

1B-106: A NOVEL SOW-PIGLET SEPARATION TECHNIQUE IN LACTATION TO ENHANCE PIGLET WELFARE AND PRODUCTION AFTER WEANING

Report prepared for the
Co-operative Research Centre for High Integrity Australian Pork

By

Professor John R. Pluske and Dr. Diana Turpin

School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA, 6150,
Australia.

January 2017



Australian Government
Department of Industry,
Innovation and Science

Business
Cooperative Research
Centres Programme

Executive Summary

The current research project aimed to mimic the natural weaning process as much as possible under commercial indoor conditions by introducing intermittent weaning (IS) and also allowing piglets to socialize with non-littermates (CoM) during lactation, to determine whether IS and/or CoM can promote positive behavior and positively affect post-weaning performance.

In Experiment 1, IS with or without the CoM of piglets was studied. Three weaning regimes using 30 multiparous sows were compared: (1) conventional weaning (CW) ($n = 10$ litters), where piglets had continuous access to the sow until weaning (d 0, farrowing = d -25 relative to weaning); (2) intermittent suckling (IS) ($n = 10$ litters), where piglets were separated from the sow for 8 h/d starting at d -7 (relative to weaning); and (3) intermittent suckling with co-mingling (ISCo) ($n = 10$ litters) where IS started at d -7 and two litters were housed together during separation and then returned to their original sow. *Ad libitum* creep feed was available from d -17 (relative to weaning). At weaning pigs were housed in pens of 11 pigs, with 27 pens in total. The ISCo treatment was divided in half to examine effects of different mixing strategies after weaning. Half of the ISCo litters were kept in familiar groups (ISCoF, familiar, $n = 4$) and the other half (ISCoNF, not familiar, $n = 5$) were mixed within treatment resulting in groups of unfamiliar pigs, the same as IS ($n = 9$) and CW ($n = 9$) treatments. The ISCo piglets ate more creep feed in the week before weaning ($P < 0.01$), but also showed more aggressive and manipulative behaviour on the first day of CoM compared with CW piglets ($P < 0.05$). IS with or without CoM increased exploratory and play behaviour on the first day of treatment intervention ($P < 0.001$) and increased sleeping behaviour on the last day of treatment intervention compared with CW ($P < 0.001$). Mixing strategy at weaning had an effect on performance with the highest growth and feed intake seen in ISCoF pigs 2 to 8 days after weaning ($P < 0.001$). The IS and ISCoNF pigs also grew faster and ate more than CW pigs 2 to 8 d after weaning ($P < 0.001$). Post-weaning injury scores suggested reduced aggression in ISCo as evidenced by reduced skin irritation ($P < 0.05$). The IS pigs slept the most and displayed less manipulative behaviours on the day of weaning; plasma haptoglobin levels remained low in IS pigs after weaning ($P \leq 0.01$).

In Experiment 2, the effect of different pre-weaning interventions on performance, aspects of behaviour, and selected neuroendocrine, inflammatory and immune indices was studied in 593 weaning pigs (59 litters, weaning age 22 ± 1.7 d). Measurements were taken at various time points 2 weeks before and after weaning. Sugar absorption tests (20% mannitol and 20% galactose) were used to assess gastrointestinal tract (GIT) absorptive capacity. One week before weaning, litters were either co-mingled (CoM) for 8 hours daily with another litter or not co-mingled (NoCoM). Half of the litters were also subjected to intermittent suckling (IS) involving separation from their sow for 8 hours daily and the other half remained with their sow (NoIS). Hence, four treatments were produced in a 2x2 factorial design; (1) CoM IS ($n = 16$ litters), (2) CoM NoIS ($n = 14$ litters), (3) NoCoM IS ($n = 16$ litters), (4) NoCoM NoIS ($n = 13$ litters). Weaning stress was evidenced by increases in cortisol, haptoglobin and N:L ratios when data were combined ($P < 0.001$). While CoM did not affect performance before weaning, growth ($P < 0.05$), feed intake ($P < 0.05$) and body weight ($P < 0.05$) were reduced in CoM pigs 7 to 14 days after weaning. One week of IS before weaning improved feed intake before weaning ($P < 0.01$), resulting in better growth ($P = 0.01$) and a tendency for a higher feed intake 2 to 7 days after weaning. A higher mannitol absorption was evident in CoM NoIS pigs 3 days after weaning ($P < 0.01$) and galactose absorption was reduced in IS pigs 3 days before weaning ($P < 0.05$) and tended to be reduced 3 days after weaning ($P < 0.1$), likely reflecting a GIT adaptive response.

In conclusion, IS during the last week of lactation stimulates a greater creep feed intake before weaning resulting in higher growth and a tendency for a greater feed intake between 2 and 7 days after weaning. Co-mingling during the last week of lactation did not improve post-weaning performance, but did reduce aggression and improve mannitol absorptive capacity after weaning.

Table of Contents

Executive Summary.....	i
1 Introduction.....	1
2 Experiment 1: Intermittent suckling with or without co-mingling of non-littermate piglets before weaning improves piglet performance in the immediate post-weaning period when compared with conventional weaning.	2
■ <i>Methodology</i>	2
2.1.1 Animals, housing and diet.....	2
2.1.2 Experimental design	3
2.1.3 Body weights, feed intake and injury scores.....	4
2.1.4 Behavioural measurements.....	4
2.1.5 Blood analysis.....	4
2.1.6 Statistical analysis.....	5
■ <i>Outcomes</i>	7
2.2.1 Piglet Mortality	7
2.2.2 Piglet performance	8
2.2.3 Behavioural measurements.....	9
2.2.3.1 Pre-weaning behaviour	9
2.2.3.2 Post-weaning behaviour	10
2.2.4 Injury scores.....	13
2.2.5 Blood analyses.....	14
3 Experiment 2: Improving welfare and production in the peri-weaning period: Effects of co-mingling and intermittent suckling on the stress response, performance, behaviour, and gastrointestinal tract carbohydrate absorption in young pigs	16
■ <i>Methodology</i>	16
3.1.1 Animals and housing.....	16
3.1.2 Experimental design	17
3.1.3 Body weights, feed intake and injury scores.....	17
3.1.4 Behavioural measurements.....	17
3.1.5 Blood analysis.....	18
3.1.6 Sugar absorption tests	18
3.1.7 Statistical analysis.....	18
■ <i>Outcomes</i>	19
3.2.1 Piglet performance	19
3.2.2 Behavioural measurements.....	21
3.2.2.1 Pre-weaning behaviour	22
3.2.2.2 Post-weaning behaviour	22
3.2.3 Injury scores.....	24
3.2.4 Blood analyses.....	24
3.2.5 Sugar absorption tests	25
4 Conclusion.....	27
5 Application of the Research	28
6 Limitations/Risks	29
7 Recommendations.....	29
8 References	30

1 Introduction

Weaning is an abrupt transition to independency that takes place at 21 to 28 d of age. Despite efforts to familiarize piglets with creep feed during lactation, there is often large within and between litter variation in intake (Pajor et al., 1991). A lack of familiarity with feed, in combination with other stressors such as a change in housing, maternal separation and mixing with unfamiliar piglets, generally causes a period of reduced nutrient intake immediately after weaning (Leibbrandt et al., 1975; Brooks and Tsourgiannis, 2003). To avoid a consequential reduction in growth and gastrointestinal tract (GIT) inflammation and dysfunction that generally results from underfeeding in combination with other weaning-associated stressors (Pluske et al., 1997; Pié et al., 2004), there has been a large focus on finding methods that stimulate feeding behaviour in this period.

Intermittent suckling (IS), a gradual weaning regime that also mimics the increasing time a sow would spend away from her piglets under natural conditions, has shown an improvement in post-weaning feed intake and growth in litters compared with conventional weaning (Kuller et al., 2004; Kuller et al., 2007). This improvement is likely mediated through increase familiarization with creep feed as piglets are forced to explore sources of nutrition other than milk. Alternatively, habituation with maternal separation may also prevent or attenuate the weaning-associated stress response (Berkeveld et al., 2009), reducing the development of altered behaviour patterns such as aggression, manipulation and a lack of play behaviour in recently weaned piglets (Dybkjær, 1992). With an interest towards housing systems that reduce sow confinement, IS has also received renewed interest from a reproductive point of view as a potential way to mate sows during lactation rather than after weaning (Kemp and Soede, 2012).

Piglets will also generally have a higher solid feed intake after weaning if they are kept in alternative housing that resembles natural conditions, such as multi-suckling and get-away systems with a communal piglet area (Pajor et al., 1999; Weary et al., 2002; van Nieuwamerongen et al., 2015). In a study that used a get-away system with a communal piglets area, sows spent 14 h/d away from the piglets by the end of lactation (d 27) and piglets spent 40% of the observation time in the pens of other litters (Weary et al., 2002). By allowing non-littermates to co-mingle (CoM) in these systems, piglets are more likely to have positive play experiences (Petersen et al., 1989) and improved social skills, which results in better acceptance of unfamiliar pigs and reduced aggression after weaning (D'Eath, 2005; Kutzer et al., 2009; Li and Wang, 2011).

In Experiment 1, we developed a novel IS system to include the opportunity for pre-weaning socialisation (CoM), where non-litter mates could interact with each other before weaning to potentially improve social development and reduce post-weaning aggression. We hypothesized that piglets subjected to IS (8 h separation per day for 7 d) would show better growth, higher feed intake, reduced negative behaviour patterns and reduced blood markers of inflammation, stress and lipid mobilisation supporting an increase in more feed directed behaviours before and after weaning. Intermittent suckling was applied in two regimes, with and without CoM (the mixing of piglets from two litters during separation) and compared with conventional weaning. It was expected that combining IS with CoM would improve post-weaning performance in an additive manner and further reduce aggressive behaviour in the immediate post-weaning period. However, since a control litter with co-mingling

was not included in this study and measures of GIT morphology and function were not examined a second Experiment was designed. In Experiment 2, IS or NoIS was applied with or without CoM to determine how IS and CoM each contribute to pre- and post-weaning performance through effects on growth, feed intake, GIT absorptive capacity, behaviour, immune, inflammatory and neuroendocrine indices compared with a conventional weaning regime in primiparous litters. The hypotheses tested were: (1) exposure to gradual weaning (IS for 8 hours per day, 7 days before weaning) and (2) the mixing of non-littermate piglets during lactation (CoM of two litters) would reduce the stress response when piglets were mixed at weaning, improving production, behaviour and GIT absorptive capacity of selected sows indicating enhanced welfare and improved GIT morphology and function.

2 Experiment 1: Intermittent suckling with or without co-mingling of non-littermate piglets before weaning improves piglet performance in the immediate post-weaning period when compared with conventional weaning.

Methodology

This study was conducted at a commercial piggery in Western Australia. At the conclusion of the study, the pigs continued on a grower/finisher facility at another location.

2.1.1 Animals, housing and diet

A total of 30 primiparous and multiparous sows (Large White x Landrace) and their offspring were selected based on their farrowing date from the farm and used in a single replicate. One week before farrowing, pregnant sows were housed individually across three adjacent rooms consisting of 5, 16 and 9 sows, respectively, with parities ranging from one to seven.

Individual housing consisted of farrowing crates (0.6 x 2.4 m) within farrowing pens (1.8 x 2.4 m) with slatted flooring. The pens consisted of a covered, heated creep area with artificial lighting provided between 7:00 and 17:00, a single feeder, and two nipple waterers. Water was provided on an *ad libitum* basis. An average litter size of 10.7 ± 0.48 (mean \pm SD) was achieved by cross fostering within the first 3 d of farrowing within each pre-allocated treatment group. Within 1 wk. of farrowing, piglets were made individually identifiable with numbered ear tags, their tails were docked, males were castrated and all piglets received a 1 ml intramuscular (IM) iron injection (PigDex100, Aventis Animal Nutrition, Carole Park, QLD, 4300) and a 2 ml IM injection of Respire One® (*Mycoplasma hypopneumoniae* vaccine; Pfizer, West Ryde, NSW, 2114). The beginning of the experiment, d -25 (25 d before weaning), was designated as the day on which most litters were born. Litters were born from 1 d before to 1 d after d -25.

