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**Rault, J-L, Ferrari, J., Pluske, J.R. and Dunshea, F.R. (2015)  
Neonatal oxytocin administration and supplemental milk  
ameliorate the weaning transition and alter hormonal  
expression in the gastrointestinal tract in pigs. Domestic Animal  
Endocrinology, 51 . pp. 19-26.**

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# Accepted Manuscript

Neonatal oxytocin administration and supplemental milk ameliorate the weaning transition and alter hormonal expression in the gastrointestinal tract in pigs

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PII: S0739-7240(14)00091-5

DOI: [10.1016/j.domaniend.2014.11.001](https://doi.org/10.1016/j.domaniend.2014.11.001)

Reference: DAE 6108

To appear in: *Domestic Animal Endocrinology*

Received Date: 23 June 2014

Revised Date: 3 November 2014

Accepted Date: 4 November 2014

Please cite this article as: Rault J-L, Ferrari J, Pluske JR, Dunshea FR, Neonatal oxytocin administration and supplemental milk ameliorate the weaning transition and alter hormonal expression in the gastrointestinal tract in pigs, *Domestic Animal Endocrinology* (2014), doi: 10.1016/j.domaniend.2014.11.001.

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1 **Neonatal oxytocin administration and supplemental milk ameliorate the**  
2 **weaning transition and alter hormonal expression in the gastrointestinal tract**  
3 **in pigs**

4  
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**12 Abstract**

13           The aim of this study was to investigate the influences of milk supplementation during  
14 lactation, over 1 wk after weaning, and oxytocin administration for the first 14 d of life on the  
15 pigs' response to weaning. Pigs from 20 litters were allocated to each of these 3 treatments in a  
16 randomized factorial design. Oxytocin was administered subcutaneously daily from 0 to 14 d of  
17 age at a rate of 10 I.U. per kg. The milk supplement consisted of a mixture of 25% skim milk  
18 powder offered either during lactation between 10 and 20 d of age or for the first wk after  
19 weaning as a transitional diet along with dry pellets. Pigs were weaned at 21 d of age. Growth  
20 rate was measured from birth to slaughter at 140 d of age and feed intake of supplemental milk  
21 or feed from 10 to 56 d of age. Organ weights (heart, liver, stomach, kidneys) and the gene  
22 expression of ghrelin, leptin, and glucagon-like peptides (GLP-1 and GLP-2) were measured in  
23 the stomach, ileum, and duodenum at 10, 21 and 28 d of age. Milk supplementation after  
24 weaning resulted in immediate feed intake and partially alleviated the depression in growth rate  
25 over the first 7 d post-weaning ( $P < 0.001$ ), but milk supplementation during lactation had no  
26 effects ( $P > 0.1$ ). However, effects were only transient and disappeared once the milk liquid diet  
27 was removed. Neonatal oxytocin administration reduced weight loss over the first 2 d after  
28 weaning ( $P = 0.03$ ), without affecting feed intake ( $P > 0.1$ ), hence possibly reducing weaning  
29 stress. Seven d after weaning, oxytocin-treated pigs had greater stomach ghrelin and leptin  
30 expression (both  $P = 0.02$ ), and pigs supplemented with milk after weaning had greater stomach  
31 leptin and GLP-2 expression ( $P = 0.02$  and  $P = 0.05$ , respectively). Hence, neonatal oxytocin  
32 administration or post-weaning milk supplementation are both effective means of enhancing  
33 gastric leptin expression and reducing weight loss at weaning, likely improving gut health during  
34 this critical period.

35

36 **Keywords:** ghrelin; GLP; gut health; leptin; stress; *sus scrofa*.

37

ACCEPTED MANUSCRIPT

## 38 1. Introduction

39 Neonatal mammals rely on their dam for survival, through protection and the provision of  
40 food. Weaning occurs gradually in natural conditions. For feral pigs, weaning takes between 8  
41 and 19 wk to complete [1]. This time allows the young pig to mature in its digestive and  
42 absorptive capacities and in its ability to cope with environmental challenges. However, in  
43 commercial pig production, weaning usually occurs abruptly between 3 and 4 wk of age, and the  
44 separation of piglets from the dam is usually associated with changes in their diet, physical and  
45 social environments. These result in nutritional, thermal, immunological and psychological  
46 challenges [2,3]. Weaning is therefore a multi-factorial stressor for pigs, and is generally  
47 associated with weight loss and increased morbidity and mortality reflective of the pigs'  
48 difficulty in coping with this challenge [4].

49 A variety of strategies have been attempted to facilitate the weaning transition on the pig,  
50 mostly aimed at alleviating the post-weaning growth lag. Dry pelleted feed is the standard diet  
51 form given to newly-weaned pigs on most farms, and represents an abrupt change from the  
52 highly digestible and palatable nutrients piglets' receive from milk. Studies have shown that  
53 specific feeding regimes, such as supplementing piglets with milk during lactation [5-7], or into  
54 the early post-weaning period while gradually introducing a dry pelleted feed [6,8-10], can  
55 alleviate weight loss and improve indices of gastrointestinal tract structure and function in the  
56 post-weaning period.

57 Hormonal interventions, such as oxytocin, a mammalian peptide, could also facilitate  
58 weaning. In rats, repeated oxytocin administration induces long-lasting metabolic and  
59 physiologic changes such as increased growth [11,12], decreased corticosterone concentrations  
60 and levels of plasma gastrin, cholecystokinin and insulin [13,14]. In addition, oxytocin may

61 reduce the stress response to weaning by reducing the psychological attachment to the dam and  
62 favouring self-oriented behaviours [15], although the precise mechanism by which oxytocin  
63 exerts its effect remains unclear [16]. The release of oxytocin can be stimulated by touch,  
64 warmth and the ingestion of food [17], which are common daily occurrences for the suckling pig  
65 but become suddenly sporadic in the post-weaning period.

