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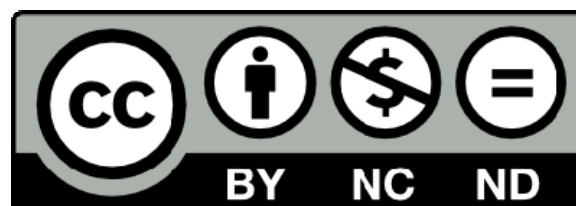
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**Turpin, D.L., Langendijk, P., Sharp, C. and Pluske, J.R. (2017) Improving welfare and production in the peri-weaning period: Effects of co-mingling and intermittent suckling on the stress response, performance, behaviour, and gastrointestinal tract carbohydrate absorption in young pigs. *Livestock Science*, 203. pp. 82-91.**

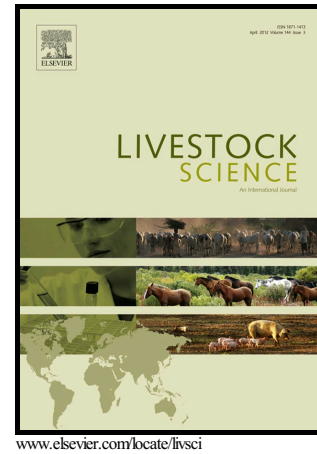
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PII: S1871-1413(17)30202-0  
DOI: <http://dx.doi.org/10.1016/j.livsci.2017.07.006>  
Reference: LIVSCI3257

To appear in: *Livestock Science*

Received date: 11 November 2016  
Revised date: 22 March 2017  
Accepted date: 10 July 2017

Cite this article as: D.L. Turpin, P. Langendijk, C. Sharp and J.R. Pluske  
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  
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# 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

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## Abstract

We investigated the effect of different pre-weaning interventions on performance, aspects of behaviour, and selected neuroendocrine, inflammatory and immune indices in 593 weanling pigs (59 litters, weaning age  $22 \pm 1.7$ ). Measurements were taken at various time points two weeks before and after weaning. Sugar absorption tests (20% mannitol and 20% galactose solutions?) 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). Measurements are compared within each of the main effects (CoM or IS) unless otherwise stated. Acute

weaning stress was evidenced by increases in cortisol, haptoglobin and N:L ratios when data were combined ( $P < 0.001$ ). However, there were tendencies ( $P < 0.1$ ) for lower cortisol in IS pigs and higher N:L ratios in CoM pigs at weaning. 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 ( $P < 0.1$ ) for a higher feed intake 2 to 7 days after weaning. Co-mingled piglets had more scratches 4 days before weaning ( $P < 0.001$ ), but tended to have fewer scratches 2 days after weaning ( $P < 0.1$ ). Pigs exposed to either IS or CoM displayed more sleeping behaviour the day after weaning ( $P < 0.01$  and  $P < 0.001$ ). 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. Overall, despite improvements in mannitol absorption and behaviour, there was no beneficial effect of CoM on performance after weaning. Alternatively, IS improved behaviour and performance during the first week after weaning.

**Keywords:** co-mingling, intermittent suckling, piglet, sugar absorption test, weaning

## Introduction

Weaning piglets involves abrupt social, environmental and dietary changes at a young age (three to four weeks) that often causes a low feed intake (McCracken et al., 1999), reduced growth (Weary et al., 2008) and predisposition to gastrointestinal tract (GIT) dysfunction and disease (Pluske et al., 1997). While it is well accepted that reduced solid

feed intake of weaned pigs is one of the major contributing factors to growth stasis at the time of weaning (Funderburke and Seerley, 1990; McCracken et al., 1995), the relative contribution mixing previously unfamiliar piglets at weaning has on behaviour and stress is also well recognised (Friend et al., 1983; Puppe et al., 1997; Parratt et al., 2006).

Allowing piglets from different litters to socialise (co-mingle, CoM) before weaning mimics natural conditions where sows and their litters live in social groups from the time the piglets are 10 days of age (Jensen and Recén, 1989). During this time, piglets come into contact with non-littermates and other sows and engage in non-aggressive and playful interactions (Jensen and Recén, 1989). Such interactions have been shown to have a positive influence later in life, particularly at the time of weaning, causing reduced aggression (van Nieuwamerongen et al., 2015; Verdon et al., 2016) and an increase in solid feed intake during the pre-weaning period (Weary et al., 2002). While studies that comprehensively examine piglets' behavioural, physiological, immunological and inflammatory responses to CoM systems do exist (Pluske and Williams, 1996; Morgan et al., 2014; van Nieuwamerongen et al., 2015), the influence weaning stress has on the up regulation of stress pathways and GIT dysfunction is now well recognised (Moeser et al., 2007b). Therefore, the potential benefit pre-weaning socialisation could have on GIT structure and function warrants further investigation.