Sows were fed a standard lactation diet *ad libitum* from entry into the farrowing house (approximately 1 week before farrowing) until weaning (14.5 MJ/kg digestible energy (DE); crude protein (CP), 19.1%). Piglets were offered creep feed (15 MJ/kg DE; CP, 23%) *ad libitum* from a single rotary hopper feeder (27 cm diameter) from 17 d before weaning (d -17). Immediately preceding weaning, piglets received a second 2 ml IM dose of Respire One® (*Mycoplasma hypopneumoniae* vaccine; Pfizer, West Ryde, NSW, 2114) as well as a 2 ml IM injection of Relsure® PCV (Porcine circovirus Type 1 vaccine, Zoetis, Florham Park, NJ, 07932). At weaning, piglets were housed in a separate building in weaner pens with slatted flooring (1.13 x 2.5 m) in a temperature-controlled building. Feed was provided on an *ad libitum* basis (14.6 MJ/kg DE; CP, 20.8%) via a 5-hole weaner hopper. Piglets remained in the weaner pens until the end of the experiment, and had unlimited access to drinking water provided by a single water nipple in each pen.

2.1.2 Experimental design

Once farrowing was completed, selected sows and their litters were randomly allocated to one of three treatments: conventional weaning (CW) ($n = 10$ litters), IS (intermittent suckling) ($n = 10$ litters) or ISCo (intermittent suckling with CoM) ($n = 10$ litters). Litters receiving different treatments were spread evenly across the three adjacent rooms and parity remained similar across the three treatments (3.6 ± 2.1 for CW, 3.5 ± 2.0 for IS and 3.6 ± 2.0 for ISCo; mean \pm SD). The piglets in the CW treatment remained with the sow continuously through lactation until weaning. The piglets in the IS and ISCo treatments were separated from their sow and housed in an empty farrowing crate in another room for 8 h/d (0700 to 1500) for 7 d before weaning. Separation was achieved by transporting piglets by litter in a trolley from one room to another. The empty farrowing pens in which separated piglets were housed were identical to those that housed the sows, but no sows were housed in the separation room. For the ISCo treatment, two litters were housed in one farrowing pen during the separation time to allow for pre-weaning socialisation. The space allowance was 0.42 m^2 and 0.21 m^2 for IS and ISCo piglets respectively. In the ISCo treatment, the same two litters were socialised each day and the original litter was returned to the sow at the end of separation. When piglets were separated from the sow, the rotary feeder with creep feed was moved with the litter into the separation pen. The separation pens housing the ISCo piglets therefore had two rotatory feeders per pen during separation.

Weaning age across treatments was equal and averaged 25.3 ± 0.7 (mean \pm SD). Piglets were mixed and housed in pens of 10 to 13 with an average of 11.1 ± 0.82 (mean \pm SD) per pen. Mixing for CW and IS was done within treatment by randomly allocating 2-3 pigs per litter into each pen. The grouping of ISCo piglets after weaning was achieved in one of two ways: (i) four litters were only grouped with piglets they had previously had contact with before weaning (called ISCoF; familiar), and (ii) six litters were mixed with unfamiliar piglets (similar to CW and IS) (called ISCoNF; not familiar). This arrangement was necessary to examine the effect of familiarity on post-weaning behaviours since reduced aggression in pigs socialised before weaning has been partly attributed to a higher number of familiar pigs in the group rather than previous social experience (Verdon et al., 2016).

2.1.3 Body weights, feed intake and injury scores

All piglets were individually weighed on d -17, -7 and -4 before weaning, at weaning, and on d 2 and 8 after weaning. Creep and weaner feed residuals were measured simultaneously with body weights. Minimal wastage was observed due to twice daily checking of the feeders by staff to ensure the pan was not too full. Therefore, disappeared creep feed was considered eaten.

During the weighing procedure on the day of weaning (d 0) and 2 d after weaning, an injury score was recorded from all piglets as an indicator of aggression. The injury scoring system was adapted from Widowski et al. (2003) and consisted of a four-point scale for scratches and redness around the head, ears and flank (Table 1).

Table 1. Injury scoring system using scratches and redness adapted from Widowski et al. (2003)

Score	0	1 (Mild)	2 (Moderate)	3 (Severe)
Scratches	No scratches were evident on the head, ears and flank	1 to 3 small (\leq 2cm) scratches or areas of abraded skin on head, ears or flank	1 to 3 large ($>$ 2cm) scratches or areas of abraded skin on head, ears or flank	More than 3 scratches or larger areas of superficial skin loss on head, ears or flank
Redness	No redness or swelling on the head, ears or flank	Redness and swelling barely detectable on head, ears and flank	Redness or swelling were obvious on head, ears and flank	Irritation easily observed as darker reddening and/or moderate to severe swelling

2.1.4 Behavioural measurements

At the beginning of the experiment, five focal piglets were randomly selected from each litter (a total of 150 piglets, 50 piglets per treatment group) and made individually identifiable with marker spray. Instantaneous scan sampling by one observer was then used to record the main activity of the individual selected piglets on days -10, -7, -1, 0, 1 and 7. All focus piglets were observed every 30 minutes for two, 2-hour periods (morning 0900 and afternoon 1400), excluding d 0 and d 1 during which only one, 2 h period was used to record behaviour. The different types of behaviour recorded during scan sampling were adapted from behaviour categories previously used by Pluske and Williams (1996) and Bolhuis et al. (2005) and are presented in Table 2.

2.1.5 Blood analysis

Blood samples were taken from two randomly selected piglets per litter or pen on d -6 and -4 before weaning and d 2 and 4 after weaning (piglets were not bled more than two times within a 7 d period). Piglets were held in dorsal recumbancy and

blood samples were collected via jugular venipuncture with the procedure lasting no more than 90 seconds. Nine millilitres of blood was collected into lithium heparin coated tubes. Blood samples were taken at the same time of day (noon) to minimise the effects of diurnal variation. Plasma was separated by centrifugation (20 minutes at 2800 x g, 4 ° C) and then stored as 0.5 mL aliquots at -20 ° C until analysis.

Plasma cortisol levels were determined using a commercial ELISA kit (Enzo Life Sciences, Cortisol ELISA kit, AD-901-071, Farmingdale, NY) in accordance with the manufacturers' instructions with the exception of optical density, which was read at 415 nm instead of the recommended 405nm. Intra-assay CV was 10.5% (low standard), 6.6% (medium standard) and 7.3% (high standard). Plasma was analysed at the Animal Health Laboratories (Department of Agriculture and Food Western Australia) for the determination of (i) haptoglobin (Hp), using an enzymatic colorimetric in-house assay based on modified methods of Eckersall et al. (1999), and (ii) glycerol, using a Randox Reagent Kit (GY105, Crumlin, United Kingdom) and Olympus AU400 analyser (Tokyo, Japan).

2.1.6 Statistical analysis

Statistical analyses were performed using SPSS (v.21; IBM). Residuals were tested for normality and data were transformed or non-parametric tests were used if needed. Analysis of post-weaning data included four treatment groups after weaning (CW, IS, ISCoF and ISCoNF; as described above). Differences between ISCoF and ISCoNF were present for post-weaning ADG (average daily gain), ADFI (average daily feed intake) and FCR (feed conversion ratio) ($P < 0.05$). Therefore, post-weaning performance data are presented with four treatment groups (CW, IS, ISCoF and ISCoNF). However, no differences between ISCoF and ISCoNF were present for behaviour, injury score or blood parameter data ($P > 0.05$; with the exception of sleeping behaviour on d 7 after weaning during which ISCoNF slept more than ISCoF, $P < 0.01$). Therefore, analysis was repeated with three treatment groups for post-weaning data for these parameters (CW, IS and ISCo).

Data for pre-weaning piglet mortality, body weight (BW), ADG, ADFI and FCR were analysed on a per litter or pen basis using a general linear model with treatment as the fixed factor. Since two feeders were included in each of the ISCo separation

Table 2. Ethogram used during instantaneous scan sampling observations adapted from Pluske and Williams (1996) and Bolhuis et al. (2005).

Behaviour	Description
Sleeping behaviour	Lying on the side or belly with eyes closed, not performing any other described behaviour
Lying/sitting behaviour	Lying on the side or belly with eyes open or passive sitting, not performing any other described behaviour
Standing behaviour	Standing without performing any other described behaviour
Aggressive behaviour	Head knocking, biting or fighting with another pen or littermate
Exploring/play behaviour	Standing up and investigating the surroundings such as nosing the floor, scrapping the floor with one of the forelegs, nosing or nibbling on fixtures. Running across the pen and pivoting with or without the gentle nudging of a pen or littermate
Manipulative behaviour	Belly nosing Mounting Oral manipulation of other pen or littermates Laying down and biting the metal frame work of the weaner pens (after weaning only)
Ingestive-related behaviour	Eating (chewing feed) Drinking from water nipple Eliminating (defecating or urinating)
Sow directed behaviour	Suckling or massaging the sow Manipulation of the sow during the pre-weaning period

pens, ADFI data for the ISCo treatment group before weaning were analysed on a per socialised group basis (i.e. $n = 5$). To normalise the distribution of pre-weaning ADFI data, feed disappearance at -7 to -4 d before weaning and -4 to 0 d before weaning were pooled together and presented as feed disappearance during the last week of lactation. Unexpectedly, feed disappearance from d -17 to d -7 for the IS treatment groups tended to be higher than that of the controls (9 ± 1.7 , 8 ± 2.3 and 3 ± 1.6 g/piglet/day for IS, ISCo and CW respectively, $P < 0.1$), therefore, ADFI from d -17 to d -7 was included as a covariate in the analysis of the pre-weaning ADFI data. Feed conversion ratio was calculated by dividing ADFI (d 2 to 8) by ADG (d 2 to 8).

Measurements for injury scores, plasma cortisol, Hp and glycerol were averaged per crate or pen and then analysed using a general linear model with treatment as a fixed factor. Redness scores before and after weaning, as well as plasma Hp concentration were not normally distributed. Data were transformed using a square root transformation to force normality. The mean values and confidence intervals were then back-transformed and expressed as least square means.

Behaviour data obtained from instantaneous scan sampling are expressed as a percentage of total observations for each behaviour on a specific day. The distribution of all behaviours observed was not normal and transformation of the data did not correct this. The percentage of scan samples for a specific behaviour was therefore compared between treatments on different days using a Kruskal-Wallis test with post-hoc analysis to determine which groups were different from one another. A non-parametric Friedman test was used to compare differences in proportion of time spent on a specific behaviour within treatment. If this test detected an overall treatment effect, data were subsequently tested pairwise using a Wilcoxon test.

All post-hoc analyses included a Bonferroni correction for pairwise comparisons and correlations were performed using a Pearson correlation test. Statistical significance was accepted at $P \leq 0.05$ and a trend was considered at $P > 0.05$ and $P \leq 0.1$. Data are presented as raw means \pm SEM, except when n is different between treatments, in which case data are presented as raw means \pm SE unless otherwise stated.

