefits of IS under various farm conditions, quantification of individual preweaning feed intake, and the development of a 'more natural' housing system for lactating sows and their litters.

#### **Intestinal hypoperfusion and weaning-associated villous atrophy**

As mentioned in the general introduction, the postweaning villous atrophy might (partly) be caused by intestinal hypoperfusion. Severe, experimentally reduced

SMA flow for 1 h was found to result in histologic lesions (Pargger et al., 1997). Moreover, TPN feeding of piglets resulted in a 30% reduction of the portal and SMA blood flow after 8 h (Niinikoski et al., 2004). This rapid decrease in intestinal blood flow preceded the villous atrophy, suppression of cell proliferation and survival in the small intestine observed after 48 h of TPN. Since weaning is associated with a period of low feed intake or fasting, we hypothesized that splanchnic hypoperfusion might play a part in the occurrence of intestinal dysfunction after weaning.

In chapter 5 of the present thesis we investigated whether the Physiogear™ I wireless flowmeter can be used to measure flow in group-housed animals without any human contact. Results of this chapter indicated that this tool enables one to measure the SMA blood flow in weaned piglets, providing the opportunity to relate flow measurements to undisturbed animal behavior and performance. Unfortunately, the experimental design and the low number of animals used in this pilot study are not appropriate to substantiate the above described hypothesis (which was evidently beyond the scope of this pilot study). Hence, more research is needed investigating whether weaning is associated with a reduced intestinal blood flow possibly (in part) responsible for the postweaning intestinal dysfunction.

A recent study of Van Dijk et al. (2008) investigated the effect of the administration of 2-iminobiotin (2-IB; a compound with neuroprotective properties in perinatal, experimentally-induced hypoxia-ischemia conditions) at birth on piglet growth rates, morbidity and mortality under farm conditions. Piglets treated with 2-IB after birth had a higher growth rate in the first 10 days after birth and tended to at weaning compared to untreated control piglets. The authors of this study postulated that the administration of 2-IB might have ameliorated the adverse effects of hypoxia and ischemia experienced during birth on the gastrointestinal tract, ultimately resulting in the improved neonatal growth rates (Van Dijk et al., 2008).

In future studies the Physiogear™ I wireless blood flow meter and the administration of a compound with neuroprotective properties, such as 2-IB, or a combination of both can be used to elucidate whether the postweaning small intestinal villous atrophy and dysfunction in piglets is (partly) mediated by intestinal hypoperfusion.

**Plasma citrulline concentration and postweaning intestinal function**

One of the aims formulated in the general introduction of this thesis was to investigate possible (biological) markers, which enable longitudinal monitoring of intestinal function in weaned piglets. Results of the current thesis demonstrated that weaning is associated with a rapid reduction of the plasma citrulline concentration in piglets (Chapter 6). Moreover, we found a distinct correlation between plasma citrulline and mannitol concentrations on day 4 postweaning (Chapter 6). In an attempt to investigate the relation between plasma citrulline concentration and small intestinal morphology, blood samples were gathered in the study of Chapter 7. Results of this study corroborated the finding of the previous study described in chapter 6 that weaning of piglets reduced the plasma citrulline concentration. Yet, no (or only a weak) correlations between plasma citrulline concentrations and the intestinal morphology were found. Although there were some distinct differences in gut morphology between IS and conventional piglets after weaning, no differences in plasma citrulline concentration were found. On the one hand, as we discussed previously (Chapter 7), the sampling location (jugular vein) might not have been adequate to detect changes in citrulline production. Moreover, it has to be stated that this was a transverse study in which piglets were not fasted before the blood sample was obtained. This might explain the higher variation in plasma concentrations generally observed in the study described in chapter 7 compared to that of chapter 6. This increased variation might have complicated the detection of a possible relation between plasma citrulline concentrations and gut morphology. On the other hand, differences in plasma citrulline concentration might not have to be correlated to changes intestinal morphology per se, in order to consider it a marker for intestinal function. Previous studies of Nabuurs et al. (1994, 1996) did not find a relationship between net absorption values of the small intestine and villous height and crypt depth after weaning. These authors suggest that the lack of a correlation could be explained by the presence of young, immature enterocytes in the intestine. Hence, further research is needed to investigate whether plasma citrulline concentration is an adequate marker of intestinal function.

**Gut related hormones and weaning-associated anorexia and villous atrophy**

We speculate that the absence of villous atrophy at day 2 postweaning, as prevented by applying one week of IS before weaning at day 26 (Chapter 7), might have been involved in the higher feed intake levels observed between day 2 and 7 postweaning compared to conventional weaned piglets. The cells lining the small

intestinal lumen are responsible for the production of quite some intestinal hormones, such as gastric inhibitory polypeptide (GIP) and glucagon-like peptide-2 (GLP-2), involved in appetite regulation. Disrupting the small intestinal integrity and function by postweaning villous atrophy, as observed in conventionally weaned piglets (Chapter 7), might also have influenced the crosstalk between gut and brain via these mediators, resulting in the lower feed intake levels observed in the conventionally weaned piglets. It would be most interesting to study the effects of weaning on plasma concentrations of gut hormones and their possible involvement in postweaning anorexia.

### **Benefits of IS under various farm conditions**

Subjecting piglets to IS during the (extended) lactation period was found to prevent the weaning-associated small intestinal villous atrophy (Chapter 7). We suggest that the beneficial effects of IS on postweaning gut characteristics reduces the risk on the development of postweaning diarrhoea. Yet, the studies of the present thesis were conducted on experimental and commercial farms without a history of postweaning diarrhoea. In a study of Nabuurs et al. (1996) piglets subjected to IS with supplementary feeding during the suckling period were found to have an attenuated reduction in small intestinal absorption after ETEC exposure. Hence, we postulate that IS could improve the postweaning intestinal function in piglets of herd with a history of postweaning diarrhoea. Moreover, it might decrease the use of therapeutic antibiotics on (these) pig husbandry farms. Future studies either in herds with a history of postweaning diarrhoea or in herds experimentally infected with ETEC and/or rotaviruses might indicate whether an IS regimen can diminish the detrimental effects of weaning on piglet performance under these circumstances.