In addition to CoM, gradual weaning regimes that familiarise piglets with maternal separation and encourage the exploration of nutrient sources other than milk have also been examined as an alternative approach to reducing stress and enhancing performance at weaning. Intermittent suckling (IS), a gradual weaning technique whereby a sow and her piglets are separated for a specified period of time each day during lactation,

stimulates pre-weaning creep feed intake, resulting in improved post-weaning solid feed intake and growth (Kuller et al., 2004; Kuller et al., 2007). However, while villous height and crypt depth can be maintained at the time of weaning if fasting can be avoided (Pluske et al., 1996a; van Beers-Schreurs et al., 1998), results from studies examining IS regimes and small intestinal morphology differ showing either an improvement in GIT morphology (Nabuurs et al., 1996) or no change at all (Berkeveld et al., 2009) compared with conventionally weaned controls.

Turpin et al. (2017) combined IS with CoM and showed an increase in eating behaviour and creep feed intake during the last week of lactation, as well as an improvement in growth and feed intake between 2 and 8 days after weaning, when compared with IS without CoM and conventional weaning. Pigs exposed to IS in combination with CoM also spent less time fighting than the controls over the immediate post-weaning period. However, since a control litter with CoM was not included in this study, the positive effects observed could not be fully attributed to the combination of IS and CoM.

Furthermore, measures of GIT morphology and function were not examined. The aim of the current study was 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 sugars indicating enhanced welfare and improved GIT morphology and function.

## Materials and methods

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).

### *Animals and housing*

A total of 59 primiparous sows (Large White x Landrace) and their offspring was 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 were identical in layout within the same farrowing building on the farm. Lights were on between 0700 and 1600.

Litter size was standardised ( $10.1 \pm 0.41$  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 weaning age was  $22 \pm 1.7$  days (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  $9.7 \pm 0.91$  pigs per pen (mean  $\pm$  SD). 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.

### *Experimental design*

Within the first week of lactation litters in each batch were assigned to one of four treatments. Treatments were arranged in a 2 x 2 factorial design, with the factors being (1) socialisation (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 separation, 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) NoCoM 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) NoCoM No IS ( $n = 13$  litters with 132 piglets), where piglets remained with their sow continuously until weaning. Due to the layout of the farrowing rooms, litters in the CoM NoIS treatment group could only be housed in the centre two (out of four) rows where a common corridor could be shared between two farrowing crates, therefore the CoM NoIS treatment group was not balanced between farrowing crate positions in the room. However, the other treatment groups were balanced between farrowing crate positions. 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  $0.42 \text{ m}^2$  and  $0.21 \text{ m}^2$  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. This arrangement meant that pigs in all treatment groups would be housed with some familiar and some unfamiliar pigs after weaning.

*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).

#### *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 previously used in Turpin et al. (2017), and are presented in Table 2.

#### *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 (SAT). The SAT 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, 2000 g 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.

#### *Blood sampling*

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 SAT 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 minutes at 2000 g at 4° C) and then 1 ml 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 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).

#### *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

SAT and behavioural observation data, which were analysed using the individual piglet as the experimental unit. Data were checked 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  $\mu$  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.

## Results

### *Blood parameters*

Neither CoM or IS influenced the plasma concentrations of Hp throughout the experiment ( $P > 0.05$ , Table 3). On the day of weaning, there was a tendency ( $P < 0.1$ ) for IS pigs to have a lower plasma cortisol concentration than NoIS pigs and a 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 3). There was no interaction between the two main effects for the concentrations of any of the blood parameters.

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 ( $r = 0.28$ ,  $P < 0.001$ ).

#### *Piglet production indices*

Before any treatment intervention, piglet BW was similar within the two main effects. Unexpectedly and 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 4). 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 4). 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 severe 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 4). 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 4) 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 4). 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 4). 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 4). 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 4). Between days 2 to 7 after weaning, pigs that were exposed to IS during lactation tended to have a higher voluntary feed intake ( $P < 0.1$ , Table 4), 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 4). 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 ( $P > 0.05$ ).

### *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 5). 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 5). Intermittent suckling had no influence on mean scratch scores before and after weaning ( $P > 0.05$ ) and neither CoM nor IS influenced redness scores ( $P > 0.05$ ).