66 This experiment aimed to determine the influence of milk supplementation during  
67 lactation, over 1 wk after weaning, and of oxytocin administration for the first 14 d life on the  
68 pigs' feed intake, growth rate, organ weights and the gene expression of hormones released from  
69 the gastrointestinal tract, with an emphasis on ghrelin, leptin, glucagon-like peptide-1 (GLP-1)  
70 and glucagon-like peptide-2 (GLP-2). The hypothesis of this study was that pigs supplemented  
71 with milk, during lactation or after weaning, or pigs administered with exogenous oxytocin  
72 would show greater feed intake in the first 7 d after weaning, greater growth rate and greater  
73 expression of these gastrointestinal hormones.

74

## 75 **2. Materials and methods**

76 The project was approved by the Victorian Department of Primary Industries Ethics  
77 Committee in accordance with the Australian Code of Practice for the Care and Use of Animals  
78 for Scientific Purposes.

79

### 80 *2.1. Animals and milk supplementation during lactation*

81 Pigs were allocated to treatments in a  $2 \times 2 \times 2 \times 2$  randomised factorial design with the  
82 respective factors being sex (male vs. female), injection (oxytocin vs. saline), pre-weaning

83 dietary treatment (supplemented with milk during lactation vs. unsupplemented) and post-  
84 weaning dietary treatment (pellets vs. pellets plus milk).

85 Twenty Large White x Landrace multiparous sows of mixed parities suckling 10-12 pigs  
86 were randomly allocated to 1 of 2 treatments: pigs were either supplemented with milk or were  
87 unsupplemented and relied solely on suckling the sow. The liquid supplement consisted of a  
88 mixture of 25 % skim milk powder (SMP) and water. The SMP was available from day 10 of  
89 lactation to weaning (day 21). The SMP was reconstituted by adding 1 part powdered SMP  
90 (Murray Goulbourn, Melbourne, VIC, Australia) to 4 parts warm tap water; 20 mL of a live  
91 probiotic (Yakult Australia Pty Ltd, Dandenong, VIC, Australia) was added per litre as a source  
92 of *Lactobacillus casei* Shirota strain to prevent diarrhoea. The SMP mixture was stored at 4 °C  
93 and used within 2 d. The supplement was delivered by a gravity feed system that was designed to  
94 minimise spillage and contamination by faeces and urine [6].

95

## 96 2.2. Oxytocin administration

97 Within each litter, pigs were allocated at birth into pairs of the same sex based on similar  
98 live weight. Within each litter, 2 female and 2 male pigs of each pair (n = 40 per sex) were  
99 injected subcutaneously with oxytocin (Ilium Syntocin, Troy Laboratories, Glendenning, NSW,  
100 Australia) daily from 0 to 14 d of age at a rate of 10 I.U. per kg of BW (equivalent to 20 µg per  
101 kg of BW). The other 2 female and 2 male pigs of the pairs (n = 40 per sex) were injected  
102 subcutaneously with 0.9 % saline in the same manner and same quantity as the control. These  
103 pigs were used for live measurements. Any additional pig in the litter also received injections of  
104 either oxytocin or saline and 117 pigs were later euthanized for the collection of tissue samples  
105 and measurements of gene expression.



106

## 107 2.3. Weaning and milk supplementation post-weaning

108 Pigs were weaned at 21 d of age into individual weaner crates in order to measure their  
109 feed intake. Forty pigs were fed *ad libitum* a high quality weaner pelleted diet, Ultrawean 75  
110 (digestible energy (DE): 16 MJ/kg, crude protein (CP): 24 %, total Lysine: 1.0 %; Ridley  
111 AgriProducts, Pakenham, VIC, Australia), whereas 40 other pigs were fed the same diet but also  
112 supplemented with the same SMP mixture as a transitional diet. Over the 7 d post-weaning, the  
113 dry matter (DM) content of the SMP mixture was gradually increased by the inclusion of less  
114 milk and more dry pelleted feed (Ultrawean 75) until pigs were completely offered the dry  
115 pelleted diet. Accordingly, for the first 2 d post-weaning, pigs were offered 1 L of SMP mixture.  
116 On the third day post-weaning, 200 g of Ultrawean 75 was added and the amount of pelleted diet  
117 increased by 100 g/d until day 7 while the amount of the SMP mixture decreased  
118 proportionately. From the end of the first wk after weaning, all pigs were fed *ad libitum*  
119 Ultrawean 100 (DE: 16 MJ/kg, CP: 24 %, Lysine content: 0.9 %; Ridley AgriProducts,  
120 Pakenham, VIC, Australia) for another week before being changed to an *ad libitum* high quality  
121 weaner mash from the third week to 56 d of age (DE: 14.5 mj/kg, CP: 23 %, total Lysine: 1.0 %;  
122 Riverbank Stockfeeds, Leongatha, VIC, Australia). At 56 d of age, pigs were returned to the  
123 herd, housed in 1 shed in groups of 10 in 3.0 × 3.4 m pens, and fed conventional grower (DE:  
124 14.3 MJ/kg, CP: 18.7 %, total Lysine: 0.6 %; Riverbank Stockfeeds, Leongatha, VIC, Australia)  
125 and finisher diets (DE: 13.0 MJ/kg, CP: 18.2 %, total Lysine: 0.5 %; Riverbank Stockfeeds,  
126 Leongatha, VIC, Australia) until slaughter.

127

## 128 2.4. Animal measurements

129 Live weight of the pigs was determined at birth and then at 10, 14, 21, 23, 25, 28, 35, 42,  
130 49 and 56 d of age. Supplemental milk intake was determined for each litter daily from day 10 to  
131 20 of lactation. All feed refusals post-weaning were weighed for each pig daily between 21 and  
132 27 d of age and weekly at 35, 42, 49 and 56 d of age to determine individual feed intake. Feed  
133 intake was only measured until 56 d of age because this study focused on feed intake around  
134 weaning, and therefore by 56 d of age all pigs are expected to have adapted to solid feed. From  
135 day 56, the pigs were returned to the herd and housed in group pens and so further observations  
136 on individual growth performance could not be made. However, live weight and back fat depth  
137 ( $P_2$ ) were measured on all individual pigs at 140 d of age (market slaughter weight). Ultrasonic  
138 back fat depth ( $P_2$ ) was measured 65 mm from the mid line at the level of the last rib, using a  
139 backfat scanner (Renco Lean-Meater Minneapolis, MN, USA).