### **Individual preweaning feed intake and postweaning piglet performance**

Preweaning creep feed intake was found to be highly variable both between litters (Okai et al., 1976; Barnett et al., 1989) and between piglets of the same litter (Pajor et al., 1991; Bruininx et al., 2002). As described by Bruininx et al. (2004) two mechanisms have been proposed that might explain this high variation in individual creep feed intake. First, it could be explained by maturation; piglets with a more matured gastrointestinal tract might consume more creep feed, since they are better capable to digest and absorb the nutrients from the solid feed. In several studies, a positive correlation was observed between weight and preweaning feed

intake, indicating that large piglets, occupying the more productive (anterior) teats, consumed more food (Pajor et al., 1991; Bøe and Jensen, 1995). On the other hand, it could be explained by the need for energy intake, suggesting that piglets with an inadequate milk intake compensate for it with an increased creep feed consumption. In a study of Algers et al. (1990) piglets with the highest gain in the preweaning week, indicating a good teat quality, were found to consume less solid feed. Irrespective of motivation, the high variation in creep feed intake of individual piglets complicate the investigations of the effects of preweaning creep feed on postweaning piglet performance. This becomes evident from the equivocal effects of creep feed supplementation during the lactation period on postweaning intestinal function, even when eaters and non-eaters could be recognized (Bruininx et al., 2004; Kuller et al., 2007b). Hence, quantification of individual creep feed intake is necessary. Moreover, the development of a method to assess individual creep feed intake of piglets would provide the opportunity to further investigate the piglet's motivation for the consumption of solid feed during the suckling period.

### **Gradual adaptation to weaning**

Confining sows and their litters to conventional housing conditions denies them the opportunity to a gradual weaning process. This becomes evident from the high and hardly undiminished nursing frequency at weaning and the concomitant low preweaning solid feed intake of the piglets. As mentioned before, the abrupt weaning of piglets is associated with detrimental effects on postweaning piglet performance, and even piglet death. Hence, in our opinion it is highly desirable, if not a necessity, to improve the adaptation of piglets to weaning under farm conditions. Several alternative management strategies, such as sow controlled housing combined with a communal piglet area, were found to improve piglet postweaning performance, as judged by piglet growth and feed intake, as well as behavior. Moreover, these improvements on piglet's adaptation to weaning coincided with substantial benefits for the sow, such as leaving the litter at will and participate in a more complex social environment of the get away area. Yet, the high variation by which the contact with the litter is reduced by the sow is a disadvantage of this alternative housing. As suggested previously, combining such sow controlled housing systems with IS, would reduce the variation of litters in their response to this housing system. Moreover, given the increasing time spent away from the litter during the lactation period when contact is controlled by the sow, one might ask to what extent a more gradual increase of the separation

interval (h/day) might further improve this combination of alternative housing systems. To conclude, more research is needed to investigate how alternative management strategies can contribute to the realization of a (required) more gradual transition of piglets to weaning, preferably without compromising the high levels of production achieved in conventional systems.

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# SUMMARY

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**INTERMITTENT SUCKLING AND EXTENDED  
LACTATION: IMPROVING ADAPTATION OF PIGLETS  
TO POSTWEANING CHALLENGES**

Under (semi-)natural conditions, piglets are gradually weaned during the long lactation period with complete weaning occurring around 12 to 17 weeks of age. During this period, piglets become acquainted with nutrient sources other than sow milk and, with nursing frequency decreasing, they start consuming an increasing amount of solid feed. In European, conventional pig husbandry weaning of piglets is very distinct from that observed under (semi-)natural conditions and consists of an abrupt separation from the sow at 3 to 4 weeks of age. At this young age, piglets are (physiologically) not fully competent to deal with this situation. As a result, weaning is associated with reduced nutrient intake, reduced growth, altered behavioral patterns, and a greater susceptibility to diarrhoea (**Chapter 1**). It is known that an older age at weaning is associated with an increased postweaning feed intake, an attenuated postweaning growth reduction, a reduction of stereotypic behavior and improved social skills later in life, and an attenuated (weaning-associated) villous atrophy and a reduction of other detrimental effects on intestinal function (**Chapter 1**). Thus, by an improved adaptation of older piglets to weaning, extending lactation length can improve piglet postweaning performance. However, extending lactation length is economically undesirable, since the persisting suckling stimulus will prevent the onset of cyclicality and hence, postpone the establishment of a next pregnancy of the sow. Inducing oestrus and ovulation in sows by intermittent suckling (IS; a management strategy in which piglets are temporarily separated from their sow for several hours on each day from a certain age until weaning) might be a possible way to retain the reproductive activity of the sow during an extended lactation period. Moreover, IS was found to improve piglet preweaning creep feed intake and postweaning performance, although the success rate was variable due to differences in applied IS regimens. At the start of this PhD study, data on combining IS with an extended lactation period were scarce and mainly focused on reproductive performance of the sow and/or stimulation of preweaning feed intake by the piglets. When an IS regimen is applied during the lactation period, piglets are involuntarily separated from their sow for several hours per day. It is well known that enforced maternal separation can cause behavioural and physiological changes, but the effects of IS on piglet behavior and physiology were largely unknown at the time.

Based on the findings above, the general aim of the current thesis was to determine whether intermittent suckling during an extended lactation improves the adaptation of piglets to weaning. More specific (research questions 1-4), we investigated how IS regimens, differing in separation interval, timing and duration, affected 1) piglet

pre- and postweaning growth and feed intake, 2) piglet behavior during the suckling period, 3) postweaning gut characteristics. Parameters currently used to investigate postweaning gut characteristics in piglets are end-point measurements, but 4) we aimed to monitor postweaning intestinal function of piglets longitudinally. In the paragraphs below we will summarize the findings of this thesis with respect to the separate research questions. An overview of the IS regimens used in the separate studies of this thesis, is given in table 8.1 of the general discussion (**Chapter 8**).