Outcomes

2.2.1 Piglet mortality

Pre-weaning piglet mortality was similar between all treatments ($P > 0.05$). Mortality mostly occurred before treatment intervention (CW: 0.4 ± 0.70 , IS: 0.3 ± 0.67 , ISCo: 0.1 ± 0.32 piglets per litter, $P > 0.05$). Litter sizes on the first day of treatment intervention did not differ (CW: 10.4 ± 0.70 , IS: 10.3 ± 0.95 , ISCo: 10.5 ± 0.53 , $P > 0.05$) and mortality after the start of IS with or without CoM was negligible, with only 1 piglet in the IS treatment group dying due to crushing on d -4 of the experiment. Therefore, litter sizes at weaning were 10.4 ± 0.70 for CW, 10.2 ± 1.23 for IS and 10.5 ± 0.53 for ISCo ($P > 0.05$). None of the treatment groups experienced post-weaning mortalities, however, 9 piglets were not weaned due to having a BW less than 4.6kg (5 piglets from CW, 1 piglet from IS and 3 piglets from ISCo) and were returned to the herd with a nurse sow as per regular farm protocol.

2.2.2 Piglet performance

Piglet BW and ADG were similar between all treatments between d -17 to -7 relative to weaning (Table 3, $P > 0.05$). During the last week of lactation (d -7 to 0), exposure to IS or IS with CoM did not affect BW or growth compared with CW litters (Table 3, $P > 0.05$). Therefore, BW did not differ between the three treatment regimens at weaning (Table 3, $P > 0.05$). However, ISCo litters ate more creep feed than IS and CW litters between d -7 and 0 (Table 3, $P < 0.01$).

After weaning, all treatment groups suffered a reduction in weight gain from d 0 to 2. Between d 2 and 8, ISCoF pens grew the fastest, CW pens grew the slowest and IS and ISCoNF pens were intermediate (Table 4, $P < 0.001$). At the end of the experiment (d 8), BW was similar across all four treatment groups (Table 4, $P > 0.05$).

Weaning markedly increased feed intake in all treatment groups relative to intake in lactation, however ADFI did not differ between treatments during the first 2 d after weaning. Between d 2 to 8 after weaning, all IS treatment pens were eating more than CW pens (Table 4, $P < 0.001$), however, ISCoF were eating more than IS pens ($P < 0.001$) and there was a tendency for ISCoF to eat more than ISCoNF pens ($P < 0.1$). Feed conversion ratio was lower for ISCoF than CW between 2 and 8 days after weaning, while IS and ISCoNF were intermediate (Table 4, $P < 0.05$).

Table 3. Mean values for piglet BW, ADG and ADFI before weaning for three different weaning treatments.

Day ¹	Treatment ²			SEM
	CW	IS	ISCo	
BW, kg				
D -17	2.8	2.9	3.0	0.14
D -7	5.4	5.7	5.6	0.22
D -4	6.3	6.4	6.4	0.22
D 0	7.3	7.4	7.5	0.23
ADG, g				
D -17 to -7	265	278	272	8.76
D -7 to -4	283	229	270	19.44
D -4 to 0	267	253	251	12.84
ADFI ³ ,g				
D -7 to 0	7 ^a ± 2.1	15 ^a ± 2.1	22 ^b ± 3.0	

¹Day = day in relation to weaning with weaning = d 0, d -25 is the day on which most the litters were born.

²CW = conventional weaning ($n = 10$), IS = intermittent suckling ($n = 10$), ISCo = intermittent suckling with co-mingling ($n = 10$).

³D -17 to -7: ADFI included as covariate in analysis.

^{ab} Values within a row not having the same superscript are significantly different.

Table 4. Mean values for piglet BW, ADG, ADFI and FCR after weaning for three different weaning treatments.

Day ¹	Treatment ²			
	CW	IS	ISCoF	ISCoNF
BW, kg				
D 2	7.5 ± 0.23	7.3 ± 0.23	7.3 ± 0.34	7.6 ± 0.31
D 8	8.4 ± 0.24	8.7 ± 0.24	9.0 ± 0.37	9.0 ± 0.33
ADG, g				
D 0 to 2	-25 ± 13.9	-56 ± 13.7	-78 ± 21.6	-48 ± 17.7
D 2 to 8	165 ± 8.6 ^a	234 ± 8.5 ^b	294 ± 13.4 ^c	242 ± 11.1 ^b
ADFI, g				
D 0 to 2	80 ± 5.3	73 ± 5.3	67 ± 7.9	72 ± 7.1
D 2 to 8	192 ± 4.5 ^a	256 ± 4.5 ^b	291 ± 6.7 ^{cy}	269 ± 6.0 ^{bcx}
FCR, g/g				
D 2 to 8	1.2 ± 0.03 ^a	1.1 ± 0.03 ^{ab}	1.0 ± 0.05 ^b	1.1 ± 0.04 ^{ab}

¹Day = day in relation to weaning with weaning = d 0, d -25 is the day on which most the litters were born.

²CW = conventional weaning ($n = 9$), IS = intermittent suckling ($n = 9$), ISCoF = intermittent suckling with co-mingling, familiar pigs ($n = 4$), ISCoNF = intermittent suckling with co-mingled, not familiar pigs ($n = 5$).

^{ab}Values within a row not having the same superscript are significantly different.

^{xy}Values within a row not having the same superscript are a trend.

2.2.3 Behavioural measurements

2.2.3.1 Pre-weaning behaviour

Before the start of IS intervention (d -10), there was no difference ($P > 0.05$) between treatments in the proportion of total observations spent per behaviour category (Table 5.1). On d -7, treatment intervention caused an increase in lying and sitting behaviour for both IS and ISCo piglets compared with d -10 behaviour observations for each treatment (Table 5.1, $P < 0.001$). Furthermore, exploratory and play behaviour also increased ($P < 0.001$) in IS piglets between d -10 to -7 whereas aggressive behaviour increased in ISCo piglets ($P < 0.001$). Compared with CW piglets, ISCo piglets showed a greater proportion of standing, manipulative and aggressive behaviours on d -7 while IS piglets were intermediate (Table 5.1, $P < 0.05$ for all behaviours). Only piglets subjected to ISCo slept more, had higher levels of other inactive behaviour (lying and sitting) and showed a greater proportion of total observations on ingestive-related behaviours compared with IS and CW piglets on d -7 (Table 5.1, $P \leq 0.001$ for all behaviours).

Over the course of treatment intervention (d -7 to -1), sleeping increased in both IS and ISCo piglets ($P < 0.001$) with piglets from both IS treatments spending over 90% of total observations during separation sleeping in their crates, which was higher than the level of sleeping for CW piglets ($P < 0.001$). Consequently, the expression of all other behaviour categories reduced over time (d -7 to -1) for ISCo ($P < 0.001$) and lying/sitting, standing and exploratory and play behaviour reduced over time (d -7 to -1) for IS piglets ($P < 0.001$). As a result, observations for lying/sitting ($P < 0.001$), standing ($P < 0.001$), exploration/ play ($P < 0.001$) and manipulation ($P < 0.01$) were lower in IS and ISCo piglets on day -1 compared with CW piglets (Table

5.1). There was no difference in the expression of ingestive-related behaviour or aggressive behaviour between treatments on day -1 (Table 5.1, $P < 0.05$).

2.2.3.2 Post-weaning behaviour

On the day of weaning (approximately 3 h after the event), a considerable proportion of observations was spent on sleeping behaviour in IS pigs with IS pigs sleeping more than CW and ISCo pigs ($P < 0.001$) and CW and ISCo pigs lying or sitting more than IS pigs (Table 5.2, $P < 0.001$). More manipulative and exploratory/play behaviour was also observed for both CW and ISCo pigs compared with IS pigs ($P < 0.001$) on the day of weaning and CW pigs displayed the highest level of standing behaviour compared with both IS treatments (Table 5.2, $P < 0.001$).

Over the initial 24 h after weaning (d 0 to d 1), sleeping and lying/sitting behaviour varied differently over time for each of the different treatments (see Table 5.2) resulting in higher levels of sleeping for ISCo than CW and IS pigs and higher levels of lying or sitting behaviour for CW and ISCo pigs than IS pigs 24 h after weaning (Table 5.2, $P < 0.001$). While the proportion of total observations spent on manipulative behaviour did not change for CW and IS pigs, there was a reduction in observations for ISCo pigs over the initial 24 h after weaning (Table 5.2, $P < 0.001$). Therefore, on d 1 after weaning, ISCo pigs expressed less manipulative behaviour than CW pigs, and IS pigs were intermediate ($P < 0.05$). Alternatively, exploratory and play behaviour increased over the initial 24 hours after weaning for IS pigs while the proportion of total observations for exploratory and play behaviour decreased over the same timeframe for ISCo and CW pigs ($P < 0.001$ for IS and ISCo and $P = 0.001$ for CW).

Apart from the day of weaning during which ISCo pigs had the highest level of eating behaviour (Table 5.2, $P < 0.001$), no difference in eating patterns were observed between treatments after weaning ($P > 0.05$).

One week after weaning, the proportion of total observations spent on sleeping in both IS treatments was more than the CW pigs ($P < 0.001$), but lying and sitting was less in both IS treatments compared with CW pigs ($P = 0.001$). In contrast, the proportion of total observations spent on the other behaviour categories was similar for all treatments 7 days after weaning (Table 5.2, $P > 0.05$), with the exception of aggressive behaviour where IS pigs fought more than CW and ISCo pigs ($P > 0.05$). However, this type of behaviour was still rarely observed.

Table 5.1. Pre-weaning behaviour (proportion of total observations, %) of selected CW, IS and ISCo piglets¹

Behaviour ² , % of total observations	D ³ -10				D -7				D -1			
	CW	IS	ISCo	SEM	CW	IS	ISCo	SEM	CW	IS	ISCo	SEM
Sleeping	50.5	44.0 ^x	39.0 ^x	3.9	54.8 ^a	55.0 ^{a,y}	24.5 ^{b,y}	2.7	47.8 ^a	91.2 ^{b,z}	95.0 ^{b,z}	2.3
Inactive	2.0 ^x	5.5 ^x	3.0 ^x	1.3	4.5 ^{a,xy}	13.5 ^{b,y}	32.5 ^{c,y}	1.6	7.0 ^{a,y}	3.0 ^{b,x}	0.5 ^{b,z}	1.6
Standing	6.0	7.5 ^x	5.5 ^x	1.8	4.3 ^a	6.3 ^{ab,x}	7.8 ^{b,x}	1.1	2.0 ^a	0.3 ^{b,y}	0.0 ^{b,y}	0.4
Aggressive behaviour	3.0	2.0	0.5 ^x	1.1	0.5 ^a	1.0 ^{ab}	3.0 ^{b,y}	0.6	0.0	0.0	0.3 ^x	0.1
Exploratory and play behaviour	6.0	10.0 ^x	12.0 ^x	2.1	7.8 ^a	17.0 ^{b,y}	16.8 ^{b,x}	1.9	7.3 ^a	1.5 ^{b,z}	0.5 ^{b,y}	0.9
Manipulative behaviour	4.5	6.0	5.5 ^x	1.7	3.0 ^a	4.5 ^{ab}	7.5 ^{b,x}	1.2	3.0 ^a	0.8 ^b	0.5 ^{b,y}	0.6
Ingestive-related behaviour	4.0	5.0	5.5	1.5	2.3 ^a	1.0 ^a	5.8 ^b	0.9	3.8	3.2	3.0	1.0
Sow-directed behaviour	24.0	20.0	29	3.1	22.3	-	-	1.2	28.5	-	-	1.3

¹CW = conventional weaning ($n = 50$), IS = intermittent suckling ($n = 50$), ISCo = intermittent suckling with co-mingling ($n = 50$).

²Results obtained by scan sampling observations. Data were tested nonparametrically.

³Day = day in relation to weaning with weaning = d 0, d -25 is the day on which most the litters were born.

^{a-c}For each day, means with different superscripts are different ($P < 0.05$), indicating differences between treatments per day.

^{x-z}For each day treatment, means with different superscripts ($P < 0.05$), indicating differences between days within each treatment.