#### *Behaviour observations*

Behaviour 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 6.1). However, unexpectedly, piglets selected for exposure to the main effect of IS spent a greater proportion of total observations engaging in sleeping behaviour ( $P < 0.01$ ) and a smaller proportion of total observations exploring/playing ( $P < 0.001$ ) compared with piglets that were selected to remain with their sow (Table 6.1). Furthermore, sow directed behaviour was greater in piglets subjected to co-mingling than piglets that remained with their littermates ( $P = 0.001$ , Table 6.1). 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.

#### *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 6.1). 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 6.1). 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 6.1).

#### *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 6.1). 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 6.1) 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 ( $P > 0.05$ ).

#### *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 6.2). Co-mingling also reduced exploring/play behaviour 7 days after weaning ( $P < 0.05$ , Table 6.2). 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 ( $P > 0.05$ ). Piglets 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 6.2).

#### *Effects of IS on post-weaning behaviours*

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 6.2). 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 6.2). Intermittent suckling did not influence aggressive or exploratory/play behaviour on days 1 and 7 after weaning ( $P > 0.05$ , Table 6.2).

#### *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 1). Hence, a CoM x IS x day effect was found (Table 7). 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 7). Three days before weaning, piglets exposed to IS had a lower plasma galactose concentration than piglets that remained continuously with their sow (Figure 1,  $P < 0.05$ ). This effect continued as a trend ( $P < 0.1$ ) 3 days after weaning (Figure 1).

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

## Discussion

The aim of the current study was to determine how pre-weaning interventions (IS and CoM) each contributed to the prevention of the detrimental effects on piglet performance, GIT adaptation and behaviour associated with conventional weaning. Overall, the study showed that exposure to IS during the last week of lactation improved growth by 17.5% and tended to improve voluntary feed intake between 2 and 8 days after weaning. In contrast, pigs that were exposed to CoM during the last week of lactation were 500 g lighter at the end of the experiment and showed slower growth and reduced feed intake in the second week after weaning compared with pigs not exposed to CoM. In this regard, it was therefore unexpected that pigs exposed to CoM without IS had the highest absorption of mannitol 3 days after weaning. Both IS and CoM increased exploratory and play behaviour on the first day of treatment interventions, which later changed to increased sleeping behaviour on the last day of intervention and the first day of weaning. Although

aggressive and manipulative behaviour observations were similar in all focus pigs, injury score results suggested that pigs exposed to CoM fought more on the first day of treatment intervention, but also tended to fight less immediately after weaning. Finally, while there were differences between treatments in plasma cortisol and N:L ratios concentrations at the time of weaning, these changes were mild and transient and not suggestive of chronic stress or inflammatory compromise.

Weaning caused a significant increase in blood measures (cortisol, Hp and N:L ratios) for all pigs when data were pooled. An increase in plasma cortisol, inflammatory and immune parameters at the time of weaning is in accordance with previous reports (Puppe et al., 1997; Sauerwein et al., 2005; Colson et al., 2006; Moeser et al., 2007b; Van der Meulen et al., 2010) and suggests, as expected, that weaning in the current study was acutely stressful. The increase in cortisol was not affected by CoM, but when IS took place during the last week of lactation, the cortisol increase tended to be limited. Rather than indicating psychological stress, increases in cortisol concentration at weaning may reflect an adaptive stress response, possibly through intestinal remodelling and maturation (Wu et al., 2000), however due to the gradual nature of IS, the small intestine of these piglets may have already started to remodel and mature during the last week of lactation. Although Hp was not influenced by IS or CoM, concentrations never reached critical levels indicative of severe inflammation (Sales et al., 2015). Haptoglobin is a major acute phase protein in the pig (Eckersall et al., 1996) and despite acute infection or inflammation not being recognised in the current study, the negative correlation to growth supports Hp being used as a tool to evaluate the general health status on a farm (Knura et al., 2000). This suggests that IS or CoM did not influence the general wellness of pigs over the weaning period, a result that is consistent with other IS and CoM studies (van

Nieuwamerongen et al., 2015; Turpin et al., 2016a; Turpin et al., 2017). In contrast to results from van Nieuwamerongen et al. (2015) where post-weaning leucocyte numbers did not differ between socialised and non-socialised piglets, pigs exposed to CoM during lactation in the current study tended to have a higher N:L ratio on the day of weaning than pigs that were only socialised with their littermates before weaning. This effect seemed to be transient and may have been a reflection of the higher level of aggression in CoM pigs on day -4 as evidenced by more scratches (Tuchscherer and Manteuffel, 2000), a change not seen by van Nieuwamerongen et al. (2015) due to an enriched environment and a lower stocking density. The reason why CoM or IS influenced one blood measure and not another is not known, but overall, the effects CoM and IS had on blood measures were transient and based on these data, it cannot be concluded that IS or CoM reduced the (albeit) mild, weaning-associated stress response.