Since the onset of IS in previous studies was found to be associated with a reduction in growth during the suckling period and, as a consequence, with lower weight at weaning, it was hypothesized that distributing the total separation time per day over two separation intervals might (partly) prevent this growth reduction, while still stimulating preweaning creep feed intake. To investigate the effect of separation interval during IS on piglet growth and feed intake (**Chapter 2**), three weaning regimens were compared: 1) conventional weaning at day 21 (CW), 2) IS with 6-h separation intervals (IS6) and 3) IS with 12-h separation intervals (IS12). In both IS treatments sow and litters were daily separated (12 h/day) from day 14 until weaning ( $43 \pm 1$  day). One week after the onset of IS (day 20), creep feed intake was increased in litters from both IS groups compared with CW litters. Both IS groups consumed considerable amounts of creep feed before weaning (day 41 to 45). Total feed intake before weaning was greater in IS12 ( $3,808 \pm 469$  g/piglet) than in IS6 ( $2,717 \pm 404$  g/piglet). In comparison, CW litters consumed  $18 \pm 9$  g/piglet before weaning (day 21). Onset of IS induced a growth check in both IS groups (34 % for IS12 and 22 % for IS6). Only a mild growth check was observed after weaning of IS litters (14 % for both IS groups). However, a serious growth check (98 %) was observed after weaning of CW litters. Body weights at the end of the experiment (day 55) were similar among weaning regimens. Results of this study indicate that IS stimulated feed intake during lactation, and combined with an extended lactation it provided a more gradual transition to weaning. Because the IS6 regimen did not prevent the growth check after the onset of IS and is rather laborious, we suggest that application of IS12 might be preferable (**Chapter 2**).

In the study described above, the effect of IS on piglet behavior was also investigated (**Chapter 3**). Since maternal separation can induce behavioral (and physiological) changes, it was questioned whether the separation intervals during an IS regimen are associated with altered piglet behavior, especially with behavior indicative for piglet distress. IS reduced the nursing frequency compared to the

continuously suckled litters of the conventional treatment, and litter activity largely coincided with the presence of the sow during an extended lactation period. Separation of the sow and piglets during 12 consecutive hours in the IS12 regimen reduced the time spent on nursing behavior. Like in weaned CT litters, it increased the time spent on exploratory and ingestive behavior during lactation, possibly improving adaptation to the postweaning state. Distribution of total separation time (12h/d) over 2 periods synchronized litter activity even more to the presence of the sow, because they spent more time on nursing and less on eating behavior. Finally, both IS regimens were not associated with any behavioral patterns indicative of piglet distress. As such, an intermittent suckling regimen may provide a gradual adaptation to the postweaning situation without causing obvious behavioral distress (**Chapter 3**).

The study described in Chapter 2 revealed that an IS regimen from 14 days of age in combination with an extended lactation (6 week; IS6 or IS12) markedly reduced the postweaning growth check. However, since feed intake is normally rather low in the first weeks of age, it was questioned whether it is sensible to subject piglets to intermittent suckling at a young age (day 14). It was postulated that piglets have a higher feed intake when first submitted to intermittent suckling at a later age (e.g. day 21). If so, the time needed to reach a level of preweaning feed intake that ensures a continued substantial nutrient intake after weaning, ultimately preventing a postweaning growth check might be shorter. Therefore, a second study was conducted to determine the effect of piglet age at onset of IS and the effect of duration of IS on pre- and postweaning piglet feed intake and growth (**Chapter 4**). Intermittent suckling started at day 14 (IS14) or day 21 (IS21) of lactation, and litters were either weaned after ~ 1 week of IS (IS14-S and IS21-S, respectively) or ~ 4 weeks of IS (IS14-L and IS21-L, respectively). One wk of IS (day 14 to 21) increased creep feed intake of IS14 litters compared to the IS21 litters that were still suckling continuously in that period. Postponing the onset of IS from 14 to 21 days of age increased feed intake during the first wk of IS considerably. Moreover, the reduction in growth when weaned after 1 week of IS was of shorter duration when IS started at day 21 (IS21-S) than at day 14 (IS14-S). In IS litters weaned after an extended lactation, postweaning growth was only slightly decreased (IS14-L) or even increased (IS21-L) compared to preweaning growth. At the end of the experiment (day 49 to 56), there were no differences in body weight, growth or feed intake between the four IS treatments. In conclusion, onset of IS at an older age (d 21) markedly improved feed intake stimulation, and shortened the period of

postweaning growth reduction when piglets were weaned after 1 wk of IS. Combining IS with an extended lactation period apparently resulted in a more gradual adaptation to weaning, irrespective of age at onset, as judged by piglet growth and feed intake (Chapter 4). One may question whether postponing the onset of IS to an older age, together with the associated higher feed intake, facilitates the adaptation to weaning. In the latter study (Chapter 4) initiation of IS at a later age coincided with a later weaning age as well, making it hard to evaluate the relative contribution of weaning age and age at the start of IS. Moreover, no comparison was made to a conventional weaning regimen. One of the aims of the study reported in **Chapter 7** was to determine how age at weaning of piglets that have been subjected to IS, and the duration of the preceding IS period, each contribute to postweaning adaptation through effects on feed intake and piglet growth. Litters were subjected to conventional weaning (CW; weaned at d 26) or to one of the following IS regimens. Litters in the IS treatments were subjected to IS 1) from day 19 onward and weaned at day 26 (IS19-7D), or 2) from day 19 onward and weaned at day 33 (IS19-14D), or 3) from day 26 onward and weaned at day 33 (IS26-7D). One wk of IS before weaning on day 26 (IS19-7D) resulted in a similar weaning-associated relative growth check within the first 2 days after weaning compared to CW litters ( $72 \pm 13\%$  and  $90 \pm 7\%$ , respectively), but in a greater piglet growth and feed intake between d 2 and 7 postweaning. In IS piglets weaned after an extended lactation (day 33), a markedly smaller weaning-associated relative growth check was observed shortly postweaning ( $11 \pm 18\%$  and  $32 \pm 19\%$  for IS19-14D and IS26-7D litters, respectively). In these litters, feed intake and growth within the first 2 d after weaning were slightly greater when piglets experienced 2 wk of IS (IS19-14D) rather than 1 wk (IS26-7D). This study indicates that 1 wk of IS before weaning at d 26 of lactation improves feed intake and growth between d 2 and d 7 postweaning compared to conventionally weaned piglets, although it did not prevent a profound growth check shortly after weaning. However, combining 1 wk of IS with an extended lactation (weaning at d 33) improved postweaning adaptation markedly in terms of growth and feed intake. Increasing the duration of IS from 1 to 2 wk slightly improved growth and feed intake shortly after weaning, but the contribution to postweaning adaptation seemed to be relatively small compared to extending lactation. In conclusion, the results of the present thesis suggest that piglets submitted to IS and weaned at a conventional age (3 to 4 weeks) are not yet fully capable to cope with weaning, as judged by piglet growth and feed intake. However, when weaned at an older age

(extended lactation) even a short (1-week) period of IS was found to result in a more gradual adaptation to weaning.