Table 5.2. Post-weaning behaviour (proportion of total observations, %) of CW, IS and ISCo pigs¹

Behaviour ² , % of total observations	D ³ 0				D 1				D 7			
	CW	IS	ISCo	SEM	CW	IS	ISCo	SEM	CW	IS	ISCo	SEM
Sleeping	20.4 ^{a,x}	91.3 ^{b,x}	22.4 ^{a,x}	2.8	53.1 ^{a,y}	58.2 ^{a,y}	78.0 ^{b,y}	4.1	39.8 ^{a,z}	58.9 ^{b,y}	54.6 ^{b,z}	3.1
Inactive	32.1 ^{a,x}	0.5 ^{b,x}	18.4 ^{c,x}	2.7	19.9 ^{a,y}	15.8 ^{a,y}	8.1 ^{b,y}	2.3	15.1 ^{a,y}	6.6 ^{b,z}	11.2 ^{ab,y}	1.4
Standing	13.3 ^{a,x}	2.6 ^b	4.1 ^b	1.9	4.6 ^y	6.6	5.1	1.9	8.7 ^x	4.8	4.8	1.3
Aggressive behaviour	2.0	0.0	1.5	0.9	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0
Exploratory and play behaviour	15.8 ^{a,x}	0.0 ^{b,x}	31.6 ^{ax}	2.5	5.6 ^y	5.1 ^y	2.6 ^y	1.5	8.2 ^y	6.6 ^y	3.1 ^y	1.5
Manipulative behaviour	10.7 ^{a,xy}	1.0 ^{b,x}	12.8 ^a	2.1	7.7 ^{ax}	4.6 ^{ab,x}	3.1 ^b	1.5	13.0 ^y	10.7 ^y	15.3	1.8
Ingestive-related behaviour	2.0 ^{a,x}	1.5 ^{a,x}	8.7 ^{b,x}	1.3	6.6 ^y	9.2 ^y	3.1 ^y	2.1	13.0 ^z	9.9 ^y	11.0 ^x	1.5

¹CW = conventional weaning ($n = 50$), IS = intermittent suckling ($n = 50$), ISCo = intermittent suckling with co-mingling ($n = 50$).

²Results obtained by scan sampling observations. Data were tested nonparametrically.

³Day = day in relation to weaning with weaning = d 0, d -25 is the day on which most the litters were born.

^{a-c}For each day, means with different superscripts are different ($P < 0.05$), indicating differences between treatments per day.

^{x-z}For each day treatment, means with different superscripts ($P < 0.05$), indicating differences between days within each treatment.

2.2.4 Injury scores

On the day of weaning, there was no difference in scratch scores between treatments (Table 6, $P > 0.05$), however CW piglets had higher redness scores than piglets in either IS treatment (Table 6, $P < 0.05$). Two days after weaning, there was a tendency for pigs in CW pens to have more scratches than pigs in ISCo pens (Table 6, $P < 0.1$). At the same point in time, higher redness scores were seen in CW and IS pigs compared with ISCo pigs (Table 6, $P < 0.001$).

Before weaning, scratch scores did not correlate with redness scores ($r = 0.11$, $P > 0.05$). However, scratch and redness scores were positively correlated after weaning ($r = 0.67$, $P < 0.001$). There was a weak positive correlation across treatments between aggressive behaviour the day before weaning (d -1) and scratch scores measured on the day of weaning (just prior to the event of weaning) ($r = 0.22$, $P < 0.01$).

Table 6. Effects of three different weaning treatments on injury score on the day of weaning (just prior to the process of weaning) and at 2 d after weaning.

	Treatment ¹			SEM	P-value
	CW	IS	ISCo		
Scratch injury score					
Weaning ²	0.48	0.30	0.51	0.09	0.237
D 2 after weaning	1.03 ^x	0.97 ^{xy}	0.55 ^y	0.15	0.074
Redness injury score ³					
Weaning ²	0.21 ^a (0.11-0.35)	0.05 ^b (0.01-0.13)	0.04 ^b (0.01-0.12)		0.016
D 2 after weaning	0.48 ^a (0.32-0.67)	0.35 ^a (0.21-0.52)	0.02 ^b (0.00-0.08)		<0.001

¹CW = conventional weaning ($n = 10$ pre-weaning, $n = 9$ post-weaning), IS = intermittent suckling ($n = 10$ pre-weaning, $n = 9$ post-weaning), ISCo = intermittent suckling with co-mingling ($n = 10$ pre-weaning, $n = 9$ post-weaning).

²Injury scores were assessed just prior to weaning.

³Data were square root transformed, then back transformed, and expressed as least square means with 95% confidence intervals.

^{ab} Values within a row not having the same superscript are significantly different.

^{xy} Values within a row not having the same superscript are a trend.

2.2.5 Blood analyses

Pre-weaning treatment did not affect plasma cortisol concentration before weaning and concentrations were still similar between treatments 2 d after weaning (Table 7, $P < 0.05$). Four days after weaning, cortisol levels for IS pigs were higher than that of ISCo pigs (Table 7, $P = 0.001$) and tended to be higher than that of CW pigs (Table 7, $P < 0.1$).

Plasma Hp concentrations were higher in IS piglets compared to CW piglets on d -6 before weaning (the second day of IS intervention), and ISCo Hp levels were intermediate (Figure 1, $P < 0.05$). Two days later, there was no difference in Hp concentration between treatments (Figure 1, $P > 0.05$). After weaning, ISCo pigs had the highest Hp concentration on d 2 ($P < 0.01$), while on d 4, Hp levels were higher in CW pigs than IS pigs and ISCo pigs were intermediate (Figure 1, $P = 0.01$).

The concentration of glycerol in plasma was highest in CW piglets on d -6 and -4 before weaning (Table 8, $P < 0.05$ and < 0.01 respectively for d -6 and -4). Two days after weaning, there was no difference in glycerol concentration between treatments (Table 8, $P > 0.05$) and on the final blood-sampling day (d 4), there was a tendency for ISCo pigs to have a lower glycerol concentration than CW pigs (Table 8, $P < 0.1$).

Table 7. Plasma cortisol (ng/ml) concentrations during three different weaning treatments before and after weaning.

Day ^{1,2}	Treatment ³			P-value
	CW	IS	ISCo	
D -6	19 (11.0-29.6)	19 (10.6-28.8)	23 (14.1-34.6)	0.724
D -4	20 (12.1-29.6)	27 (18.0-38.5)	22 (13.4-31.7)	0.467
D 2	28 (14.9-45.5)	20 (9.2-35.0)	24 (12.2-40.7)	0.672
D 4	25 ^{abx} (17.9-32.6)	32 ^{axy} (24.4-41.1)	14 ^{by} (8.9-19.8)	0.001

¹Day in relation to weaning with 0 representing weaning (e.g. 2 is 2 d after weaning).

²Data has been transformed via square root and then back transformed and expressed as least square means with 95% confidence intervals.

³CW = conventional weaning ($n = 10$ pre-weaning, $n = 9$ post-weaning), IS = intermittent suckling ($n = 10$ pre-weaning, $n = 9$ post-weaning), ISCo = intermittent suckling with co-mingling ($n = 10$ pre-weaning, $n = 9$ post-weaning).

^{ab}Values within a row not having the same superscript are significantly different.

^{xy}Values within a row with different superscripts are trends.

Figure 1. Plasma haptoglobin (mg/ml) concentrations in the three different weaning treatments before and after weaning. Values are presented as actual means \pm SE. Analysis involved transformation of the data using a square root calculation; CW = conventional weaning ($n = 10$ pre-weaning, $n = 9$ post-weaning), IS = intermittent suckling ($n = 10$ pre-weaning, $n = 9$ post-weaning), ISCo = intermittent suckling with co-mingling ($n = 10$ pre-weaning, $n = 9$ post-weaning). ^{a,b} On each experimental day, values not having the same superscript are significantly different.

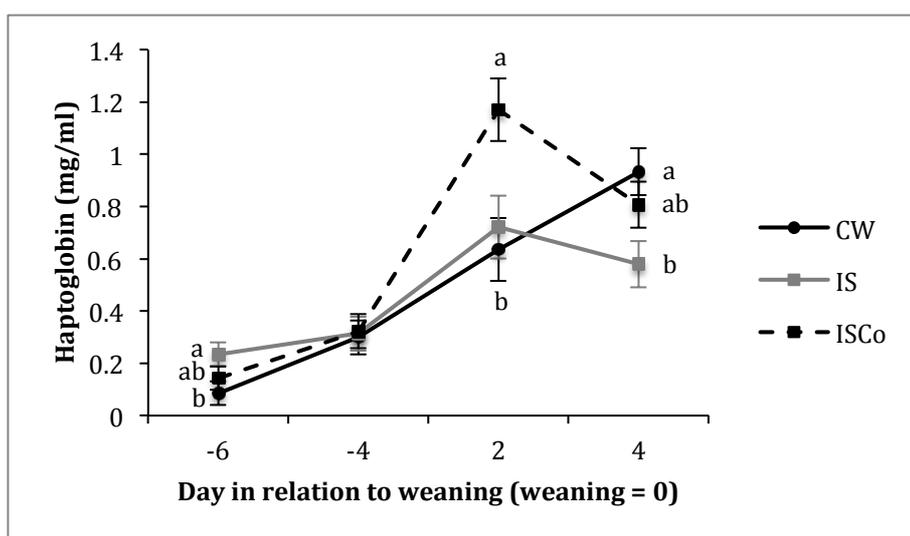


Table 8. Plasma glycerol concentrations in three different weaning regimens before and after weaning

Day ^{1,2}	Treatment ³			P-value
	CW	IS	ISCo	
D -6	81 ^a (68.0-95.7)	57 ^b (46.1-69.3)	53 ^b (42.8-65.2)	0.004
D -4	78 ^a (61.7-95.3)	44 ^b (32.4-57.8)	45 ^b (33.2-59.0)	0.002
D 2	20 (12.4-29.5)	27 (16.4-40.1)	12 (6.7-19.9)	0.613
D 4	20 ^x (13.9-28.1)	12 ^{xy} (6.7-19.9)	10 ^y (5.7-15.7)	0.053

¹Day in relation to weaning with 0 representing weaning (e.g. -6 is 6 d before weaning).

²Data has been transformed via square root and then back transformed and expressed as least square means with 95% confidence intervals.

³CW = conventional weaning ($n = 10$ pre-weaning, $n = 9$ post-weaning), IS = intermittent suckling ($n = 10$ pre-weaning, $n = 9$ post-weaning), ISCo = intermittent suckling with co-mingling ($n = 10$ pre-weaning, $n = 9$ post-weaning).

^{ab}Values within a row not having the same superscript are significantly different.

^{xy}Values within a row with different superscripts are trends.

3 Experiment 2: Improving welfare and production in the peri-weaning period: Effects of co-mingling and intermittent suckling on the stress response, performance, behaviour, and gastrointestinal tract carbohydrate absorption in young pigs

Methodology

The experiment was conducted at a commercial pork operation in Western Australia and was approved by the Animal Ethics Committee at Murdoch University (permit number R2765/15).

3.1.1 Animals and housing

A total of 59 primiparous sows (Large White x Landrace) and their offspring used between September and November 2015 over five batches consisting of 12, 13, 11, 13 and 10 sows. Immediately before farrowing and during lactation sows were housed individually in farrowing crates (0.6 x 2.4 m) within farrowing pens (1.8 x 2.4 m). The farrowing pen consisted of a slatted floor with a sow feeder and two nipple drinkers (one for the sow and one for the piglets). Water was available *ad libitum*. To the side of the pen was a covered, heated creep area for the piglets. Sows were fed a commercial lactation diet *ad libitum* from entry into the farrowing house (approximately 1 week before farrowing) until weaning (14.5 MJ/kg digestible energy (DE); crude protein (CP), 19.1%). Each batch was housed in a separate room, which was identical in layout within the same farrowing building on the farm. Lights were on between 0700 and 1600.