140

#### 141 *2.5. Euthanasia and tissue sample collection*

142 For organ weights and gene expression, pigs were euthanized at 10 (n = 15), 21  
143 (weaning; n = 39), 28 (n = 31) or 35 (n = 32) d of age. Pigs were euthanized via a lethal injection  
144 of pentobarbital (Lethobarb<sup>®</sup>, Virbac, Milperra, New South Wales, Australia) intracardially at a  
145 dose of 0.3 mL/kg. Immediately following death, a ventral incision was made from the sternum  
146 to the pubis and the gastrointestinal tract removed. The gastrointestinal tract was tract removed.  
147 Stomach, small intestine, large intestine, liver, kidneys and heart were individually weighed.  
148 Following flushing with cold 0.9 % physiological saline solution, a mucosal sample from the  
149 fundic region of the stomach and mucosal samples from the proximal duodenum and ileum were  
150 collected for subsequent gene expression analysis at 10, 21 and 28 d of age. Samples collected

151 for gene expression analysis were wrapped in aluminium foil and snap frozen in liquid nitrogen.  
152 Samples were later transferred to the -80 °C freezer and stored until analysis.

153

#### 154 *2.6. Real-Time polymerase chain reaction (RT-PCR) analysis of gene expression*

155 The RNA was extracted from tissue samples using Trizol<sup>®</sup> Reagent (Invitrogen, Life  
156 Technologies, Mulgrave, VIC, Australia) according to the protocol of the manufacturer. The  
157 RNA quality and quantity was determined using an Experion automated electrophoresis station  
158 (Bio-Rad Laboratories Inc, Hercules, California, USA) along with the Experion StdSens  
159 Analysis Kit (Bio-Rad Laboratories Inc, Hercules, California, USA) according to the protocol of  
160 the manufacturer. Electropherograms for each sample were analysed for the concentration of  
161 RNA and the ratio of 28S to 18S and samples with 2 clean peaks and a ratio close to 1.8 were  
162 accepted. Samples with background 'noise' and/or low ratios were not accepted and the original  
163 tissue sample was re-extracted.

164 The RNA of suitable quality was then reverse-transcribed into cDNA in triplicate using  
165 the SuperScript<sup>™</sup> III First Strand Synthesis System for RT-PCR (Invitrogen, Life Technologies,  
166 Mulgrave, VIC, USA), random hexamers method. Briefly, 8 µL of RNA from each sample was  
167 aliquoted in triplicate to a 96 well plate and combined with 1 µL 50 ng/µl random hexamer and 1  
168 µL 10 mM dNTP mix. The plate was incubated at 65 °C for 5 min using the Corbett Palm Cycler  
169 PCR (Corbett Research, Mortlake, New South Wales, Australia). The following cDNA synthesis  
170 mix was prepared per sample in the following order: – 2 µL 10x RT buffer, 4 µL 25 mM MgCl<sub>2</sub>,  
171 2 µL 0.1 M DTT, 1 µL 40 U/µL RnaseOUT, 1 µL 200 U/µL SuperScript III RT. To each of the  
172 sample wells, 10 µL of this cDNA synthesis mix was added and the plate centrifuged briefly  
173 (Eppendorf, Hamburg, Germany). The plate was then incubated as follows: 10 min at 25 °C, 50

174 min at 50 °C, 5 min at 85 °C. The samples were then chilled on ice for 2 min before the addition  
175 of 1 µL RNase H to each of the sample wells and the final incubation at 37 °C for 20 min. For  
176 each tissue type, 2 µL from each of the sample wells was combined and aliquoted into 10 µl lots  
177 for later use as a positive control for polymerase chain reaction (PCR) investigations. These  
178 positive control samples were also used to optimise primer conditions before PCR analyses of  
179 the experimental samples. All cDNA was stored frozen at -80 °C until required for polymerase  
180 chain reaction (PCR) analysis.

181 Primers for the genes of interest were designed using the pig (*Sus scrofa*) genome  
182 database. The messenger RNA sequence of the gene of interest was obtained using the National  
183 Center for Biotechnology Information (NCBI) nucleotide database. This sequence was then  
184 copied into Invitrogen's OligoPerfect™ Designer web page. The oligoPerfect Designer finds  
185 primer sequences from the given mRNA sequence that meets individual specifications of size,  
186 annealing temperature, GC content, region of analysis, product size, salt concentration and  
187 primer concentration. Potential forward and reverse primer sequences were then transferred to  
188 Premier Biosoft International's NetPrimer program to determine if the primers had any of the  
189 following adverse characteristics: hairpins, dimers, palindromes or repeats. The 2 forward and  
190 reverse primer pairs with the least number of these adverse characteristics were selected (Table  
191 1). These custom primers were ordered through GeneWorks Pty Ltd (Hindmarsh, South  
192 Australia, Australia).