Parameters currently used to investigate characteristics of the gut during the postweaning period in piglets are end-point measurements. In the present thesis we aimed to monitor postweaning intestinal function of piglets longitudinally. One might hypothesize that splanchnic hypoperfusion plays a part in the occurrence of intestinal dysfunction after weaning (**Chapter 1**). Therefore, we investigated whether the Physiogear<sup>TM</sup> I wireless flowmeter can be used to measure flow in group-housed animals without any human contact (**Chapter 5**). Four piglets of about 7 kg were instrumented with a 3-mm flowprobe around the superior mesenteric artery (SMA) and SMA flow was measured pre- and post-weaning. During measurements, behaviour was recorded. The piglets did not show any abnormal behaviour and were not restrained by the flowmeter. Severe reductions (>75%) in SMA flow coincided with nursing (pre-weaning) and aggressive behaviour (post-weaning) and were only short-lived. It was demonstrated that this tool enables one to measure the SMA blood flow in weaned piglets, providing the opportunity to relate flow measurements to undisturbed animal behavior and performance. Unfortunately, the experimental design and the low number of animals used in this pilot study were not appropriate to substantiate the above described hypothesis. Hence, more research is needed to investigate whether weaning is associated with a reduced intestinal blood flow, which might be (partly) responsible for the postweaning intestinal dysfunction. In the study of **Chapter 6** we investigated whether plasma citrulline and intestinal fatty acid binding protein (I-FABP) concentrations might be used as longitudinal markers for small intestinal function in piglets after weaning. Since elevated I-FABP levels are associated with intestinal diseases in man and with experimentally induced intestinal ischemia in pigs, it was suggested that plasma I-FABP concentration might be used as a sensitive marker of damage to the intestinal mucosa in pigs. Plasma citrulline concentration was previously reported to be a simple and reliable marker of absorptive bowel length and absorptive function. Therefore we hypothesized that plasma citrulline might be a promising marker for monitoring postweaning intestinal function in piglets. Plasma citrulline and I-FABP levels were measured longitudinally in weaned and unweaned piglets, and related to intestinal absorption values, i.e. plasma mannitol and D-xylose concentrations in a sugar absorption test. Piglets were either weaned conventionally (CW) or remained with the sow (UNW). Plasma I-FABP concentration showed a great variation within treatments, and no

difference in plasma I-FABP levels between the CW and UNW treatment was observed. D-xylose absorption was not different between treatments. Mannitol absorption, however, was lower in the weaned CW piglets compared to the UNW piglets, with the lowest value on day 4 postweaning. Weaning also reduced plasma citrulline concentrations in the piglets of the CW treatment compared to those of the UNW treatment. On day 4 and 7 postweaning plasma citrulline levels of CW piglets were lower compared to preweaning values. Furthermore, in the CW treatment plasma citrulline concentrations correlated to plasma mannitol levels at day 4 postweaning ( $r = 0.89$ ,  $P = 0.008$ ) and overall ( $r = 0.76$   $P = 0.001$ ). In conclusion, based on the results of the study of Chapter 6, plasma citrulline concentration seems to be a possible marker for monitoring intestinal function in pigs after weaning.

In the study described in Chapter 7, results of the preceding studies were integrated. The effect of IS regimens on postweaning gut characteristics (besides their effects on piglet growth and feed intake, as already described above) were investigated. Conventional weaning of piglets was found to result in reduced plasma citrulline concentrations in piglets (**Chapter 6**). We anticipated that the villous atrophy often associated with weaning might play a role in these reduced plasma citrulline values. Therefore, another aim of the study described in **Chapter 7** was to investigate the possible relation between postweaning small intestinal morphology and plasma citrulline concentrations. Although one week of IS before weaning on day 26 resulted in a similar weaning-associated relative growth check within the first 2 days after weaning compared to conventionally weaned litters, it prevented the weaning-associated villous atrophy observed at day 2 postweaning in conventionally weaned litters. Similarly, in IS piglets weaned after an extended lactation (weaning on day 33), no villous atrophy was observed after weaning, irrespective of duration of IS (1 or 2 week). Irrespective of treatment, plasma citrulline concentrations (cardiac blood samples) were reduced at day 2 and day 8 postweaning compared to the values at weaning. No correlations were observed between postweaning plasma citrulline concentrations and postweaning small intestinal villous height. This study indicates that 1 week of IS before weaning prevents the weaning-associated villous atrophy observed in conventionally weaned piglets. Moreover, to gain more insight in the relation between small intestinal citrulline production and morphological characteristics, a sampling location closer to the site of citrulline production, would be desirable.

In conclusion, the results of the studies of this thesis suggest that IS is a promising management strategy to improve the adaptation of piglets to weaning, by preventing postweaning detrimental effects on piglet nutrient intake, growth, and small intestinal morphology. These effects are most profound when IS is combined with an extended lactation. In addition, the periods of maternal separation during an IS regimen were not associated with the development of behavioral patterns indicative for piglet distress. Based on the findings of this thesis, we postulate that plasma citrulline might be a possible marker for postweaning small intestinal function in pigs. However, more research is needed to elucidate the effects of weaning on citrulline metabolism in pigs.

The studies of the current thesis demonstrated that IS during an extended lactation improved postweaning piglet performance. Yet, as stated above, implementation of IS as an alternative management system can only be profitable if reproductive performance of sows is not compromised. Although promising results of IS on sow reproductive performance have been reported in a concurrent PhD-study, more insight is still needed in the factors affecting the proportion of sows responsive to IS (**Chapter 8**).

Finally, it is highly desirable and necessary to improve the adaptation of piglets to weaning under farm conditions. More research is needed to investigate how alternative management strategies can contribute to the realization of a (required) more gradual transition of piglets to weaning, preferably without compromising the high levels of (re)production achieved in conventional systems.