Litter size was standardised (average of 10.1 ± 0.41 piglets per litter (mean \pm SD) within the first 3 days of farrowing by cross-fostering piglets from large litters onto sows with smaller litters. Within the first week of farrowing, piglets were made individually identifiable with numbered ear tags, their tails were docked, males were castrated and all piglets received a 1 ml intramuscular (IM) iron injection (PigDex100, Aventis Animal Nutrition, Carole Park, QLD, 4300). Creep feed (15 MJ/kg DE; CP, 23%) was offered to all piglets *ad libitum* in a rotary hopper feeder (27cm diameter) from 13 days before weaning up until weaning.

To synchronise the start of IS and/or CoM within a batch, day -22 (22 days before weaning) was designated as the start of data collection. Intermittent suckling and/or CoM always started on day -7. Weaning took place 7 days later on day 0. Piglets were born between 2 days before and 4 days after day -22, and average weaning age was 22 ± 1.7 (mean \pm SD). Immediately preceding weaning all piglets received a 2 ml IM injection of Relsure® PCV (Porcine circovirus Type 1 vaccine, Zoetis, Florham Park, NJ, USA). Weaning involved transporting pigs a small distance to a different building where they were housed in temperature controlled rooms consisting of 18 pens. Each batch was housed in a separate room with an average of 9.7 ± 0.91 pigs per pen. Weaner pens consisted of slatted flooring, one nipple drinker per pen and one feeder with five feeding places. A commercial weaner diet (14.6 MJ/kg DE; CP, 20.8%) was available *ad libitum* until the end of the experiment, after which time all pigs returned to the commercial herd.

3.1.2 Experimental design

Within the first week of lactation litters were assigned to one of four treatments depending on the position of their farrowing crate within the room. Treatments were arranged in a 2 x 2 factorial design, with the factors being (1) socialization (CoM) or no socialization (NoCoM) in lactation, and (2) separation (IS) versus no separation (NoIS) in lactation. Four treatment groups were therefore formed: (1) CoM IS ($n = 16$ litters with 161 piglets), where piglets were separated from their sow for 8 hours per day, 7 days before weaning and during the separation time, two litters were housed in one farrowing pen to allow for pre-weaning socialisation; (2) CoM NoIS ($n = 14$ litters with 140 piglets), where piglets remained with their sow (i.e. no separation), but the barrier between two farrowing pens across a corridor was removed for 8 hours per day 7 days before weaning allowing piglets opportunity to socialise with another sow and her litter; (3) No CoM IS ($n = 16$ litters with 160 piglets), where piglets were separated from their sow into an empty farrowing pen next to their sow's pen for 8 hours per day 7 days before weaning; (4) No CoM No IS ($n = 13$ litters with 132 piglets), where piglets remained with their sow continuously until weaning. The IS and CoM practices took place between 0730 and 1530, and all piglets were returned to their original sow at the end of the day. For the CoM groups, the same two litters were socialised everyday. Separation for IS involved individually lifting each piglet into the allocated empty farrowing pen. The rotary feeder with creep feed was also moved with the litter into the separation pen. Therefore, the separation pens housing the CoM and IS piglets had two rotary feeders per pen during separation. The space allowance during separation was 0.42m^2 and 0.21m^2 for CoM IS and NoCoM IS piglets respectively. At weaning, pigs were mixed within treatment by randomly allocating 2-3 piglets per litter into each pen (average 9.7 ± 0.91 pigs per pen). This arrangement meant that pigs in all treatment groups would be housed with some familiar and some unfamiliar pigs after weaning.

3.1.3 Body weights, feed intake and injury scores

Piglets were weighed individually on days -13, -4, 0 (weaning), 2, 7 and 14 (subsample of pens from batches 3, 4 and 5) and average daily gain (ADG) was calculated. Batches 1 and 2 could not be included in the day 14 measurements because of limited pen numbers. These batches were returned to the herd after day 7. Average daily feed intake (ADFI) was determined by measuring feed residuals simultaneously with body weight (BW). Minimal wastage was observed due to twice daily checking of the feeders by staff to ensure the pan was not too full. Therefore, disappeared creep feed was considered eaten.

To subjectively estimate level of aggression, injury in the form of scratches or redness on the head, ears and flank was scored individually 4 days before weaning and 2 days after weaning using a four point scale adapted from Widowski et al. (2003) (Table 1).

3.1.4 Behavioural measurements

On day -9 of the experiment, five piglets from 16 randomly selected litters from the last three batches of the experiment were marked with stocker marker spray to allow for individual identification from a distance. A total of 80 piglets (20 piglets per treatment group) were selected. Instantaneous scan sampling by one observer was then used to record the main activity of the individual piglet on days -8, -7, -1, 1 and 7. All focus piglets were observed every 30 minutes for two, 2-hour periods (morning 0900 and afternoon 1400) thus providing 8 observations per piglet per measurement day. The different types of behaviours recorded during the sampling were adapted from behaviour categories previously described by Pluske and Williams (1996) and Bolhuis et al. (2005) and are presented in Table 2.

3.1.5 Blood analysis

At 1100 on days -7, -1 and 0 (2 hours after weaning), a blood sample was collected from 2 piglets per litter and, on day 3 after weaning, a blood sample was collected from 1 pig per pen. Samples were taken from piglets that had not been subjected to the sugar absorption test and the same piglet was not bled more than 2 times within a 7-day period. The procedure lasted no more than 90 seconds and involved holding the piglet in dorsal recumbancy while collecting 6 ml of blood into both a lithium heparin-coated and EDTA-coated tubes. The EDTA samples were kept on ice until they were delivered to the Murdoch University laboratory for further processing on the same day. The lithium heparin samples were centrifuged on the day of collection (20 min at 2800 rpm at 4° C) and then 1ml of plasma aliquots were stored at -80° C.

The EDTA whole blood samples obtained were used to determine white blood cell differential. These counts were measured manually at Murdoch University (Murdoch, Western Australia) and Vetpath Laboratories (Belmont, Western Australia). Blood smears were made and then a differential white blood cell count was achieved by counting 100 cells per slide. Only the neutrophil: lymphocyte ratios (N: L ratios) are presented.

The lithium heparin plasma samples were used to determine plasma cortisol and haptoglobin (Hp) concentrations. Plasma cortisol levels were determined using a commercial ELISA test kit. Samples were analysed in duplicate in accordance with the manufacturers' instructions with the exception of optical density, which was read at 415 nm instead of the recommended 405 nm. Plasma was also analysed at the Animal Health Laboratories (Department of Agriculture and Food Western Australia) for the determination of Hp using an enzymatic colorimetric in-house assay based on modified methods of Eckersall et al. (1999).

3.1.6 Sugar absorption tests

Three days before weaning (day -3) and 3 days after weaning one piglet per litter was subjected to a sugar absorption test. The sugar absorption test was longitudinal in that the same piglets were used before and after weaning. Selected piglets were fasted for 3 hours by separation from the sow (before weaning only) and removal of solid feed. Water was permitted. Thereafter, an oral dose of 2.5 ml/kg sugar solution containing 20% mannitol ($\geq 98\%$; Sigma Aldrich, St Louis, MO, USA) and 20% galactose ($\geq 99\%$; Sigma Aldrich, St Louis, MO, USA) dissolved in Baxter sterile water (Baxter Healthcare Pty Ltd, Old Toongabbie, NSW, Australia) was administered via an orogastric tube. Twenty minutes after administration of the sugar solution, a blood sample was collected into a lithium heparin tube via jugular venepuncture. Lithium heparin tubes were stored on ice until plasma was collected from each tube after centrifugation (20 minutes, 2800 rpm at 4° C) and stored as 1 ml aliquots at -80° C. The lithium heparin plasma samples obtained 20 minutes after administration of the sugar solutions were then used to determine plasma mannitol and galactose concentrations as markers for GIT absorption. Commercial kits (Abcam, ab 155890 D-Mannitol colorimetric assay kit; ab83382 Galactose assay kit, Waterloo, NSW, Australia) were used for both sugars in accordance with the manufacturers' instructions. A one in three dilution was used for all samples for both sugars.

3.1.7 Statistical analysis

Data were analysed with SPSS (IBM Corp, Version 21, Armonk, NY, USA). Variables were averaged per litter (before weaning) or pen (after weaning), with the exception of sugar absorption test and behavioural observation data, which were analysed using the individual piglet as the experimental unit. We checked data for normality and square root transformed

if needed. Data are presented as raw means \pm SE, unless otherwise stated, due to the uneven number of observations per treatment. Interpretation of batch variation was not reliable due to the small numbers of litters per treatment per batch, and therefore batch was not included in any of the statistical models. Statistical significance was accepted at $P \leq 0.05$ and a trend was considered at $P \leq 0.1$ and $P > 0.05$. All post-hoc analyses included a Bonferroni correction and correlations were performed using a Pearson correlation test.

A GLM procedure to examine differences within main effects (IS and CoM) and interactions between main effects (IS x CoM) was used for the following variables: BW, ADG, ADFI, FCR, blood parameters and injury scores, using the following model:

$Y = \mu + \text{CoM} + \text{IS} + \text{CoM} \times \text{IS} + e$, where μ is overall mean and e is the residual error, with square root transformation of data on ADFI and all blood parameters.

Samples for blood parameters were not taken from the same piglets on each of the sampling days; therefore, data were pooled to examine the effect of time over the experimental period.

A two way mixed ANOVA was used to examine the effect of time and the interaction of time with the main effects (CoM and IS) for sugar absorption test data using the following model: $Y = \text{CoM} + \text{IS} + \text{CoM} \times \text{IS} + t + \text{CoM} \times t + \text{IS} \times t + \text{CoM} \times \text{IS} \times t + e$, where t is time and e is residual error.

All behaviours before and after weaning were abnormally distributed and transformation of the data failed to force normality. As a result, the proportion of total observations piglets spent on a specific behaviour was compared within main effects (CoM and IS) using a Kruskal-Wallis test. Interactions between the main effects for behavioural observations could not be performed due to the distribution of the data. Non-parametric Friedman (before weaning) and Wilcoxon (after weaning) tests were used to examine the effect of time within each of the main effects. If the Friedman test detected an overall time effect, data were subsequently tested pairwise. Since post-weaning data only included two repeated values, subsequent pairwise testing was not required.

Since no interactions between CoM and IS were detected (with the exception of mannitol absorption), all tables and figures present results within the main effects only unless otherwise stated.

Outcomes

3.2.1 Piglet performance

Before any treatment intervention, piglet BW was similar within the two main effects. Unexpectedly, before any treatment intervention (days -13 to -7), piglets selected for exposure to IS grew slower than piglets that were to remain continuously with their sow ($P < 0.01$, Table 9). This difference was still apparent throughout the entire IS treatment intervention (days -7 to -4, $P < 0.001$ and days -4 to 0, $P < 0.01$) (Table 9). Co-mingling with non-littermates for 8 hours per day did not influence piglet BW or ADG before weaning. Piglets not exposed to IS during the last week of lactation tended to be 0.5 kg heavier than piglets that were exposed to IS at the time of weaning. There was a marked reduction in growth in the immediate post-weaning period (day 0 to 2) for all pigs, but there were no differences within the main effects ($P > 0.05$, Table 9). Between 2 and 7 days after weaning, pigs exposed to IS during lactation had a higher growth rate than pigs not exposed to IS ($P = 0.01$), however, NoIS pigs still maintained a higher ($P < 0.01$) BW on day 2 after weaning

and tended ($P < 0.1$) to have a higher BW on day 7 after weaning (Table 9) compared with IS pigs. Subjecting pigs to CoM before lactation only had an effect on growth performance during the second week after weaning, with NoCoM pigs growing faster than CoM pigs. This resulted in CoM pigs weighing on average 0.8 kg less than NoCoM pigs at the end of the experiment (day 14). Intermittent suckling had no effect on growth or BW during the second week after weaning.