193 The cDNA samples were analysed via RT-PCR using primer sets for *18s* rRNA as the  
194 housekeeper gene, together with ghrelin, leptin, GLP-1 and GLP-2 at optimised temperatures and  
195 concentrations (Table 1). Primers for each gene of interest were optimised to determine the  
196 appropriate annealing temperature and the most efficient concentration of primer to include in

197 the reaction. Temperature optimisation of each primer was undertaken using a temperature  
198 gradient between 50 to 65 °C depending on the primer set. The temperature at which the product  
199 amplified with good repeatability above 10 threshold cycles (Ct), and that had a clean melt curve  
200 with no evidence of primer-dimer formation was chosen. All PCR reactions were undertaken in  
201 real time (RT-PCR) on a BioRad MyIQ Single Colour Real Time PCR Detection System  
202 (BioRad Laboratories Inc., Hercules, California, USA). Reactions were made up in 0.2 mL  
203 iCycler 96 well PCR plates (BioRad Laboratories Inc, Hercules, California, USA) and sealed  
204 with iCycler iQ optical tape (BioRad Laboratories Inc, Hercules, California, USA). Each PCR  
205 reaction mix consisted of 12.5 µL Sybr Green Supermix (BioRad Laboratories Inc, Hercules,  
206 California, USA), 1-3 µL forward primer (depending on optimised concentration), 1–3 µL  
207 reverse primer (depending on optimised concentration), 2 µL cDNA from the appropriate tissue,  
208 with the remaining volume made up to 25 µL with RNase free H<sub>2</sub>O. Each 96 well plate also  
209 contained 2 wells as a positive control (cDNA used in these wells was from the control batch to  
210 ensure consistent results across plates), 2 wells as a blank (containing only 25 µL of RNase free  
211 H<sub>2</sub>O) and 2 wells as a negative control (the 2 µL of cDNA was replaced in these wells by 2 µL  
212 of RNase free H<sub>2</sub>O). The cDNA amplification program included step 1: 95 °C for 3 min followed  
213 by 60 cycles; step 2: 90 °C for 10 sec and 55 to 63 °C for 45 sec (temperature dependent upon  
214 primer, see Table 1); step 3: 95 °C for 60 sec; step 4: 90 °C for 60 sec; step 5: 55 °C for 60 sec;  
215 and finally 60 cycles of step 6: 55 °C for 10 sec. A melt curve was produced for each reaction  
216 run in order to detect if any primer-dimers were produced. All samples had a Ct value for both  
217 the gene of interest and the housekeeper gene, with the difference between the two Ct values  
218 evaluated as the  $\Delta$  Ct. When using RT-PCR to evaluate gene expression in samples obtained  
219 from multi-factorial experiments, the  $\Delta$  Ct method is required to statistically analyze the data

220 arising from such experiments [18]. Using this method, a lower  $\Delta Ct$  value indicates an earlier  
221 amplification of the product and hence greater gene expression, whereas the reverse is true for a  
222 greater  $\Delta Ct$  value.

223

## 224 *2.7. Statistical analysis*

225 Data was checked for normality and homogeneity of variance and statistically analysed  
226 using ANOVA with Genstat software 13<sup>th</sup> edition (VSN International Ltd, Hemel Hempstead,  
227 United Kingdom). The experimental unit was the pig and sow was used as the blocking factor.  
228 The model was used for performance, feed intake, and gene expression data and included sex,  
229 oxytocin administration, pre- and post- weaning milk supplementation as the main factors and all  
230 interaction between these factors. As there were no significant interactions (all  $P > 0.05$ ), the  
231 data are presented as main effects.

232

## 233 **3. Results**

### 234 *3.1. Pre-weaning growth performance and supplemental milk intake*

235 Oxytocin- and saline-administered pigs did not differ in birth weight at the onset of the  
236 experiment (1.7 kg vs. 1.7 kg, SED = 0.1 kg,  $P = 0.33$ ), nor was there any effect of sex on birth  
237 weight (1.7 vs. 1.7 kg, SED = 0.1 kg,  $P = 0.96$ ). Between birth and 10 d of age, females had a  
238 lower average weight gain than males (214 g/d vs. 231 g/d, SED = 8.6 g/d,  $P = 0.05$ ).

239 Supplemental milk intake during lactation increased steadily from 848 g/d to 2516 g/d per litter  
240 over the 10 d of supplementation, or the equivalent of  $\approx 85$  to 252 g/d per pig (Figure 1). Pigs  
241 supplemented with milk from day 10 of lactation had greater average weight gain between day  
242 10 and weaning than non-supplemented pigs (303 g/d vs. 262 g/d, SED = 17.5 g/d,  $P = 0.03$ ), and

243 this tended to be greater in females than males (291 g/d vs. 275 g/d, SED = 9.6 g/d,  $P = 0.09$ ).

244 The administration of oxytocin had no effect on average weight gain between birth and weaning,  
245 and did not differ between males and females ( $P > 0.1$ ).

246

247 *3.2. Post-weaning growth performance and feed intake over the first week post-weaning (21-28 d*  
248 *of age)*

249 The DM feed intake was over 10 times greater in the first 2 d after weaning in pigs  
250 weaned onto the gruel diet consisting of pellets supplemented with milk compared with pigs  
251 weaned on pellets alone ( $P < 0.001$ ; Table 2). This greater feed intake continued for 7 d after  
252 weaning during which time these pigs had their feed gradually changed from the SMP mixture to  
253 a solid diet ( $P < 0.001$ ). Pigs weaned onto the SMP mixture plus the dry pelleted feed lost 2.5  
254 times less weight over the initial 2 d post-weaning than those weaned onto pellets alone ( $P <$   
255  $0.001$ ), and started regaining weight faster during the first 7 d after weaning (at 4 d and 7 d post-  
256 weaning; both  $P < 0.001$ ). However, the feed conversion efficiency was never affected ( $P >$   
257  $0.10$ ).

258 Supplemental milk offered during lactation did not affect feed intake, live weight,  
259 average weight gain nor feed conversion efficiency over the first 7 d after weaning ( $P > 0.10$ ).

260 The administration of oxytocin for the first 2 wk of life had no effect on feed intake for  
261 the first 7 d after weaning ( $P > 0.10$ ), but resulted in the pigs losing less weight over the first 2 d  
262 post-weaning ( $P = 0.03$ ). There was no effect of oxytocin on the feed conversion efficiency ( $P >$   
263  $0.10$ ).