# SAMENVATTING

**TIJDELIJK SPENEN IN COMBINATIE MET EEN  
VERLENGDE LACTATIE: EEN GRADUALE OVERGANG  
NAAR HET SPENEN VAN BIGGEN**

Biggen in de vrije natuur worden naarmate ze ouder worden steeds onafhankelijker van hun moeder en worden definitief gespeend – dat wil zeggen: niet langer gezoogd - op een leeftijd variërend van 12 tot 17 weken. In de periode voorafgaand aan het spenen raken de biggen geleidelijk gewend aan andere voedingsbronnen dan zeugenmelk en beginnen zij, terwijl de zoogfrequentie afneemt, steeds meer vast voer te consumeren. Het spenen van biggen, zoals dat in de conventionele varkenshouderij in Europa geschiedt, is niet goed vergelijkbaar met dit langdurige speenproces onder natuurlijke omstandigheden. De biggen worden namelijk op een leeftijd van 3 tot 4 weken gescheiden van de moeder en moeten voor wat betreft hun voedingsbron abrupt overschakelen van zeugenmelk naar vast voer. Op deze jonge leeftijd zijn de biggen (fysiologisch gezien) nog niet goed in staat om zich aan te passen aan de vele veranderingen die gepaard gaan met het spenen, zoals de scheiding van de zeug, het mengen met biggen van andere tomen, de veranderde huisvestingscondities en de genoemde omschakeling naar vast voer. Het spenen van biggen resulteert dan ook in een tijdelijk verminderde inname van nutriënten, een daling van de groei, veranderingen in het gedrag en een hoger risico op het ontstaan van diarree (**Hoofdstuk 1**). Wanneer biggen op een latere leeftijd worden gespeend, resulteert dat vaak in een verbetering van de voeropname en de groei na spenen, minder stereotiep gedrag en een verbetering van de darmfunctie van de gespeende biggen (Hoofdstuk 1). Het leek dan ook aannemelijk dat het verlengen van de zoogperiode een beter functioneren van de biggen na het spenen tot gevolg zou kunnen hebben. Vanuit economisch perspectief is het verlengen van de zoogperiode echter niet wenselijk. De aanhoudende zoogstimulus resulteert in het uitblijven van follikelontwikkeling en ovulaties bij de zeug, waardoor uiteindelijk het tot stand komen van een volgende dracht vertraagd wordt. De worpindex wordt dus verlaagd.

Een manier om bronst en ovulaties tijdens de lactatie te induceren, is het verlagen van de frequentie van de zoogstimulus door het toepassen van tijdelijk spenen: Intermittent Suckling (IS). Dit is een management systeem waarbij zeug en biggen gedurende de zoogperiode dagelijks voor een aantal uren van elkaar gescheiden worden. Naast het induceren van een bronst en ovulaties tijdens de zoogperiode, kan IS, afhankelijk van het toegepaste protocol, de voeropname van biggen tijdens de lactatie stimuleren en (daarmee) de prestaties van de biggen na het spenen verbeteren. Bij de aanvang van het promotieonderzoek was echter nauwelijks literatuurinformatie beschikbaar over de effecten van IS, in combinatie met een verlengde zoogperiode, op de prestaties van biggen na het spenen. Bovendien was

niet goed gedocumenteerd of en, zo ja, op welke wijze IS het gedrag van biggen beïnvloedt. De periodes tijdens een IS regime waarin biggen gescheiden zijn van de zeug, zouden fysiologische en ethologische veranderingen in de jonge big kunnen induceren, zoals eerder beschreven in studies naar de effecten van (kortstondige) maternale isolatie.

De algemene doelstelling van dit proefschrift was dan ook om te onderzoeken of IS, in combinatie met een verlengde lactatie, de adaptatie van biggen aan de situatie na het spenen kan verbeteren. Hiertoe is een viertal specifieke vraagstellingen geformuleerd. In dit proefschrift wordt beschreven welke effecten verschillende IS regimes, die variëren wat betreft separatie-interval, startmoment en duur, hebben op 1) de groei en voeropname van biggen vóór en na het spenen, 2) het gedrag van biggen tijdens de zoogperiode, 3) een aantal kenmerken van de darmfunctie na het spenen. Omdat de gangbare parameters om veranderingen in darmfunctie te kunnen meten euthanasie van de biggen vereisen, hebben we ons in dit proefschrift ten doel gesteld om 4) de kwaliteit van de darmfunctie van biggen na het spenen *in vivo* longitudinaal te kunnen vervolgen. In de onderstaande paragrafen zal per onderzoeksdoel een samenvatting gegeven worden van de resultaten. Een overzicht van de IS regimes, zoals die in de afzonderlijke studies zijn toegepast, wordt weergegeven in tabel 8.1. van de algemene discussie (Hoofdstuk 8).

Uit eerdere studies was gebleken dat kort na de aanvang van een IS regime een verminderde groei van de biggen optrad, wat uiteindelijk leidde tot een lager lichaamsgewicht bij het spenen. Er werd verondersteld dat een IS regime, waarbij de totale duur van de dagelijkse scheiding van zeug en biggen gelijk zou blijven maar verdeeld zou worden over meerdere periodes gedurende het etmaal, deze groeidaling zou kunnen voorkomen, met behoud van de stimulatie van de voeropname. Deze hypothese is door ons onderzocht in een studie (**Hoofdstuk 2**) waarin drie management systemen werden vergeleken: 1) conventioneel spenen op dag 21 (CS), 2) IS met een interval van 6 uur (IS6), en 3) IS met een interval van 12 uur (IS12). In beide IS behandelingen werden de biggen in totaal gedurende 12 uren per dag van de zeug gescheiden, vanaf dag 14 tot aan spenen (dag  $43 \pm 1$  d). De voeropname van IS biggen was tijdens de 1<sup>e</sup> week van IS groter dan die van CS biggen waarbij de zeug continue aanwezig was. De totale voeropname tijdens de zoogperiode was groter bij IS12 biggen ( $3,808 \pm 469$  g/big) dan bij IS6 biggen ( $2,717 \pm 404$  g/big). De CS biggen daarentegen hadden tijdens de zoogperiode (met spenen op dag 21) een voeropname van slechts  $18 \pm 9$  g/big. De eerste 2 dagen IS