Creep feed intake before the onset of treatment intervention (between days -13 and -7) was negligible ($P < 0.05$, Table 9). Intermittent suckling during the last week of lactation improved creep feed intake by 63% between days -7 and -4 and 42% between days -4 to 0 ($P < 0.01$, Table 9). Co-mingling of non-littermates for 8 hours per day during the last week of lactation had no effect on creep feed intake ($P > 0.05$, Table 9). Weaning markedly increased solid feed intake in all groups, but exposure to IS and CoM before weaning did not improve solid feed intake within the first 2 days after weaning ($P > 0.05$, Table 9). Between days 2 to 7 after weaning, pigs that were exposed to IS during lactation tended to have a higher voluntary solid feed intake ($P < 0.1$, Table 9), but there was no effect for CoM ($P > 0.05$). In contrast, pigs that were not exposed to CoM during lactation had an improved solid feed intake between 7 and 14 days after weaning, but IS had no effect ($P < 0.01$, Table 9). Co-mingling of non-littermates or IS during lactation did not improve food conversion efficiency during the 14 days after weaning ($P > 0.05$), and no interactions between main effects were observed for any of the production parameters ($P > 0.05$).

Table 9. Effects of co-mingling and intermittent suckling on piglet performance before and after weaning.

Item	CoM ¹		IS ²	
	CoM	NoCoM	IS	NoIS
BW, kg				
Day ³ -13	2.9 ± 0.12	3.0 ± 0.13	2.9 ± 0.12	3.0 ± 0.13
Day -7	4.5 ± 0.15	4.6 ± 0.15	4.5 ± 0.14	4.7 ± 0.16
Day -4	5.2 ± 0.16	5.4 ± 0.17	5.1 ± 0.16	5.5 ± 0.17
Day 0	6.2 ± 0.19	6.4 ± 0.19	6.1 ^x ± 0.18	6.6 ^y ± 0.20
Day 2	6.4 ± 0.15	6.5 ± 0.14	6.2 ^a ± 0.14	6.7 ^b ± 0.15
Day 7	7.1 ± 0.16	7.2 ± 0.16	7.0 ^x ± 0.16	7.3 ^y ± 0.16
Day 14 ⁵	8.5 ^a ± 0.29	9.3 ^b ± 0.24	8.6 ± 0.30	9.2 ± 0.24
ADG, g/piglet				
Day -13 to -7	262 ± 7.1	277 ± 7.3	258 ^a ± 6.9	281 ^b ± 7.5
Day -7 to -4	246 ± 9.6	248 ± 9.8	220 ^a ± 9.3	275 ^b ± 10.1
Day -4 to 0	246 ± 10.4	263 ± 10.6	232 ^b ± 10.0	277 ^a ± 10.9
Day 0 to 2	6 ± 15.4	21 ± 14.9	25 ± 14.9	2 ± 15.4
Day 2 to 7	148 ± 7.3	132 ± 7.1	154 ^a ± 7.1	127 ^b ± 7.3
Day 7 to 14 ⁵	263 ^a ± 13.1	300 ^b ± 11.1	289 ± 13.4	274 ± 10.7
ADFI⁴, g/day				
Day -13 to -7	1.7 (0.94-2.76)	1.2 (0.70-1.78)	1.6 (0.95-2.41)	1.3 (0.68-2.10)
Day -7 to -4	3.0 (2.00-4.31)	2.5 (1.83-3.36)	4.3 ^a (3.20-5.49)	1.6 ^b (0.95-2.47)
Day -4 to 0	5.7 (3.89-7.88)	5.2 (3.93-6.68)	7.2 ^a (5.48-9.25)	3.9 ^b (2.57-5.57)
Day 0 to 2	94 (68.6-124.0)	101 (75.5-131.1)	84 (60.6-111.2)	113 (84.5-145.1)
Day 2 to 7	149 (122.7-177.9)	142 (116.9-169.0)	165 ^x (137.7-193.8)	127 ^y (103.2-154.1)
Day 7 to 14 ⁵	303 ^a (272.9-335.1)	349 ^b (321.3-377.6)	341 (307.7-375.1)	311 (285.9-337.3)
Food: gain ratio, g food per g live-weight gain⁶				
Day 2 to 7	1.1 ± 0.08	1.1 ± 0.08	1.1 ± 0.08	1.1 ± 0.08
Day 7 to 14 ⁵	1.2 ± 0.03	1.2 ± 0.03	1.2 ± 0.03	1.1 ± 0.02

^{a,b} Within main effects, values not followed by a common superscript differ ($P < 0.05$).

^{x,y} Within main effects, values not followed by a common superscript are a trend ($P < 0.1$).

¹ CoM: co-mingling with another litter for 8 hours per day during the last week of lactation, NoCoM: no co-mingling during the last week of lactation.

² IS: intermittent suckling involving separation from the sow for 8 hours per day during the last week of lactation, NoIS: no intermittent suckling.

³ Day in relation to weaning with weaning representing 0 (e.g. day -13 is 13 days before weaning).

⁴ Data were subjected to square root transformation before GLM analysis. Values were then back transformed and expressed as least square means with 95% confidence intervals (in parentheses).

⁵ Batches 3,4 and 5 only. ⁶ Day 0 to 2 FCR not included due to negative growth.

3.2.2 Behavioural measurements

Behavioural observations recorded on day -8 were taken as a baseline (i.e. before any IS or CoM intervention). On day -8 the proportion of total observations piglets spent on lying/sitting, standing, aggression, manipulation and ingestive-related behaviours was

similar for all piglets ($P > 0.05$, Table 10.1). However, unexpectedly, piglets selected for exposure to the main effect of IS spent a greater proportion of total observations sleeping ($P < 0.01$) and a smaller proportion of total observations exploring/playing ($P < 0.001$) compared with piglets that were selected to remain with their sow (Table 10.1). Furthermore, sow directed behaviour was greater in piglets subjected to co-mingling than piglets that remained with their littermates ($P = 0.001$, Table 10.1). Sow directed behaviour was not subjected to statistical analysis after day -8 since both main effects included piglets that were separated from their sow during the behaviour observation times.

3.2.2.1 Pre-weaning behaviour

Effects of CoM on pre-weaning observations

Co-mingling caused an increase ($P < 0.001$) in the proportion of total observations piglets spent exploring/playing between days -8 to -7, resulting in a higher level of exploring/playing than piglets that were not mixed with another litter on day -7 ($P < 0.05$, Table 10.1). This difference disappeared by day -1 ($P > 0.05$) with exploring and play behaviour decreasing ($P < 0.001$) and sleeping behaviour increasing ($P < 0.01$) between days -7 to -1 for CoM piglets (Table 10.1). Co-mingling also influenced ingestive-related behaviours over time, with a greater proportion of total observations for eating/drinking/eliminating on day -1 compared with baseline, day -8 ($P < 0.05$). Co-mingling did not influence the expression of lying/sitting, standing, aggressive or manipulative behaviours ($P > 0.05$, Table 10.1).

Effect of IS on pre-weaning observations

Between days -8 to -7, IS caused a decrease ($P = 0.001$) in sleeping behaviour and an increase in standing ($P < 0.05$), exploring/playing ($P < 0.001$) and eating/drinking/eliminating ($P < 0.05$) behaviours (Table 10.1). On the first day of intervention (day -7), piglets exposed to IS spent a greater proportion of total observations on standing ($P < 0.001$) and exploring/playing ($P < 0.001$) behaviours than NoIS piglets. In contrast, NoIS piglets spent a greater proportion of total observations sleeping ($P < 0.01$, Table 10.1) on day -7. Between days -7 and -1, exploring/playing decreased ($P < 0.001$) and sleeping increased ($P < 0.001$) for IS piglets, which resulted in higher sleeping levels than piglets not exposed to IS ($P < 0.01$) on the last day of intervention (day -1). Intermittent suckling did not influence lying/sitting, aggressive or manipulative behaviours during the week before weaning ($P > 0.05$).

3.2.2.2 Post-weaning behaviour

Effects of CoM on post-weaning behaviour

Co-mingling piglets with another litter for 8 hours per day 1 week before weaning, increased sleeping behaviour and reduced standing on the first day after weaning ($P < 0.001$ and $P < 0.05$ respectively, Table 10.2). Co-mingling also reduced exploring/play behaviour 7 days after weaning ($P < 0.05$, Table 10.2). Co-mingling did not influence inactive behaviours such as lying and sitting, aggression, manipulation or eating/drinking/eliminating on either day 1 or 7 after weaning ($P > 0.05$), and the expression of behaviours over time was not influenced by CoM ($P > 0.05$). Pigs that were not exposed to CoM before weaning (NoCoM) exhibited an increase ($P = 0.001$) in proportion of total observations for sleep and a decrease ($P = 0.001$) in proportion of total observations for standing behaviour over time (days 1 to 7) (Table 10.2).

Effects of IS on post-weaning behaviour

On the first day after weaning, pigs that spent time away from their sow during the last week of lactation (IS) slept more ($P < 0.001$) and lay/sat less ($P < 0.05$) than pigs that remained with their sow during lactation (NoIS) (Table 10.2). Over time (days 1 to 7), sleeping increased ($P < 0.01$) while standing decreased ($P < 0.05$) for both IS and NoIS pigs. Feed-directed behaviour as well as lying/sitting also increased for IS pigs over the same timeframe ($P < 0.05$ and $P < 0.01$ respectively). Seven days after weaning, the proportion of total observations spent on sleeping remained higher for IS pigs than NoIS pigs ($P < 0.001$), but manipulative behaviour was higher in NoIS pigs ($P < 0.05$, Table 10.2). Intermittent suckling did not influence aggressive or exploratory/play behaviour on days 1 and 7 after weaning ($P > 0.05$, Table 10.2).

Table 10.1 Effects of co-mingling and intermittent suckling on the proportion of total behavioural observations displayed by piglets before weaning.

	CoM ¹		IS ²		SEM
	CoM	NoCoM	IS	NoIS	
Day³ -8					
Sleep	0.222 ^x	0.372	0.372 ^{ax}	0.222 ^b	0.046
Lie/sit	0.106	0.163	0.169	0.100	0.026
Standing	0.116	0.103	0.097 ^x	0.122	0.023
Aggressive	0.000	0.000	0.000	0.000	0.000
Explore/play	0.100 ^x	0.072	0.031 ^{ax}	0.141 ^b	0.017
Manipulative	0.050	0.047	0.053	0.044	0.013
Ingestive-related	0.025 ^x	0.016	0.016 ^x	0.025	0.011
Sow	0.381 ^a	0.228 ^b	0.263	0.347	0.033
Day -7					
Sleep	0.209 ^{ax}	0.347 ^b	0.200 ^{ay}	0.356 ^b	0.030
Lie/sit	0.094	0.072	0.078	0.088	0.021
Standing	0.109	0.119	0.169 ^{ay}	0.059 ^b	0.018
Aggressive	0.003	0.003	0.003	0.003	0.003
Explore/play	0.341 ^{ay}	0.244 ^b	0.450 ^{ay}	0.134 ^b	0.019
Manipulative	0.047	0.028	0.050	0.025	0.012
Ingestive-related	0.047 ^{xy}	0.038	0.050 ^y	0.034	0.010
Sow	0.150	0.150	-	0.3	0.017
Day -1					
Sleep	0.359 ^y	0.397	0.453 ^{ax}	0.303 ^b	0.037
Lie/sit	0.119	0.106	0.119	0.106	0.022
Standing	0.109	0.119	0.175 ^{ay}	0.053 ^b	0.022
Aggressive	0.006	0.000	0.006	0.000	0.003
Explore/play	0.134 ^x	0.153	0.153 ^z	0.134	0.025
Manipulative	0.034	0.025	0.031	0.028	0.009
Ingestive-related	0.050 ^y	0.038	0.056	0.031	0.012
Sow	0.172	0.163	-	0.33	0.018

^{a,b} Within main effects, values not followed by a common superscript within a row differ ($P < 0.05$).