264 Females seemed to better adapt to their new diet better than males, eating about 14 %  
265 more over the first 7 d after weaning ( $P < 0.05$ ). However, this greater feed intake in females did

266 not translate into immediate differences in terms of average weight gain nor feed conversion  
267 efficiency ( $P > 0.10$ ).

268

269 *3.3. Growth performance and feed intake from week 2 post-weaning to market slaughter weight*  
270 *(28-140 d of age)*

271 The pigs fed the post-weaning transitional diet (SMP mixture with the pellets) during the  
272 first 7 d after weaning showed a lower feed intake in the second wk post-weaning ( $P = 0.04$ ;  
273 Table 3). The pigs fed the pellets plus milk diet were heavier than the pigs fed pellets alone 7 d  
274 after weaning ( $P = 0.02$ ), but this difference disappeared once all pigs were offered dry pelleted  
275 feed ( $P > 0.10$ ).

276 Surprisingly, pigs that received supplemental milk during lactation showed heavier body  
277 weight than non-supplemented pigs at 49 and 56 d of age ( $P = 0.02$  and  $P = 0.05$  respectively).  
278 However, they did not differ in terms of feed intake, average weight gain nor feed conversion  
279 efficiency ( $P > 0.10$ ).

280 The administration of oxytocin during the first 2 wk of life resulted in heavier pigs at 49  
281 d of age ( $P = 0.04$ ), with a greater average weight gain from 42 to 49 d of age ( $P = 0.01$ ) and a  
282 better feed conversion efficiency ( $P = 0.03$ ). This increase in growth rate was associated with a h  
283 greater feed intake observed in the following wk from 49 to 56 d of age in pigs given oxytocin ( $P$   
284  $= 0.04$ ).

285 Females maintained an almost 15 % greater feed intake compared with males in the  
286 second wk after weaning ( $P = 0.01$ ), and had heavier body weight than males from 35 to 49 d of  
287 age ( $P < 0.05$ ). However, they did not show differences in terms of feed conversion efficiency ( $P$   
288  $> 0.10$ ).



289 The greater live weight observed in pigs supplemented with milk during lactation seen at  
290 49 and 56 d of age was still present at 140 d of age compared with pigs that were not  
291 supplemented ( $P = 0.05$ ). No differences were observed in P2 back fat across treatments ( $P >$   
292 0.10).

293

#### 294 3.4. Organ weights

295 For the euthanized pigs, administering oxytocin from 0 to 14 d of age caused a lower  
296 body weight at 10 d of age compared with saline-administered pigs (3.1 kg vs. 3.3 kg, SED = 0.2  
297 kg;  $P = 0.004$ ); however, this was likely an artifact of the small sample size at that time point ( $n$   
298 = 15: 7 oxytocin- and 8 saline-administered pigs) as this result did not appear for the pigs  
299 followed up throughout the experiment ( $n = 160$ ).

300 Oxytocin administration, milk supplementation during lactation or after weaning, or sex  
301 had no effect on any of the organ weights at any time point ( $P > 0.10$ ).

302

#### 303 3.5. Gene expression

304 At 10 d of age, leptin gene expression was greater in the stomach of oxytocin-treated  
305 pigs than those receiving saline during that period (9.4  $\Delta$  Ct vs. 15.0  $\Delta$  Ct; SED = 3.3  $\Delta$  Ct,  $P =$   
306 0.03). GLP-2 gene expression was greater in the ileum of female compared with male pigs (3.4  $\Delta$   
307 Ct vs. 7.5  $\Delta$  Ct, SED = 1.0  $\Delta$  Ct,  $P < 0.001$ ). No alterations in gene expression were observed in  
308 the duodenum (leptin, GLP-1, GLP-2) or for ghrelin in the stomach or leptin and GLP-1 in the  
309 ileum ( $P > 0.10$ ).

310 At 21 d of age, on the day of weaning, leptin gene expression was greater in the ileum of  
311 pigs that had not received supplemental milk in comparison to those that had been provided with

312 supplemental milk from day 10 of lactation ( $4.3 \Delta \text{ Ct}$  vs.  $9.4 \Delta \text{ Ct}$ ,  $\text{SED} = 2.2 \Delta \text{ Ct}$ ,  $P = 0.04$ ).  
313 GLP-2 gene expression was still greater in the ileum of female compared with male pigs ( $7.4 \Delta$   
314  $\text{Ct}$  vs.  $10.6 \Delta \text{ Ct}$ ,  $\text{SED} = 0.1 \Delta \text{ Ct}$ ,  $P < 0.001$ ). No alterations in gene expression were observed in  
315 either the stomach or duodenum ( $P > 0.10$ ).

316 At 28 d of age, 1 wk after weaning, both ghrelin and leptin gene expression were greater  
317 in the stomach of oxytocin-treated pigs compared with saline-treated pigs (both  $P = 0.02$ ; Table  
318 4). Leptin gene expression was also greater in the stomach of pigs that were weaned onto the  
319 pelleted diet supplemented with milk rather than onto pellets alone ( $P = 0.02$ ), and GLP-2 gene  
320 expression was greater in the ileum ( $P = 0.05$ ). The expression of GLP-1 gene was greater in the  
321 duodenum of male compared with female pigs ( $P = 0.03$ ).

322

#### 323 4. Discussion

324 Weaning pigs onto a milk-liquid-based diet and through a gradual transition to the dry  
325 pelleted diet in the first week after weaning resulted in immediate feed intake and partially  
326 alleviated the depression in growth rate commonly observed during this critical period. Weaning  
327 is a highly stressful period to the young animal, resulting in nutritional, thermal, immunological  
328 and psychological challenges [2,3,19]. Therefore, offering a gradual rather than an abrupt change  
329 in the diet after weaning is likely to have the most beneficial effect in reducing stressful effects  
330 on growth, possibly assisting in maintaining gut structure and function around that period [4,20].  
331 However, this effect was only transient and disappeared once the milk liquid diet was removed.  
332 The pigs not offered any of the SMP mixture compensated in the second week after weaning  
333 when all pigs were fed exclusively the dry pelleted diet, which coincided with the pigs

334 previously supplemented with milk for the first week reducing their intake once exclusively fed  
335 the dry pellet diet at 28 d of age.