regimes was geassocieerd met een verminderde groei van de biggen in beide IS groepen (34 % bij IS12 and 22 % bij IS6). Na het spenen van IS biggen werd slechts een geringe afname van de groei in beide IS groepen (beide 14 %) waargenomen. Dit in tegenstelling tot de CS biggen, waarbij een aanzienlijke afname van de groei werd vastgesteld (98%). Aan het einde van het experiment (dag 55) was er geen verschil in lichaamsgewicht van de biggen uit verschillende behandelingsgroepen. Deze studie toont aan dat IS de voeropname van biggen stimuleert en dat IS, gecombineerd met een verlengde lactatie, resulteert in een geleidelijke overgang naar het spenen. Ook in de IS6 behandelingsgroep werd in de aanvangsfase een vermindering van de groei waargenomen. Omdat een dergelijk regime niet veel winst oplevert maar wel veel arbeidsintensiever is, werd geconcludeerd dat het IS12 regime te verkiezen is boven het IS6 regime (**Hoofdstuk 2**).

In de hierboven genoemde studie is ook het effect van de IS regimes op het gedrag van de biggen onderzocht (**Hoofdstuk 3**). Daarbij ging het met name om de vraag of de herhaalde periodes van scheiding tijdens IS regimes geassocieerd zijn met veranderingen in het gedrag van de biggen, in het bijzonder stressgerelateerd gedrag. Het toepassen van IS resulteerde in een vermindering van de zoogfrequentie en in een synchronisatie tussen de activiteit van biggen en de aanwezigheid van de zeug. Bij de IS12 tomen werd, naast een verminderd zooggedrag ook een stijging waargenomen in de tijd besteed aan exploratie en voeropname, vergelijkbaar met dat van CS biggen na het spenen. In de IS6 tomen was de activiteit nog nauwer verbonden aan de aanwezigheid van de zeug, waarbij zij meer tijd besteedden aan zogen en minder aan voeropname. Beide IS regimes waren niet geassocieerd met gedragspatronen die een indicatie vormen voor stress. Samenvattend kan geconcludeerd worden dat het toepassen van een IS regime resulteert in een geleidelijke overgang naar het spenen van biggen, zonder dat er stressgerelateerd gedrag wordt geïnduceerd.

De studie beschreven in hoofdstuk 2 laat zien dat een IS regime vanaf dag 14, in combinatie met een verlengde lactatie, een drastische vermindering in groei na het spenen, zoals die optreedt bij het conventioneel spenen van biggen, grotendeels kan voorkomen. Omdat er tijdens de eerste week van IS (nog) geen hoge voeropname maar wel een vermindering van de groei van de biggen wordt waargenomen, kan worden betwijfeld of het starten met een IS regime vanaf een dergelijk jonge leeftijd opgelegd verstandig is. Het lijkt aannemelijk dat op latere leeftijd aanvangen met een IS regime resulteert in een hogere voeropname per dag,

waardoor de tijdsduur die nodig is om een substantiële opname van vast voer te bereiken mogelijk ook korter zou kunnen zijn.

Om deze hypothese te toetsen is een tweede studie uitgevoerd waarin het effect van de dag van aanvang en de duur van IS op de voeropname en de groei van biggen zijn bestudeerd (**Hoofdstuk 4**). De aanvang van IS vond plaats op een leeftijd van 14 of 21 dagen (IS14 of IS21) en de IS tomen werden vervolgens gespeend na blootstelling aan een korte periode (1 week; IS14-K of IS21-K), dan wel een lange periode (4 weken; IS14-L of IS21-L) met IS. Eén week IS vanaf dag 14 resulteerde in een hogere voeropname in vergelijking met biggen die nog conventioneel gehouden werden. Wanneer één week later begonnen werd met het IS regime (op een leeftijd van 21 dagen) werd een hogere voeropname in de eerste week van IS waargenomen. Daarnaast was de afname in groei na het spenen bij biggen uit deze twee behandelingsgroepen van kortere duur wanneer met IS werd gestart op 21 dagen (IS21-K). Er werd geen (IS21-L) of slechts een geringe reductie (IS14-L) gevonden in groei na het spenen bij biggen die 4 weken blootgesteld waren aan het IS regime. Aan het einde van het experiment (dag 56) waren er geen verschillen in lichaamsgewicht, groei en voeropname tussen de vier IS behandelingen. Samenvattend: door het IS regime op latere leeftijd (dag 21) te starten wordt de voeropname meer gestimuleerd en de periode van groeireductie na het spenen verkort. IS gecombineerd met een verlengde lactatieduur lijkt, gezien de groei en voeropname van deze biggen, te resulteren in een graduele overgang naar het spenen van biggen.

Een volgende vraag was hoe de leeftijd bij aanvang van IS en de duur van het IS regime afzonderlijk bijdragen aan bovengenoemde adaptatie aan het spenen. Om deze vraag te kunnen beantwoorden is een experiment uitgevoerd waarbij drie IS regimes zijn vergeleken met een conventioneel management regime (CS; spenen op dag 26). De IS regimes werden toegepast 1) vanaf dag 19 tot het spenen op dag 26 (IS19-7D), 2) vanaf dag 19 tot het spenen op dag 33 (IS19-14D), of 3) vanaf dag 26 tot het spenen op dag 33 (IS26-7D). Het spenen van biggen op dag 26 resulteerde in een afname van de groei, die in de eerste 2 dagen vergelijkbaar was voor biggen van de conventioneel gespeende en de IS19-7D groep (respectievelijk  $90 \pm 7\%$  en  $72 \pm 13\%$ ). Echter, tussen dag 2 en 7 na het spenen vertoonden biggen in de IS groep een betere groei en voeropname dan de CS biggen. Het verlengen van de lactatie naar dag 33 resulteerde in een kleinere reductie van de groei na het spenen bij IS tomen (respectievelijk  $11 \pm 18\%$  en  $32 \pm 19\%$  in IS19-14D en IS26-7D tomen). De voeropname en groei waren iets hoger bij het spenen van biggen

(dag 33) wanneer daar 2 weken met IS, in plaats van 1 week, aan waren voorafgegaan. Uit deze studie kan geconcludeerd worden dat het gedurende één week toepassen van een IS regime voor het spenen op dag 26 de afname in groei direct na het spenen weliswaar niet kan voorkomen, maar dat het wel leidt tot een verbeterde prestatie van de biggen tussen dag 2 en 7 na het spenen. Het combineren van 1 week IS met een verlengde lactatie (tot dag 33) resulteert in een substantiële verbetering van de prestaties van de biggen (direct) na het spenen. Het verlengen van de IS periode vóór het spenen (op dag 33) had in dit opzicht slechts een klein additioneel effect. De resultaten van de studies, die in dit proefschrift beschreven worden, laten zien dat het toepassen van een IS regime in combinatie met spenen op een conventionele leeftijd, nog niet tot een voldoende verbetering leidt van de adaptatie aan het spenen. Wanneer echter het toepassen van een IS regime gedurende één week wordt gecombineerd met een verlenging van de zoogperiode, dan resulteert dat voor de biggen in een graduele overgang naar de situatie na het spenen.