Studies on the socialisation of non-littermates during lactation have either shown an improvement in feed intake and growth performance after weaning in the socialised treatment groups (Weary et al., 1999; Hessel et al., 2006; van Nieuwamerongen et al., 2015), or no detrimental effect on production parameters (Pluske and Williams, 1996; D'Eath, 2005; Morgan et al., 2014). To our knowledge, this is the first study to show CoM having a negative influence on BW, growth and ADFI after weaning (days 7 to 14). While previous studies have varied in the timing of mixing, group size, environment (enriched versus not enriched) and the proportion of piglets that are familiar at mixing, none of the studies exposed piglets to CoM intermittently (e.g. for 8 hours per day). This point of difference might be the reason why CoM pigs in the current study did not perform as well as expected. Blackshaw et al. (1987) reported that levels of aggression are highest in the first 90 minutes after mixing. By exposing piglets to CoM

intermittently during lactation, each day was a new mixing day. This could have made it harder for CoM groups to form a stable hierarchy, resulting in a higher level of daily aggression (D'Eath, 2005), and extra stress during the last week of lactation may have harmed piglet performance in the long-term. In saying this, however, apart from an increase in scratches in CoM piglets 4 days before weaning and a mild increase in N:L ratios on the day of weaning, there is little evidence to suggest that aggression was increased during lactation and the CoM piglets were distressed. Alternatively, the reduced BW, growth and ADFI seen in CoM pigs between 7 and 14 days after weaning could also be a result of the reduced pen numbers (batches 3,4 and 5 only) for these measurement days. Regardless of the reason(s)?, the results of the current study suggest that CoM did not improve post-weaning performance as measured by BW, growth, feed intake and FCR.

Despite a decrease in growth at the start of IS due to reduced nursing frequency and a tendency to have a lower weaning weight than their continuously suckled counterparts, IS piglets ate more creep feed during the last week of lactation. Feed intake during lactation stimulates feed intake after weaning (Bruininx et al., 2002), and the higher creep feed intake for IS piglets during lactation was the likely cause of better growth and a tendency for better feed intake in IS pigs 2 to 7 days after weaning. This result is akin to results from previous IS studies where 10 or 12 hour separation times were used (Kuller et al., 2004; Kuller et al., 2007; Berkeveld et al., 2009). Similar results have also been achieved in sow “get-away” housing where the sows can leave their litter by choice after a certain time point during lactation, denying piglet access (Weary et al., 1999; Pajor et al., 2002; Weary et al., 2002). However, the benefits of IS and get-away systems only seem to be apparent in the immediate post-weaning period (i.e. the first week after weaning) with no

differences in production indices at the end of the experiment. Moreover, a lack of difference in BW at the end of the experiment (14 days after weaning) between pigs exposed to IS and pigs not exposed to IS, in combination with a reduction in growth at the start of IS, suggests that the higher growth rate between 2 and 5 days after weaning in IS pigs was compensatory and did not improve overall performance. It may be postulated, however, that a period of reduced growth might be more advantageous when the piglets can be supported by milk-borne growth factors, hormones and other bioactive substances from the sow (Cera et al., 1987; Jaeger et al., 1987) rather than after weaning when antibody synthesis and cellular immunity are reduced (Blecha and Kelley, 1981; Blecha et al., 1983).

As well as physiological measures of stress, behaviour measurements were also recorded since psychological stress might not necessarily be associated with reduced health and productivity (Dybkjær, 1992). Intermittent suckling and CoM caused a 70% and 28% increase respectively in exploratory/play behaviour on the first day of treatment intervention. This is in accordance with Weary et al. (2002) who reported a 45% increase in piglet activity on day 14 of lactation, the first day piglets were allowed to mix with two unfamiliar litters while being housed in a sow get-away system. Berkeveld et al. (2007) also observed a greater total activity at the start of IS (12 hours per day separation). Similar to the hypothesis suggested by Berkeveld et al. (2007), the high exploratory/play behaviour in the current study is likely to be due to restlessness associated with a new environment and sudden and previous unexperienced separation from the sow (IS) or interaction with new piglets (CoM), because the exploration and play reduced over time in conjunction with an increase in sleeping behaviour as the piglets became familiar with the process. Since enriched environments facilitate early eating and foraging behaviour



(Oostindjer et al., 2011; van Nieuwamerongen et al., 2015), the addition of straw or other chewing material in combination with IS and/or CoM could be considered for future experiments to stimulate exploring and play behaviour for longer. This may even encourage more creep feed intake throughout the entire lactation.