336 Neonatal oxytocin administration given daily from 0 to 14 d of life reduced the extent of  
337 the weight loss in the first 2 d after weaning, suggesting a possible adaptive role for this hormone  
338 in the immediate post-weaning period. Weaning is a multi-factorial stressor for pigs in  
339 commercial settings. Oxytocin has been shown to be involved in the stress response of pigs  
340 [16,21], and may reduce the stress response associated with weaning since these pigs lost less  
341 weight without showing greater feed intakes. Similarly, repeated administration of oxytocin to  
342 rats for the first 14 d of life caused increased weighed gain [12] without changes in feed intake  
343 [11]. Other studies have shown that oxytocin administration caused a decrease in plasma  
344 corticosterone concentration, and increased circulatory levels of gastric hormones and insulin-  
345 like growth factors [12-14]. In the present study, oxytocin administration early in life did not  
346 influence live weight until 49 d of age. It has previously been demonstrated that increased weight  
347 gain observed in rats does not occur immediately after injection, and instead coincides with the  
348 onset of puberty and consequently with rises in circulating levels of growth hormone and steroid  
349 hormones [12]. However, the increased live weight gain observed with the pig does not coincide  
350 with the onset of puberty and therefore the reason for this delayed effect requires further  
351 research.

352 Milk supplementation or oxytocin administration did not generally affect feed conversion  
353 efficiency, back fat depth nor organ weights. Hence, the feed supplementations or administration  
354 of oxytocin did not appear to cause any metabolic or gross anatomical physiological changes.  
355 Notwithstanding, both oxytocin administration and milk supplementation increased leptin gene  
356 expression in the stomach 1 wk after weaning. Gastric leptin gene expression has been shown to

357 be under the influence of feed intake, with gastric leptin mRNA decreasing in fasting conditions  
358 and increasing rapidly after a short period of feed intake in rats [22]. Leptin acts on hypothalamic  
359 neurons involved in feed intake regulation and energy metabolism. However, little is known  
360 about leptin action in the small intestine, but possible roles have been deduced such as intestinal  
361 lipid handling or intestinal sugar absorption [23-25]. Our results also showed that, on the day of  
362 weaning, leptin was lower in the ileum of pigs that were supplemented with milk during lactation  
363 as compared with their counterparts suckling the sow alone. Leptin is secreted from the  
364 mammary gland and excreted along with milk and consumed by the young. Indeed, pigs feeding  
365 on sows' milk have greater intestinal leptin expression than pigs fed on milk replacer alone [26],  
366 explaining why pigs receiving supplemental milk during lactation in our experiment showed  
367 lower leptin expression as they may have reduced their sow milk intake by substituting some for  
368 supplemental milk. Oxytocin administration also increased ghrelin expression in the stomach 1  
369 wk after weaning. Opposite to leptin expression, ghrelin expression increases in the stomach in  
370 response to fasting and reduces within 45 min of re-feeding [27,28].

371 Milk supplementation after weaning also increased GLP-2 gene expression in the  
372 stomach 1 wk after weaning. Enhanced expression of intestinal GLP-2 has been coupled with  
373 increases in intestinal mucosal mass, increased villous height, crypt depth and brush border  
374 enzyme activity [29,30], which are all intestinal markers of small intestinal maturity and  
375 development. The influence of GLP-2 on intestinal growth and health occurs independently of  
376 feed intake and theoretically through suppression of proteolysis and apoptosis [29]. The greater  
377 intestinal mass induced by GLP-2 suggests that pigs submitted to a gradual diet transition over  
378 the first wk after weaning have enhanced digestive and absorptive functional capacity, which  
379 may in part explain the greater growth rate of these animals.

380 Pigs readily consumed a substantial amount of supplemental milk between day 10 and 20  
381 of lactation, from approximately 85 g/d to 252 g/d per pig over the 10 d of supplementation.  
382 However, there were no differences in pre-weaning growth performance. Nonetheless, and  
383 interestingly, providing supplemental milk to pigs during lactation influenced lifelong growth  
384 performance with pigs being heavier at 49 d of age and also upon reaching market slaughter  
385 weight. Other authors have observed similar effects [6-8], as the long-term benefits conferred by  
386 additional milk early in life may be mediated by specific components contained in the milk.

387 Females adapted better to weaning than males, with a greater feed intake over the 2 wk  
388 post-weaning period and a greater growth rate in subsequent weeks. This phenomenon has  
389 previously been demonstrated [31-33]. The better adaptation to weaning by females in our study  
390 appeared independent of changes in organ weight or organ development. However, our results  
391 show a greater expression of GLP-2 in the ileum of females at 10 d of age and at weaning, which  
392 may explain the better ability of females to cope with weaning since GLP-2 improves  
393 gastrointestinal tract development and function. Similarly, Pluske *et al.* [4] reported that females  
394 have a more developed gastrointestinal tract system, greater pancreatic enzymatic capacity and  
395 greater mean villous height than males at 2 and 4 wk of age. Males showed greater GLP-1 gene  
396 expression in the duodenum at 1 wk after weaning. Unabsorbed nutrients in the lumen of the  
397 small intestine appears to be an important stimulus for GLP-1 secretion [34], and this is thought  
398 to induce satiety along with delayed gastric emptying [35]. Therefore, the delayed maturation of  
399 the males' gut may explain their lower feed intake after weaning and slower digestion, which  
400 ultimately impacts their growth.