Het bestuderen van het functioneren van de (dunne) darm bij biggen geschiedt meestal aan de hand van parameters, die verkregen worden na euthanasie van de dieren. Een van de onderzoeksdoelen van dit proefschrift was om te onderzoeken of er parameters zijn die het mogelijk maken om de werking van de darm van biggen *in vivo* te bestuderen. Dit biedt grote voordelen omdat biggen niet langer opgeofferd hoeven te worden. Bovendien kan het verloop in de tijd bestudeerd worden bij dezelfde biggen in een longitudinaal onderzoek. Op grond van informatie uit de literatuur leek het aannemelijk dat hypoperfusie van het splanchnisch gebied een rol speelt bij het ontstaan van de verminderde darmfunctie na het spenen van biggen (Hoofdstuk 1). In een van onze studies (**Hoofdstuk 5**) hebben we een daarom nieuwe apparatuur getest, de draadloze Physiogear™ I flowmeter, die gebruikt kan worden om de bloedstroom in de bloedvaten van dieren in een groepshuisvesting te meten, zonder dat daar humane interventie bij nodig is. Er zijn daartoe 4 biggen van 7 kg onder narcose geïnstrumenteerd met een 3-mm flowprobe rond de arterie mesenterica cranialis (AMC). Na de operatie werden de bloedstroom in de AMC en het gedrag van deze biggen zowel vóór als na het spenen vastgelegd. De biggen vertoonden geen afwijkend gedrag en leken niet gehinderd te worden door het dragen van de telemetrische meetapparatuur. Er werden sterke reducties (>75%) van de bloedstroom in de AMC waargenomen, zowel tijdens het zogen vóór als tijdens agressieve interacties na het spenen. De periode, gedurende welke een dergelijke reductie werd waargenomen, was echter

altijd van korte duur. Deze studie toont aan dat het mogelijk is om bij (on)gespeende biggen in een groepshuisvesting de bloedstroom in de AMC zonder ongewenste humane interventies longitudinaal te meten. Nader onderzoek zal moeten uitwijzen in welke mate spenen geassocieerd is met een verminderde bloedstroom in de AMC en welke rol dit proces eventueel speelt bij de verminderde darmfunctie van biggen na het spenen.

In de studie van Hoofdstuk 6 hebben we onderzocht of plasma concentraties van citrulline en intestinal fatty acid binding protein (I-FABP) gebruikt kunnen worden als indicatoren voor de mate van darmschade die geïnduceerd wordt bij het spenen van biggen. Zowel bij (humane) patiënten met darmaandoeningen als bij experimenteel geïnduceerde ischemie bij biggen zijn verhoogde niveaus van I-FABP waargenomen. Daarom werd verondersteld dat I-FABP ook een indicator zou kunnen zijn het meten van darmschade in biggen rond het spenen. Uit literatuurgegevens was bekend dat de plasma concentratie van citrulline een betrouwbare indicator is voor de mate van absorptie in de (dunne) darm. Daarom veronderstelden wij dat ook plasma citrulline een veelbelovende parameter zou kunnen zijn om de darmfunctie van biggen na het spenen longitudinaal te kunnen vervolgen.

In de studie die wordt beschreven in Hoofdstuk 6 zijn plasma citrulline en I-FABP concentraties longitudinaal gemeten in gespeende (G) en ongespeende (NG) biggen. Bovendien werden zij gerelateerd aan absorptie vermogen van de darm door middel van een suikerabsorptie test met D-xylose en mannitol. Bij beide behandelingsgroepen werd een hoge variatie in de plasma I-FABP concentraties gemeten, en de plasma I-FABP concentratie van G en NG biggen bleken niet significant van elkaar te verschillen. Ook de mate van absorptie van D-xylose was vergelijkbaar in beide behandelingsgroepen. De absorptie van mannitol was echter lager in de G biggen, in vergelijking met die in de NG biggen, en bereikte een minimum op dag 4 na het spenen. Het spenen van de G biggen resulteerde ook in een daling van de plasma citrulline concentraties ten opzichte van die in de NG biggen. In de gespeende biggen waren de plasma citrulline- en mannitol concentraties met elkaar gecorreleerd, zowel op dag 4 na het spenen ( $r = 0.89$ ,  $P = 0.008$ ) als over alle tijdstippen heen ( $r = 0.76$   $P = 0.001$ ). Samenvattend: de resultaten van deze studie laten zien dat de plasma citrulline concentratie een marker zou kunnen zijn voor het longitudinaal meten van de darmfunctie bij gespeende biggen.