From the selected behaviour categories used for observations, oral manipulation and aggression were thought to be the most effective behavioural indicators of stress (Dybkjær, 1992; Moore et al., 1994). In the current study, IS or CoM did not affect the level of aggression or manipulative behaviour before weaning. This result is surprising, since a higher number of scratches were reported in CoM piglets compared with piglets that were only socialised with their littermates. Furthermore, an increased level of aggression resulting in more injury would be expected in the CoM treatments since a lack of familiarity can induce fighting (Puppe, 1998; Stookey and Gonyou, 1998). This discrepancy between behaviour observations and injury scores suggest that either the CoM pigs fought more intensely when they did fight or a single aggressive piglet could have had more victims, a hypothesis suggested van Nieuwamerongen et al. (2015) for a similar outcome between single litter and multi-litter pre-weaning housing. Alternatively, this discrepancy could also highlight a potential limitation of live scan sampling, i.e., 10 seconds might not be long enough to interpret the context of a behaviour, and perhaps continuous observations may be more reliable (Verdon et al. (2016).

With regard to post-weaning behaviour outcomes, multiple studies using a variety of pre-weaning socialisation techniques have reported a reduction in aggression following mixing at weaning compared with piglets that remained in their litter groups until the time of weaning (Weary et al., 1999; Weary et al., 2002; D'Eath, 2005; Li and Wang, 2011;

Verdon et al., 2016). In the present study, the overall incidence of aggressive behaviours after weaning was very low and therefore the main effects did not influence these results, however CoM tended to reduce scratch injury scores on the second day after weaning while IS had no effect. The high level of sleeping displayed by IS and CoM pigs on the first day after weaning may suggest a positive influence of these main effects on post-weaning behaviour. While resting behaviours such as sitting or lying have been considered a symptom of stress (Dybkjær, 1992; Colson et al., 2006), sleeping often reflects relaxed or contented piglets (Morgan et al., 2014). Therefore, more sleeping observations on days 1 and 7 after weaning in combination with less lying and sitting on day 1 and less manipulative behaviours on day 7 after weaning suggests that IS piglets were comfortable with their surroundings after weaning. Increased sleeping behaviour for CoM pigs on day 1 after weaning also suggests these pigs were settled, but perhaps to a lesser extent since CoM did not influence lying or sitting behaviour and sleeping or manipulative behaviour on day 7 after weaning.

In the current study, mannitol and galactose absorption across the GIT were measured to assess GIT absorptive capacity. Mannitol and galactose are both low molecular weight sugars that are absorbed for the most part via transcellular pathways across the epithelium (Cox et al., 1999; Murray et al., 2003). Mannitol is absorbed passively (i.e., along its concentration gradient) via water-filled pores in the enterocyte membrane (Menziez et al., 1979; Travis and Menziez, 1992; Bjarnason et al., 1995), whereas galactose is absorbed actively via a sodium-glucose linked transporter (SGLT1) (Wright, 1998). During weaning, as a result of a low feed intake, villous atrophy occurs which affects the absorptive capacity of the small intestine (Pluske et al., 1997). Additionally, weaning piglets at a young age (19 days) has been shown to activate stress signalling pathways,

which contribute to disturbances in intestinal mucosal health measured by elevated secretory activity and increased intestinal permeability in the pig jejunum and colon (Moeser et al., 2007b). However, weaning at an older age (23 to 28 days) did not induce these changes (Moeser et al., 2007a; Smith et al., 2010). The interpretation of SAT results can be challenging, since basic mechanisms that control absorption pathways and rates of permeation of different markers remain to be elucidated. Furthermore, when there is disruption of the membrane integrity the pathways of marker absorption become more complex and it is likely that all markers including mannitol and galactose permeate via paracellular junctions as well as transcellular pathways (Bjarnason et al., 1995). In the case of *in vivo* SAT, intestinal permeability is normally measured by calculating the excretion ratio of two carbohydrates of different size together administered together, e.g., a monosaccharide that is absorbed across the epithelium and a disaccharide that is absorbed paracellularly through the epithelial tight junctions (Menzies et al., 1979; Travis and Menzies, 1992). Since the presence of weaning-induced intestinal barrier dysfunction in the current study is unlikely due to the selected weaning age (22 days of age) and because only monosaccharides were used in the SAT, only GIT absorptive capacity was measured in the current study, not intestinal permeability.