401

402 **Conclusions**

403 Providing pigs with a gradual change in diet after weaning is likely to have the most  
404 beneficial effect in reducing stressful effects on growth and preserving gut health, partly though  
405 increasing ghrelin and GLP-2 expression in the stomach. Oxytocin administration daily for the  
406 first 2 wk of life also conferred advantages by reducing weight loss over the first 2 d after  
407 weaning and increasing both ghrelin and leptin. However, the provision of supplemental milk  
408 during lactation had no effect on the response to weaning.

409 **References**

- 410 1 Jensen P, Recen B. When to wean: observations from free-ranging domestic pigs. *Appl.*  
411 *Anim. Behav. Sci.* 1989;23:49-60.
- 412 2 Pluske JR, Williams IH, Aherne FX. Nutrition of the neonatal pig. In: *The neonatal pig*  
413 *development and survival*, Varley MA (Ed.), CAB International, 1995, pp 187-235.
- 414 3 Weary DM, Jasper J, Hotzel MJ. Understanding weaning distress. *Appl. Anim. Behav.*  
415 *Sci.* 2008;110:24-41.
- 416 4 Pluske JR, Hampson DJ, Williams IH. Factors influencing the structure and function of  
417 the small intestine in the weaned pig: A review. *Livest. Prod. Sci.* 1997;51:215-236.
- 418 5 Azain MJ, Tomkins T, Sowinski JS, Arentson RA, Jewell DE. Effect of supplemental pig  
419 milk replacer on litter performance: Seasonal variation in response. *J. Anim. Sci.*  
420 1996;74:2195-2202.
- 421 6 Dunshea FR, Kerton DJ, Eason PJ, King RH. Supplemental skim milk before and after  
422 weaning improves growth performance of pigs. *Aust. J. Agr. Res.* 1999;50:1165-1170.
- 423 7 Wolter BF, Ellis M, Corrigan BP, DeDecker JM. The effect of birth weight and feeding  
424 of supplemental milk replacer to piglets during lactation on preweaning and postweaning  
425 growth performance and carcass characteristics. *J. Anim. Sci.* 2002;80:301-308.
- 426 8 King RH, Boyce JM, Dunshea FR. Effect of supplemental nutrients on the growth  
427 performance of suckling pigs. *Aust. J. Agr. Res.* 1998;49:883-887.
- 428 9 Pluske JR, Williams IH, Aherne FX. Villous height and crypt depth in piglets in response  
429 to increases in the intake of cows' milk after weaning. *Anim. Sci.* 1996;62:145-158.

- 430 10 Zijlstra RT, Whang KY, Easter RA, Odle J. Effect of feeding a milk replacer to early-  
431 weaned pigs on growth, body composition, and small intestinal morphology, compared  
432 with suckled littermates. *J. Anim. Sci.* 1996;74:2948-2959.
- 433 11 Uvnas-Moberg K, Alster P, Petersson M. Dissociation of oxytocin effects on body weight  
434 in two variants of female sprague-dawley rats. *Int. Physiol. Behav. Sci.* 1996;31:44-55.
- 435 12 Uvnas-Moberg K, Alster P, Petersson M, Sohlstrom A, Bjorkstrand E. Postnatal oxytocin  
436 injections cause sustained weight gain and increased nociceptive thresholds in male and  
437 female rats. *Pediatr. Res.* 1998;43:344-348.
- 438 13 Petersson M, Hulting A, Andersson R, Uvnäs-Moberg K. Long-term changes in gastrin,  
439 cholecystokinin and insulin in response to oxytocin treatment. *Neuroendocrinol.*  
440 1999;69:202-208.
- 441 14 Petersson M, Hulting A-L, Uvnäs-Moberg K. Oxytocin causes a sustained decrease in  
442 plasma levels of corticosterone in rats. *Neurosci. Lett.* 1999;264:41-44.
- 443 15 Kavushansky A, Leshem M. Role of oxytocin and vasopressin in the transitions of  
444 weaning in the rat. *Dev. Psychobiol.* 2004;45:231-238.
- 445 16 Rault J-L, Carter CS, Garner JP, Marchant-Forde JN, Richert BT, Lay Jr DC. Repeated  
446 intranasal oxytocin administration in early life dysregulates the hpa axis and alters social  
447 behavior. *Physiol. Behav.* 2013;112–113:40-48.
- 448 17 Uvnas-Moberg K. Oxytocin may mediate the benefits of positive social interaction and  
449 emotions. *Psychoneuroendocrinol.* 1998; 23:819-835.
- 450 18 Yuan J, Reed A, Chen F, Stewart CN. Statistical analysis of real-time pcr data. *BMC*  
451 *Bioinformatics* 2006;7:85.



- 452 19 Carroll JA, Veum TL, Matteri RL. Endocrine responses to weaning and changes in post-  
453 weaning diet in the young pig. *Domest. Anim. Endocrin* 1998;15:183-194.
- 454 20 Spreeuwenberg MAM, Verdonk JMAJ, Gaskins HR, Verstegen MWA. Small intestine  
455 epithelial barrier function is compromised in pigs with low feed intake at weaning. *J.*  
456 *Nutr.* 2001;131:1520-1527.
- 457 21 Rault J-L, Mack LA, Carter CS, Garner JP, Marchant-Forde JN, Richert BT, Lay Jr DC.  
458 Prenatal stress puzzle, the oxytocin piece: Prenatal stress alters the behaviour and  
459 autonomic regulation in piglets, insights from oxytocin. *Appl. Anim. Behav. Sci.*  
460 2013;148:99-107.
- 461 22 Pico C, Oliver P, Sanchez J, Palou A. Gastric leptin: A putative role in the short-term  
462 regulation of food intake. *Br. J. Nutr.* 2003;90:735-741.
- 463 23 Macajova M, Lamosova D, Zeman M. Role of leptin in farm animals: A review. *J. Vet.*  
464 *Med.* 2004;51:157-166.
- 465 24 Morton NM, Emilsson V, Liu YL, Cawthorne MA. Leptin action in intestinal cells. *J.*  
466 *Biol. Chem.* 1998;273:26194-26201.
- 467 25 Lostao MP, Urdaneta E, Martinez-Anso E, Barber A, Martinez JA. Presence of leptin  
468 receptors in rat small intestine and leptin effect on sugar absorption. *FEBS Lett.*  
469 1998;423:302-306.
- 470 26 Wolinski J, Biernat M, Guilloteau P, Westrom BR, Zabielski R. Exogenous leptin  
471 controls the development of the small intestine in neonatal piglets. *J. Endocrinol.*  
472 2003;177:215-222.