^{x,y} Within each column within each behaviour, values not sharing a common superscript differ across time ($P < 0.05$).

¹ CoM: co-mingling with another litter for 8 hours per day during the last week of lactation, NoCoM: no co-mingling during the last week of lactation.

² IS: intermittent suckling involving separation from the sow for 8 hours per day during the last week of lactation, NoIS: no intermittent suckling.

³ Day in relation to weaning with weaning representing 0 (e.g. day -8 is 8 days before weaning).

Table 10.2 Effects of co-mingling and intermittent suckling on the proportion of total behavioural observations displayed by pigs after weaning.

	CoM ¹		IS ²		SEM
	CoM	NoCoM	IS	NoIS	
Day³ 1					
Sleep	0.353 ^a	0.147 ^{bx}	0.331 ^{ax}	0.169 ^{bx}	0.023
Lie/sit	0.231	0.259	0.147 ^{ax}	0.344 ^b	0.031
Standing	0.134 ^a	0.216 ^{bx}	0.172 ^x	0.178 ^x	0.022
Aggressive	0.009	0.009	0.009	0.009	0.006
Explore/play	0.084	0.147	0.100	0.131	0.022
Manipulative	0.081	0.084	0.088	0.078	0.016
Ingestive-related	0.106	0.138	0.153 ^x	0.091	0.021
Day 7					
Sleep	0.378	0.309 ^y	0.422 ^{ay}	0.266 ^{by}	0.026
Lie/sit	0.284	0.263	0.256 ^y	0.291	0.025
Standing	0.100	0.122 ^y	0.100 ^y	0.122 ^y	0.019
Aggressive	0.000	0.006	0.006	0.000	0.003
Explore/play	0.041 ^a	0.088 ^b	0.056	0.072	0.016
Manipulative	0.094	0.094	0.066 ^a	0.122 ^b	0.018
Ingestive-related	0.097	0.119	0.088 ^y	0.128	0.022

^{a,b} Within main effects, values not followed by a common superscript within a row differ ($P < 0.05$).

^{x,y} Within each column within each behaviour, values not sharing a common superscript differ across time ($P < 0.05$).

¹ CoM: co-mingling with another litter for 8 hours per day during the last week of lactation, NoCoM: no co-mingling during the last week of lactation.

² IS: intermittent suckling involving separation from the sow for 8 hours per day during the last week of lactation, NoIS: no intermittent suckling.

³ Day in relation to weaning with weaning representing 0 (e.g. day 1 is 1 day after weaning).

3.2.3 Injury scores

When measured 4 days before weaning (3 days after the start of treatment intervention), higher mean scratch scores were evident when piglets were subjected to CoM ($P < 0.001$, Table 11). In contrast, by 2 days after weaning, there was a trend ($P < 0.1$) for pigs co-mingled during lactation to have a lower scratch score than pigs that were only socialised with their littermates during lactation (Table 11). Intermittent suckling had no influence mean scratch scores before and after weaning ($P > 0.05$) and neither CoM nor IS influenced redness scores ($P > 0.05$).

3.2.4 Blood analyses

Neither CoM or IS influenced the plasma concentrations of Hp throughout the experiment ($P > 0.05$, Table 12). On the day of weaning, there was a strong tendency ($P < 0.1$) for IS pigs to have a lower plasma cortisol concentration than NoIS pigs and a strong tendency ($P < 0.1$) for pigs exposed to CoM to have a higher N:L ratio than pigs not exposed to CoM during lactation (Table 12). There was no interaction between the two main effects for the concentrations of any of the blood parameters.

Table 11. Effects of co-mingling and intermittent suckling on mean scratch scores and redness at 4 days before and 2 days after weaning.

Item	CoM ¹		IS ²	
	CoM	NoCoM	IS	NoIS
Scratch				
Day ³ -4	0.81 ^a ± 0.04	0.57 ^b ± 0.04	0.73 ± 0.04	0.65 ± 0.04
Day 2	0.84 ^x ± 0.06	1.00 ^y ± 0.06	0.88 ± 0.06	0.96 ± 0.07
Redness				
Day -4	0.69 ± 0.06	0.67 ± 0.06	0.62 ± 0.06	0.74 ± 0.06
Day 2	0.68 ± 0.07	0.83 ± 0.07	0.75 ± 0.06	0.77 ± 0.07

^{a,b} Within main effects, values not followed by a common superscript differ ($P < 0.05$).

^{x,y} Within main effects, values not followed by a common superscript are a trend ($P < 0.1$).

¹ CoM: co-mingling with another litter for 8 hours per day during the last week of lactation, NoCoM: no co-mingling during the last week of lactation.

² IS: intermittent suckling involving separation from the sow for 8 hours per day during the last week of lactation, NoIS: no intermittent suckling.

³ Day in relation to weaning with weaning representing 0 (e.g. day -4 is 4 days before weaning).

Data were pooled to examine the effect of time. Plasma cortisol did not change between days -7 and -1, however there was an increase ($P < 0.001$) between day -1 and day 0 (2 hours after weaning) with values remaining similar between days 0 and 3. Plasma Hp showed a similar pattern with increasing concentrations from day -7 to day 3 ($P < 0.001$). Neutrophil: lymphocyte ratios were highest on the day of weaning and returned to pre-weaning values on day 3 after weaning ($P < 0.001$).

Growth before and after weaning was negatively correlated to plasma Hp concentrations ($r = 0.28$, $P < 0.001$).

3.2.5 Sugar absorption tests

Three days after weaning, pigs that were exposed to CoM without IS (CoM NoIS) showed the same level of mannitol concentration as 3 days before weaning (day -3), whereas the other animals experienced a decrease in mannitol concentration between days -3 and 3 (Figure 2). Hence, a CoM x IS x day effect was found (Table 13). Furthermore, an interaction ($P < 0.01$) occurred for CoM and IS 3 days after weaning. Mean plasma mannitol concentrations were higher ($P < 0.01$) in CoM NoIS pigs compared with NoCoM NoIS and CoM IS pigs.

The concentration of galactose changed ($P < 0.01$) with time and this effect did not depend on IS or CoM (Table 13). Three days before weaning, piglets exposed to IS had a lower plasma galactose concentration than piglets that remained continuously with their sow (Figure 2, $P < 0.05$). This effect continued as trend ($P < 0.1$) 3 days after weaning (Figure 2).

No relationships between growth and the plasma concentration of mannitol and galactose 20 minutes after oral application were identified.

Table 12. Effects of co-mingling and intermittent suckling on plasma cortisol (ng/ml), N:L ratios and plasma Hp (mg/ml) concentrations before and after weaning in piglets.

Item	CoM ¹		IS ²	
	CoM	NoCoM	IS	NoIS
Cortisol ³ , ng/ml				
Day ⁴ -7	13.0 (10.19-16.26)	11.5 (8.84-14.55)	13.7 (10.84-16.84)	10.9 (8.25-14.00)
Day -1	13.0 (10.54-15.77)	13.1 (10.59-15.91)	12.9 (10.40-15.69)	13.2 (10.72-15.99)
Day 0	19.8 (15.82-24.26)	19.8 (15.62-24.38)	16.9 ^x (13.26-21.06)	22.8 ^y (18.39-27.80)
Day 3	20.8 (16.47-25.69)	20.8 (16.43-25.68)	21.2 (16.92-25.92)	20.4 (15.98-25.45)
Haptoglobin ³ , mg/ml				
Day -7	0.17 (0.12-0.21)	0.16 (0.12-0.20)	0.18 (0.13-0.22)	0.15 (0.11-0.19)
Day 0	0.47 (0.30-0.69)	0.35 (0.21-0.52)	0.38 (0.23-0.57)	0.44 (0.28-0.64)
Day 3	0.66 (0.48-0.86)	0.60 (0.43-0.80)	0.6 (0.43-0.78)	0.70 (0.48-0.88)
N:L ratio ³				
Day -7	1.7 (1.24-2.16)	1.7 (1.30-2.20)	1.8 (1.33-2.30)	1.6 (1.21-2.06)
Day -1	1.0 (0.60-1.44)	1.2 (0.73-1.67)	1.3 (0.84-1.77)	0.9 (0.51-1.35)
Day 0	4.0 ^x (2.48-5.77)	2.2 ^y (1.22-3.47)	2.4 (1.32-3.71)	3.7 (2.34-5.48)
Day 3	1.0 (0.52-1.68)	1.6 (1.00-2.44)	1.7 (1.09-2.47)	1.0 (0.47-1.65)

^{x,y} Within main effects, values not followed by a common superscript are a trend ($P < 0.1$).

¹ CoM: co-mingling with another litter for 8 hours per day during the last week of lactation, NoCoM: no co-mingling during the last week of lactation.

² IS: intermittent suckling involving separation from the sow for 8 hours per day during the last week of lactation, NoIS: no intermittent suckling.

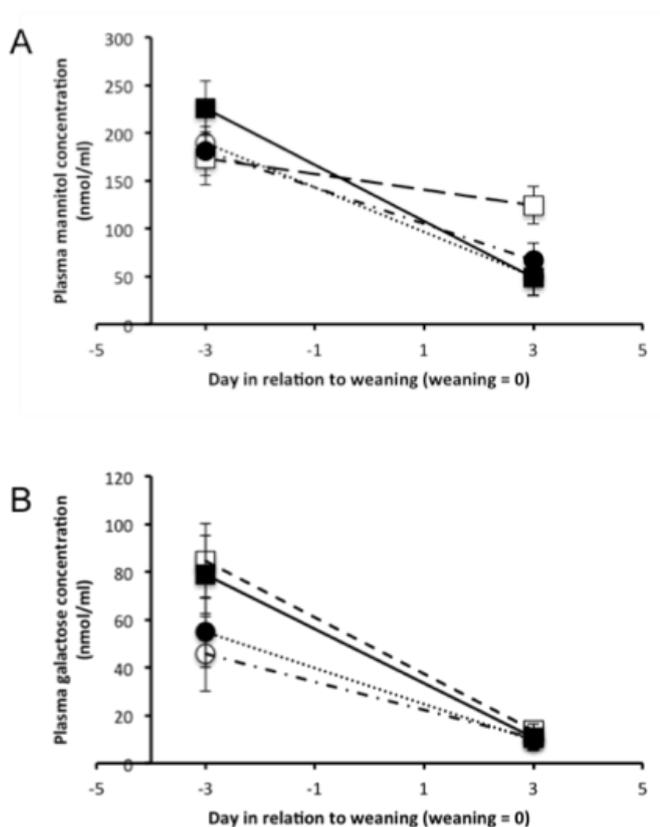
³ Data were subjected to square root transformation before GLM analysis. Values were then back transformed and expressed as least square means with 95% confidence intervals (in parentheses).

⁴ Day in relation to weaning with 0 representing weaning (e.g. day -7 is 7 days before weaning).

Table 13. Effect of time and interactions with co-mingling (CoM or NoCoM) and intermittent suckling (IS or NoIS) on plasma mannitol and galactose concentration 20 minutes after oral administration 3 days before and 3 days after weaning

	Mannitol Significance	Galactose Significance
Time	0.001	0.001
CoM x time	0.057	0.593
IS x time	0.478	0.195
CoM x IS x time	0.015	0.855

Figure 2. Plasma mannitol (A) and plasma galactose (B) concentrations (nmol/ml) of pigs before and after weaning, CoM IS, $n=16$ (■), CoM NoIS: $n=14$ (□), NoCoM IS $n=16$ (●), NoCoM NoIS: $n=13$ (○). Data are raw means \pm SE



4 Conclusion

Results show that an IS regime (without CoM) involving an 8 h/d separation for 7 d before weaning improved ADG and ADFI in the immediate post-weaning period compared with conventional weaning. However, this improvement did not seem to occur through increased familiarisation with creep feed, but rather through the

prevention or attenuation of the weaning-associated stress response as evidenced by increased sleeping behaviour and reduced manipulative behaviour immediately after weaning as well as reduced post-weaning Hp levels. Moreover, IS reduces plasma cortisol concentration on the day of weaning, possibly reflecting 1) the advancement of adaptive GIT changes during lactation, as supported by a reduction in galactose absorption before weaning or 2) less stress at the time of weaning as supported by more observations of positive behaviour such as sleeping.