Nevertheless the pattern of mannitol absorption observed over the weaning period in the current study is consistent with previously reported values (Berkeveld et al., 2008; Turpin et al., 2016b) and likely reflects a reduction in intestinal surface area. The fact that CoM NoIS pigs did not experience a reduction in mannitol absorption could suggest some improvement in adaption to the changes at weaning, however the exact mechanism by which this occurs is not immediately obvious from the results of the current study. The improvement in mannitol absorption for CoM NoIS pigs did not affect feed conversion

ratio. It is surprising that mannitol absorption was reduced 3 days after weaning compared with 3 days before weaning in IS piglets since a previous experiment by Berkeveld et al. (2009) showed the prevention of villous atrophy after weaning in IS pigs (7 days of IS starting at day 19 of age). This difference could be a reflection of some limitations in the accuracy of the mannitol sugar absorption test. While galactose absorption has a positive correlation with villous height (Pluske et al., 1996b), it is important to consider that galactose is actively absorbed via a SGLT-1 transporter, which relies on the activity of a  $\text{Na}^+\text{-K}^+$  ATP pump (Wright, 1998). A change from a milk-based diet to solid feed can increase  $\text{Na}^+\text{-K}^+$  ATP pump activity (Boudry et al., 2002), however psychological stress has been shown to decrease  $\text{Na}^+\text{-K}^+$  ATP pump activity in rats (Boudry et al., 2007). Since all treatment groups experienced a decrease in ADG at the time of weaning as well as increases in cortisol, Hp and N:L ratios, it is likely that weaning to some extent was psychologically stressful, possibly causing a decrease in  $\text{Na}^+\text{-K}^+$  ATP pump activity and therefore a decrease in galactose absorption across all treatments at the time of weaning. The reduced absorption of galactose before and after weaning for IS is somewhat surprising given a previous study did not find a difference in galactose absorption between IS and conventionally weaned pigs 4 days after weaning (Turpin et al., 2016b). However, it may reflect an adaptive GIT response made more evident by the gradual nature of IS.

## Conclusions

Results of the current study demonstrated that IS during the last week of lactation stimulated 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. Moreover, it reduced 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 acute stress at the time of weaning as supported by more observations of positive behaviour such as sleeping. 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. 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).

#### Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

#### Acknowledgments

The technical assistance of Josie Mansfield, Ingunn Stensland, Amy Kwee and Robert Pluske is gratefully acknowledged. Appreciation is extended to the Cooperative Research Centre for High Integrity Australian Pork (Pork CRC) for funding and provision of a postgraduate scholarship to the first author.

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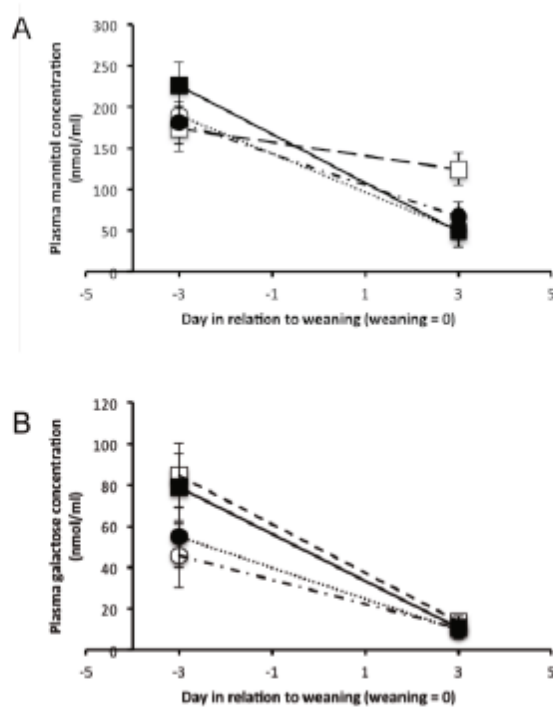


Figure 1. Plasma mannitol (A) and plasma galactose (B) concentrations (nmol/ml) of pigs before and after weaning, CoM IS,  $n=16$  ( $\circ$ ), CoM NoIS:  $n=14$  ( $\square$ ), NoCoM IS  $n=16$  ( $\bullet$ ), NoCoM NoIS:  $n=13$  ( $\blacksquare$ ). Data are raw means  $\pm$  SE

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 or 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 or flank	Redness or swelling were obvious on head, ears or flank	Irritation easily observed as darker reddening and/or moderate to severe swelling on head, ears or flank

Table 2. Ethogram used during instantaneous scan sampling observations

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 * In the case of the CoM NoIS treatment, the sow towards which the behaviour was directed (i.e. the piglet's own sow or the other sow) was not recorded.

Table 3. 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 <sup>1</sup>		IS <sup>2</sup>	
	CoM	NoCoM	IS	NoIS
Cortisol <sup>3</sup> , ng/ml				
Day <sup>4</sup> -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 <sup>x</sup> (13.26-21.06)	22.8 <sup>y</sup> (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 <sup>3</sup> , 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 <sup>3</sup>				
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 <sup>x</sup> (2.48-5.77)	2.2 <sup>y</sup> (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)

<sup>x,y</sup> Within main effects, values not followed by a common superscript are a trend ( $P < 0.1$ ).