In de hierboven genoemde studie van Hoofdstuk 7 zijn de vraagstellingen naar de effecten van IS regimes op de prestaties van biggen en de darmfunctie geïntegreerd. In deze studie werd daarom tevens het effect onderzocht van verschillende IS regimes op een aantal karakteristieken van de darm na het spenen. Uitgangspunt was de hypothese dat de atrofie van de darmvlokken, die dikwijls optreedt na het spenen van biggen, een rol zou kunnen spelen bij de door ons in hoofdstuk 6 waargenomen daling in de citrulline concentratie na het conventioneel spenen van biggen. Een van de doelstellingen van de studie was daarom na te gaan of er een relatie bestaat tussen de plasma citrulline concentratie en de morfologie van de dunne darm. Het toepassen van een IS regime gedurende één week vóór het spenen op dag 26 resulteerde niet in betere prestaties van de biggen direct na spenen, in vergelijking met de prestaties van conventioneel gespeende dieren. Het voorkwam echter wel een atrofie van de darmvlokken, zoals die bij conventioneel gespeende biggen werd waargenomen op dag 2 na het spenen. Bovendien werd bij biggen die op dag 33 werden gespeend (na het toepassen van een IS regime gedurende 1 of 2 weken) ook geen villus atrofie waargenomen. De plasma citrulline concentraties van bloedmonsters waren lager op dag 2 en 8 na het spenen, ongeacht de behandeling van de biggen. Er werden geen significante correlaties gevonden tussen de plasma citrulline concentraties na het spenen en de morfologie van de dunne darm na het spenen. Samenvattend: deze studie toont aan dat het toepassen van een IS regime gedurende 1 week vóór het spenen de atrofie van de darmvlokken, zoals die na conventioneel spenen werden gevonden, kan voorkomen. Meer onderzoek is nodig om de relaties tussen de citrulline productie en de morfologie van de dunne darm na het spenen te ontrafelen. Tot besluit: de resultaten van dit proefschrift suggereren dat IS een veelbelovende management strategie is om de adaptatie van biggen aan de situatie na het spenen te verbeteren. IS voorkomt de nadelige effecten van het spenen op de groei, de voeropname en de darmmorfologie van biggen. Deze effecten worden vooral manifest wanneer IS wordt gecombineerd met een verlenging van de zoogperiode. Daarnaast is uit onze gedragsobservaties gebleken dat het toepassen van een IS regime bij biggen niet leidt tot gedrag dat geassocieerd kan worden met stress. Op basis van de resultaten van dit proefschrift wordt verondersteld dat de plasma citrulline concentratie een marker kan zijn voor het meten van de darmfunctie na het spenen bij biggen. Er is echter meer onderzoek nodig om de effecten van het spenen op het citrulline metabolisme volledig in kaart te brengen en de relatie met de darmmorfologie te achterhalen. Uit dit proefschrift blijkt dat het toepassen van een IS regime bij

biggen in combinatie met een verlengde zoogperiode, de prestaties van biggen na het spenen positief beïnvloed. Zoals hierboven al werd opgemerkt, zal het toepassen van een dergelijk managementsysteem echter alleen in de praktijk worden toegepast wanneer de reproductieve prestaties van zeugen er niet onder leiden. Hoewel ook in dat opzicht de eerste resultaten hoopgevend zijn, dient meer inzicht verkregen te worden in de complexe interacties, die hierbij een rol spelen (Hoofdstuk 8).

Het is niet alleen wenselijk maar ook noodzakelijk om de adaptatie van biggen aan de situatie na het spenen te verbeteren. Het in dit proefschrift beschreven onderzoek beoogt hieraan een bijdrage te leveren. Toekomstig onderzoek zal onder andere gericht moeten zijn op het verkrijgen van meer inzicht in de mechanismen waarlangs alternatieve management strategieën, zoals IS, bijdragen aan het realiseren van een meer geleidelijke overgang naar het spenen van biggen.





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studies hebben er mede voor gezorgd dat we ook een kijkje konden nemen 'in' het varken. Het was een genoegen om met jou, een door-de-wol-geverfde onderzoeker met een gezonde dosis humor, de handen uit de mouwen te steken! Beste heren, bedankt voor de mooie tijd en de vele dingen die ik de afgelopen jaren van jullie heb mogen leren!

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# CURRICULUM VITAE

## ABOUT THE AUTHOR

Marieke Berkeveld was born on March 24<sup>th</sup> 1978 in Boskoop, and spent her childhood in the villages Heukelum, Kollum and Warnsveld. In 1996 she graduated from high school, the Isendoorn College, in Warnsveld. Subsequently, she started to study Biology at Wageningen University and Researchcentre (WUR). In March 2001, she graduated with a thesis on neurobiology (Human and Animal Physiology, WUR) and a traineeship in evolutionary genetics (Institute for molecular and cell biology, University of Porto, Portugal). Moreover, she worked as a student-assistant at the departments Human and Animal Physiology, and Experimental Zoology. Between January 2001 and Februari 2004, Marieke worked as junior scientist in celbiology at Numico Research BV, in a research project focussed on adipocyte metabolism. From February 2004 onward, she started her PhD at the department of Farm Animal Health at the Faculty of Veterinary Medicine of the University of Utrecht, resulting in the current dissertation. In June 2006 she married to Sander Abrahamse. From August 2008 onward Marieke Abrahamse-Berkeveld is scientist in baby nutrition research at Danone Research in Wageningen.

## OVER DE AUTEUR

Marieke Berkeveld werd geboren op 24 Maart 1978 te Boskoop en groeide op in de dorpen Heukelum, Kollum en Warnsveld. In 1996 behaalde zij het VWO diploma aan het Isendoorn College te Warnsveld. Zij startte in datzelfde jaar met de studie Biologie aan de toenmalige Landbouw Universiteit te Wageningen. Tijdens een afstudeervak bij de vakgroep Fysiologie van Mens en Dier heeft ze onderzoek gedaan naar het effect van omgevingsverrijking op de morfologie van de dentate gyrus in varkens. Daarnaast heeft ze tijdens een stage bij het Instituto de Biologia Molecular e Cellular (IBMC, Universiteit van Porto, Portugal) de genetische verwantschap van verscheidene diersoorten betreffende twee niet-klassieke MHC-I genen onderzocht. Daarnaast is ze student-assistent geweest bij de vakgroepen Experimentele Zoölogie en Fysiologie van Mens en Dier. Van januari 2001 tot februari 2004 was zij werkzaam als junior onderzoeker celbiologie bij Numico Research BV te Wageningen in een onderzoek gericht op vetcelmetabolisme. In februari 2004 begon zij als promovenda bij de afdeling Gezondheidszorg

Landbouwhuisdieren van de Faculteit Diergeneeskunde te Utrecht aan het onderzoek dat beschreven is in dit proefschrift.

In juni 2006 is zij met Sander Abrahamse getrouwd. Sinds augustus 2008 werkt Marieke Abrahamse-Berkeveld als onderzoeker bij Danone Research te Wageningen in een onderzoek gericht op babyvoeding.

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