The addition of CoM to the IS regime also improved post-weaning performance in multiparous litters, most likely due to social learning facilitating more eating before weaning and reduced aggression after weaning also reducing stress, but did not have an effect on performance with or without IS in primiparous litters. There was strong evidence from both experiments that CoM does reduce aggression, but this did not seem to impact on selected measures of stress, inflammation or immune status. CoM also improved mannitol absorptive capacity after weaning. Overall, these results suggest that mimicking certain aspects of weaning under natural conditions, such as gradual maternal separation and the opportunity to mix with non-litter mates in lactation, can positively affect post-weaning performance and highlights opportunities for potential housing systems that enhance piglet welfare whilst also potentially facilitating mating in lactation.

5 Application of the Research

The current project aimed to mimic the natural weaning process as much as possible under commercial indoor conditions by introducing gradual weaning (IS) and also allowing a subsample of piglets to socialise with non-littermates before weaning (CoM). Two different methods of separation were used in this project. The first involved moving litters to empty farrowing crates in a separate room while the second involved moving litters to an empty crate next-door to their own sow's crate, which allowed for auditory and visual contact with their sow. Both techniques required a significant amount of labour, however the second method was much quicker and resulted in similar outcomes. Co-mingling (without IS) was achieved by removing the barrier at the back of two farrowing crates allowing piglets to move between two sows and interact with piglets from another litter. This CoM method was easy to implement and required minimal labour. However, from a performance perspective, it seems that IS was the most effective pre-weaning intervention at improving performance in the first week after weaning. This improvement however, was not necessarily mediated by an increase in creep feed intake, but possibly through piglets feeling more habituated with the weaning process as demonstrated by more observations of relaxed or positive behaviours such as sleeping and less observations of negative behaviours such as manipulation. Co-mingling on the other hand did improve pre and post-weaning performance in multiparous litters when it was combined with IS, but did not have an effect on performance with or without IS in primiparous litters. There was strong evidence from both experiments that CoM does reduce aggression, but this did not seem to impact on selected measures of stress, inflammation or immune status.

The results of this project also identified the pronounced buffering effect the presence of familiar pigs can have on social support as evidenced by the improvement in post-weaning feed conversion ratio and growth and possibly if piglets subjected to CoM without IS had remained in familiar groups after weaning

in the second experiment of this project, the performance outcome may have been different. Furthermore, it is possible that greater benefits could have been achieved if CoM was used as a continuous treatment rather than intermittently (i.e. 8 hours per day).

The current project also further demonstrated the use of mannitol and galactose as single marker probes, which have been shown to be effective measures of gastrointestinal tract (GIT) absorptive function (project 1B-104). While markers of GIT structure and function are not necessarily relevant to producers, other research projects could consider the use of sugar absorption tests as welfare biomarkers, reducing the necessity for terminal experiments. Results demonstrated that IS potentially advances the adaptive GIT changes during lactation whereas CoM improved mannitol absorptive capacity after weaning suggesting a greater surface area available for the absorption of nutrients. However, the exact mechanism by which CoM influences the GIT adaptive response over weaning was not immediately obvious from the results of the second experiment.

6 Limitations/Risks

From the point of view of IS, although there were improvements in performance and welfare, these were only in the short term (i.e. the first week after weaning) and given the impracticality of physically separating the piglets from the sow each day, the commercial application of such a regime is probably limited. Further work on housing design to overcome the increased need for labour is needed.

Co-mingling would be a much easier pre-weaning intervention to implement on farm, however, this project only examined “intermittent co-mingling” (i.e. socialisation with another litter for 8 hours per day), which may have disrupted the formation of hierarchies in groups before weaning, influencing pre-weaning performance and ultimately post-weaning results.

7 Recommendations

The results from the current project suggest that while IS may improve pig welfare and performance in the immediate post-weaning period, labour associated with moving the piglets was significant. A logical progression from the work completed in this project would be to explore a group lactation system where piglets (or sows) could be separated on a daily basis as a group to mimic the gradual nature of weaning under natural conditions. Due to the reduction of the suckling stimulus, sows may begin to cycle allowing them to be mated while still nursing their piglets. This system would allow for pre-weaning socialisation, an older weaning age and gradual weaning without the loss of sow reproductive efficiency.

Alternatively, since CoM is relatively easy to implement on farms (i.e. the removal of barriers between farrowing crates rather than major changes to infrastructure), further studies examining optimal group size and methods to keep the group together after weaning could also be explored.

8 References

- Berkeveld, M. et al. 2009. Improving adaptation to weaning: Effect of intermittent suckling regimens on piglet feed intake, growth, and gut characteristics. *Journal of Animal Science* 87: 3156-3166.
- Bolhuis, J. E., W. G. P. Schouten, J. W. Schrama, and V. M. Wiegant. 2005. Behavioural development of pigs with different coping characteristics in barren and substrate-enriched housing conditions. *Applied Animal Behaviour Science* 93: 213-228.
- Brooks, P., and C. Tsourgiannis. 2003. Factors affecting the voluntary feed intake of the weaned pig. *Weaning the Pig, Concepts and Consequences*. Wageningen, The Netherlands: Wageningen Academic Publishers: 81-116.
- D'Eath, R. B. 2005. Socialising piglets before weaning improves social hierarchy formation when pigs are mixed post-weaning. *Applied Animal Behaviour Science* 93: 199-211.
- Dybkjær, L. 1992. The identification of behavioural indicators of 'stress' in early weaned piglets. *Applied Animal Behaviour Science* 35: 135-147.
- Eckersall, P. et al. 1999. An automated biochemical assay for haptoglobin: prevention of interference from albumin. *Comparative Haematology International* 9: 117-124.
- Friend, T., D. Knabe, and T. Tanskley. 1983. Behavior and performance of pigs grouped by three different methods at weaning. *Journal of Animal Science* 57: 1406-1411.
- Funderburke, D., and R. Seerley. 1990. The effects of postweaning stressors on pig weight change, blood, liver and digestive tract characteristics. *Journal of Animal Science* 68: 155-162.
- Jensen, P., and B. Recén. 1989. When to wean—Observations from free-ranging domestic pigs. *Applied Animal Behaviour Science* 23: 49-60.
- Kemp, B., and N. Soede. 2012. Should Weaning be the Start of the Reproductive Cycle in Hyper-prolific Sows? A Physiological View. *Reproduction in domestic animals* 47: 320-326.
- Kuller, W. et al. 2007. Effects of intermittent suckling and creep feed intake on pig performance from birth to slaughter. *Journal of Animal Science* 85: 1295-1301.
- Kuller, W. et al. 2004. Intermittent suckling: Effects on piglet and sow performance before and after weaning. *Journal of Animal Science* 82: 405-413.
- Kutzer, T., B. Bünger, J. B. Kjaer, and L. Schrader. 2009. Effects of early contact between non-littermate piglets and of the complexity of farrowing conditions on social behaviour and weight gain. *Applied Animal Behaviour Science* 121: 16-24.
- Leibbrandt, V. D., R. C. Ewan, V. C. Speer, and D. R. Zimmerman. 1975. Effect of Weaning and Age at Weaning on Baby Pig Performance. *Journal of Animal Science* 40: 1077-1080.
- Li, Y., and L. Wang. 2011. Effects of previous housing system on agonistic behaviors of growing pigs at mixing. *Applied Animal Behaviour Science* 132: 20-26.
- McCracken, B. A., H. R. Gaskins, P. J. Ruwe-Kaiser, K. C. Klasing, and D. E. Jewell. 1995. Diet-dependent and diet-independent metabolic responses underlie growth stasis of pigs at weaning. *Journal of Nutrition* 125: 2838-2845.
- McCracken, B. A., M. E. Spurlock, M. A. Roos, F. A. Zuckermann, and H. R. Gaskins. 1999. Weaning anorexia may contribute to local inflammation in the piglet small intestine. *The Journal of Nutrition* 129: 613-619.

- Moeser, A. J. et al. 2007. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 292: G173-G181.
- Morgan, T. et al. 2014. Socialising piglets in lactation positively affects their post-weaning behaviour. *Applied Animal Behaviour Science* 158: 23-33.
- Nabuurs, M., A. Hoogendoorn, and A. Van Zijderveld-Van Bommel. 1996. Effect of supplementary feeding during the sucking period on net absorption from the small intestine of weaned pigs. *Research in Veterinary Science* 61: 72-77.
- Pajor, E. A., D. Fraser, and D. L. Kramer. 1991. Consumption of solid food by suckling pigs: individual variation and relation to weight gain. *Applied Animal Behaviour Science* 32: 139-155.
- Pajor, E. A., D. M. Weary, D. Fraser, and D. L. Kramer. 1999. Alternative housing for sows and litters: 1. Effects of sow-controlled housing on responses to weaning. *Applied Animal Behaviour Science* 65: 105-121.
- Parratt, C. A. et al. 2006. The fighting behaviour of piglets mixed before and after weaning in the presence or absence of a sow. *Applied Animal Behaviour Science* 101: 54-67.
- Petersen, H., K. Vestergaard, and P. Jensen. 1989. Integration of piglets into social groups of free-ranging domestic pigs. *Applied Animal Behaviour Science* 23: 223-236.
- Pié, S. et al. 2004. Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. *The Journal of Nutrition* 134: 641-647.
- Pluske, J., and I. Williams. 1996. Reducing stress in piglets as a means of increasing production after weaning: administration of amperozide or co-mingling of piglets during lactation? *Animal Science* 62: 121-130.
- Pluske, J., I. Williams, and F. Aherne. 1996. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. *Animal Science* 62: 131-144.
- Pluske, J. R., D. J. Hampson, and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: A review. *Livestock Production Science* 51: 215-236.
- Puppe, B., M. Tuchscherer, and A. Tuchscherer. 1997. The effect of housing conditions and social environment immediately after weaning on the agonistic behaviour, neutrophil/lymphocyte ratio, and plasma glucose level in pigs. *Livestock Production Science* 48: 157-164.
- van Beers-Schreurs, H. M. et al. 1998. Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. *The Journal of Nutrition* 128: 947-953.
- van Nieuwamerongen, S., N. Soede, C. van der Peet-Schwering, B. Kemp, and J. Bolhuis. 2015. Development of piglets raised in a new multi-litter housing system vs. conventional single-litter housing until 9 weeks of age. *Journal of Animal Science* 93: 5442-5454.
- Verdon, M., R. S. Morrison, and P. H. Hemsworth. 2016. Rearing piglets in multi-litter group lactation systems: Effects on piglet aggression and injuries post-weaning. *Applied Animal Behaviour Science* 183: 35-41.
- Weary, D. M., J. Jasper, and M. J. Hötzel. 2008. Understanding weaning distress. *Applied Animal Behaviour Science* 110: 24-41.
- Weary, D. M., E. A. Pajor, M. Bonenfant, D. Fraser, and D. L. Kramer. 2002. Alternative housing for sows and litters.: Part 4. Effects of sow-controlled housing combined with a communal piglet area on pre-and post-weaning behaviour and performance. *Applied Animal Behaviour Science* 76: 279-290.

Widowski, T., T. Cottrell, C. Dewey, and R. Friendship. 2003. Observations of piglet-directed behavior patterns and skin lesions in eleven commercial swine herds. *Journal of Swine Health and Production* 11: 181-185.