<sup>1</sup> 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.

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

<sup>3</sup> 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).

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

Table 4. Effects of co-mingling and intermittent suckling on piglet performance before and after weaning

Item	CoM <sup>1</sup>		IS <sup>2</sup>	
	CoM	NoCoM	IS	NoIS
<b>BW, kg</b>				
Day <sup>3</sup> -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 <sup>x</sup> ± 0.18	6.6 <sup>y</sup> ± 0.20
Day 2	6.4 ± 0.15	6.5 ± 0.14	6.2 <sup>a</sup> ± 0.14	6.7 <sup>b</sup> ± 0.15
Day 7	7.1 ± 0.16	7.2 ± 0.16	7.0 <sup>x</sup> ± 0.16	7.3 <sup>y</sup> ± 0.16
Day 14 <sup>5</sup>	8.5 <sup>a</sup> ± 0.29	9.3 <sup>b</sup> ± 0.24	8.6 ± 0.30	9.2 ± 0.24
<b>ADG, g/piglet</b>				
Day -13 to -7	262 ± 7.1	277 ± 7.3	258 <sup>a</sup> ± 6.9	281 <sup>b</sup> ± 7.5
Day -7 to -4	246 ± 9.6	248 ± 9.8	220 <sup>a</sup> ± 9.3	275 <sup>b</sup> ± 10.1
Day -4 to 0	246 ± 10.4	263 ± 10.6	232 <sup>b</sup> ± 10.0	277 <sup>a</sup> ± 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 <sup>a</sup> ± 7.1	127 <sup>b</sup> ± 7.3
Day 7 to 14 <sup>5</sup>	263 <sup>a</sup> ± 13.1	300 <sup>b</sup> ± 11.1	289 ± 13.4	274 ± 10.7
<b>ADFI<sup>4</sup>, g/day</b>				
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 <sup>a</sup> (3.20-5.49)	1.6 <sup>b</sup> (0.95-2.47)
Day -4 to 0	5.7 (3.89-7.88)	5.2 (3.93-6.68)	7.2 <sup>a</sup> (5.48-9.25)	3.9 <sup>b</sup> (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 <sup>x</sup> (137.7-193.8)	127 <sup>y</sup> (103.2-154.1)
Day 7 to 14 <sup>5</sup>	303 <sup>a</sup> (272.9-335.1)	349 <sup>b</sup> (321.3-377.6)	341 (307.7-375.1)	311 (285.9-337.3)
<b>Food: gain ratio, g food per g live-weight gain<sup>6</sup></b>				
Day 2 to 7	1.1 ± 0.08	1.1 ± 0.08	1.1 ± 0.08	1.1 ± 0.08
Day 7 to 14 <sup>5</sup>	1.2 ± 0.03	1.2 ± 0.03	1.2 ± 0.03	1.1 ± 0.02

<sup>a,b</sup> Within main effects, values not followed by a common superscript differ ( $P < 0.05$ ).

<sup>x,y</sup> Within main effects, values not followed by a common superscript are a trend ( $P < 0.1$ ).

<sup>1</sup> 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.

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

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

<sup>4</sup> 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).

<sup>5</sup> Batches 3,4 and 5 only.

<sup>6</sup> Day 0 to 2 FCR not included due to negative growth.



Table 5. Effects of co-mingling and intermittent suckling on mean scratch scores and redness at 4 days before and 2 days after weaning

Item	CoM <sup>1</sup>		IS <sup>2</sup>	
	CoM	NoCoM	IS	NoIS
Scratch				
Day <sup>3</sup> -4	0.81 <sup>a</sup> ± 0.04	0.57 <sup>b</sup> ± 0.04	0.73 ± 0.04	0.65 ± 0.04
Day 2	0.84 <sup>x</sup> ± 0.06	1.00 <sup>y</sup> ± 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

<sup>a,b</sup> Within main effects, values not followed by a common superscript differ ( $P < 0.05$ ).

<sup>x,y</sup> Within main effects, values not followed by a common superscript are a trend ( $P < 0.1$ ).

<sup>1</sup> 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.

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

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

Table 6.1 Effects of co-mingling and intermittent suckling on the proportion of total behavioural observations displayed by piglets before weaning

	CoM <sup>1</sup>		IS <sup>2</sup>		SEM
	CoM	NoCoM	IS	NoIS	
Day <sup>3</sup> -8					
Sleep	0.222 <sup>x</sup>	0.372	0.372 <sup>ax</sup>	0.222 <sup>b</sup>	0.046
Lie/sit	0.106	0.163	0.169	0.100	0.026
Standing	0.116	0.103	0.097 <sup>x</sup>	0.122	0.023
Aggressive	0.000	0.000	0.000	0.000	0.000
Explore/play	0.100 <sup>x</sup>	0.072	0.031 <sup>ax</sup>	0.141 <sup>b</sup>	0.017
Manipulative	0.050	0.047	0.053	0.044	0.013
Ingestive-related	0.025 <sup>x</sup>	0.016	0.016 <sup>x</sup>	0.025	0.011
Sow	0.381 <sup>a</sup>	0.228 <sup>b</sup>	0.263	0.347	0.033
Day -7					
Sleep	0.209 <sup>ax</sup>	0.347 <sup>b</sup>	0.200 <sup>ay</sup>	0.356 <sup>b</sup>	0.030
Lie/sit	0.094	0.072	0.078	0.088	0.021
Standing	0.109	0.119	0.169 <sup>ay</sup>	0.059 <sup>b</sup>	0.018
Aggressive	0.003	0.003	0.003	0.003	0.003
Explore/play	0.341 <sup>ay</sup>	0.244 <sup>b</sup>	0.450 <sup>ay</sup>	0.134 <sup>b</sup>	0.019
Manipulative	0.047	0.028	0.050	0.025	0.012
Ingestive-related	0.047 <sup>xy</sup>	0.038	0.050 <sup>y</sup>	0.034	0.010
Sow	0.150	0.150	-	0.3	0.017
Day -1					
Sleep	0.359 <sup>y</sup>	0.397	0.453 <sup>ax</sup>	0.303 <sup>b</sup>	0.037
Lie/sit	0.119	0.106	0.119	0.106	0.022

## ACCEPTED MANUSCRIPT

Standing	0.109	0.119	0.175 <sup>ay</sup>	0.053 <sup>b</sup>	0.022
Aggressive	0.006	0.000	0.006	0.000	0.003
Explore/play	0.134 <sup>x</sup>	0.153	0.153 <sup>z</sup>	0.134	0.025
Manipulative	0.034	0.025	0.031	0.028	0.009
Ingestive-related	0.050 <sup>y</sup>	0.038	0.056	0.031	0.012
Sow	0.172	0.163	-	0.33	0.018

<sup>a,b</sup> Within main effects, values not followed by a common superscript within a row differ ( $P < 0.05$ ).

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

<sup>1</sup> 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.

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

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

Table 6.2 Effects of co-mingling and intermittent suckling on the proportion of total behavioural observations displayed by pigs after weaning

	CoM <sup>1</sup>		IS <sup>2</sup>		SEM
	CoM	NoCoM	IS	NoIS	
Day <sup>3</sup> 1					
Sleep	0.353 <sup>a</sup>	0.147 <sup>bx</sup>	0.331 <sup>ax</sup>	0.169 <sup>bx</sup>	0.023
Lie/sit	0.231	0.259	0.147 <sup>ax</sup>	0.344 <sup>b</sup>	0.031
Standing	0.134 <sup>a</sup>	0.216 <sup>bx</sup>	0.172 <sup>x</sup>	0.178 <sup>x</sup>	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 <sup>x</sup>	0.091	0.021
Day 7					
Sleep	0.378	0.309 <sup>y</sup>	0.422 <sup>ay</sup>	0.266 <sup>by</sup>	0.026
Lie/sit	0.284	0.263	0.256 <sup>y</sup>	0.291	0.025
Standing	0.100	0.122 <sup>y</sup>	0.100 <sup>y</sup>	0.122 <sup>y</sup>	0.019
Aggressive	0.000	0.006	0.006	0.000	0.003
Explore/play	0.041 <sup>a</sup>	0.088 <sup>b</sup>	0.056	0.072	0.016
Manipulative	0.094	0.094	0.066 <sup>a</sup>	0.122 <sup>b</sup>	0.018
Ingestive-related	0.097	0.119	0.088 <sup>y</sup>	0.128	0.022

<sup>a,b</sup> Within main effects, values not followed by a common superscript within a row differ ( $P < 0.05$ ).

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

<sup>1</sup> 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.

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

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

Table 7. 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

	<u>Mannitol</u>	<u>Galactose</u>
	<u>Significance</u>	<u>Significance</u>
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

### Highlights

- Co-mingling of unfamiliar piglets before weaning did not improve performance.
- Intermittent suckling improved pre-weaning creep feed intake and post-weaning growth.
- Co-mingling and intermittent suckling increased positive behaviour after weaning.
- Intermittent suckling likely advanced gastrointestinal changes in the peri-weaning period.