- 473 27 Sanchez J, Oliver P, Palou A, Pico C. The inhibition of gastric ghrelin production by food  
474 intake in rats is dependent on the type of macronutrient. *Endocrinol.* 2004;145:5049-  
475 5055.
- 476 28 Salfen BE, Carroll JA, Keisler DH, Strauch TA. Effects of exogenous ghrelin on feed  
477 intake, weight gain, behavior, and endocrine responses in weanling pigs. *J. Anim. Sci.*  
478 2004;82:1957-1966.
- 479 29 Shin ED, Drucker DJ, Brubaker PL. Glucagon-like peptide 2: An update. *Curr. Opin.*  
480 *Endocrinol. Diabetes* 2005;12:63-71.
- 481 30 Drucker DJ. Gut adaption and the glucagon-like peptides. *Gut* 2002;50:428-435.
- 482 31 Power GN, Pluske JR, Campbell RG, Cranwell PD, Kerton DJ, King RH, Dunshea FR.  
483 Effect of sex, weight and age on post-weaning growth of pigs. *Proc. Nut. Soc. Aust.*  
484 1996;20:137.
- 485 32 Dunshea FR, Brown WG, Gough CD, Eason PJ. Female pigs better handle weaning than  
486 male pigs. *Proc. Nut. Soc. Aust.* 1998;22:103.
- 487 33 Dunshea FR, Kerton DJ, Eason PJ, King RH. Supplemental skim milk before and after  
488 weaning improves growth performance of pigs. *Aust. J. Agr. Res.* 1999;50:1165-1170.
- 489 34 Van Ginneken C, Verlinden K, Van Meir F, Sys S, Weyns A. A stereologic evaluation of  
490 glucagon-like peptide-1 (glp-1) mucosal cells in the small intestine of the developing pig.  
491 *Anat. Embryol.* 2002;205:153-157.
- 492 35 Larsen PJ, Holst JJ. Glucagon-related peptide 1 (glp-1): Hormone and neurotransmitter.  
493 *Regul. Peptides* 2005;128:97-107.

494 **Table 1.** List of primers and optimised conditions to quantify genes of interest.

495

496 **Table 2.** Effect of oxytocin administration (OT), sex (S), and supplemental milk feeding during lactation (L), and pellets  
497 supplemented with milk diet post-weaning (W), on growth and dry matter (DM) feed intake from 21 d of age (weaning) to 28 d of age  
498 (1 wk after weaning)

499

500 **Table 3.** Effects of oxytocin administration (OT), sex (S), supplemental milk feeding during lactation (L), and pellets supplemented  
501 with milk diet post-weaning (W), on growth and dry matter (DM) feed intake from 28 d of age (1 wk after weaning) to 140 d of age  
502 (market slaughter weight).

503

504 **Table 4.** Effects of oxytocin administration (OT), sex (S), supplemental milk feeding during lactation (L), and pellets supplemented  
505 with milk diet post-weaning (W), on gene expression according to the RT-PCR  $\Delta$ CT of pigs slaughtered at 28 d of age.

506 **Figure 1.** Liquid skim milk intake of nursing litters offered supplemental milk between 10 and  
507 20 d of age (pre-weaning).

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**Table 1.** List of primers and optimised conditions to quantify genes of interest.

Gene (Abbreviation)	Accession number	Primer sequence	Optimum annealing temperature (°C)	Primer concentration (nM)
<i>18s</i> Ribosomal RNA (r18S1)	AY265350	Forward 5' GAA CGC CAC TTG TCC CTC TA 3' Reverse 5' GAC TCA ACA CGG GAA ACC TC 3'	61.2	60
Ghrelin	NM213807	Forward 5' CAC CAG AAA GTG CAG CAG AG 3' Reverse 5' GAA CAG AGG TGG CTG GTC TC 3'	57.0	200
Leptin (Lep1)	NM213840	Forward 5' CCT CTG AAT GGT CTG GGT TG 3' Reverse 5' GGA CTT GGG ACC ATC TGC TA 3'	57.5	400
Proglucagon (GLP-1)	AY242124	Forward 5' ACC ATT TAC TTT GTG GCT GGA 3' Reverse 5' GAG CTG GGA ATG ATC TGG ATT 3'	58.2	200
Glucagon-like peptide 2 (GLP-2)	NM214324	Forward 5' GCT GAC CAG TGA CAA TGA CC 3' Reverse 5' GGC ACC GGA ATC TCC TAG TC 3'	58.5	300

**Table 2.** Effect of oxytocin administration (OT), sex (S), and supplemental milk feeding during lactation (L), and pellets supplemented with milk diet post-weaning (W), on growth and dry matter (DM) feed intake from 21 d of age (weaning) to 28 d of age (1 wk after weaning)

	Administration		Sex		Pre-weaning		Post-weaning			Significance (P-values)			
	Oxytocin	Saline	Female	Male	Milk	Unsuppl- emented	Unsuppl- emented	Pellets + milk	SED	OT	S	L	W
<b>Liveweight (kg)</b>													
d 21	6.5	6.5	6.5	6.5	6.7	6.4	6.5	6.5	0.16	0.92	0.86	0.32	-
d 23	6.2	6.5	6.7	6.5	6.6	6.6	6.3	6.9	0.18	0.49	0.36	0.74	<0.01
d 25	6.9	6.9	7.0	6.8	7.0	6.8	6.7	7.1	0.17	0.88	0.48	0.39	<0.01
d 28	7.4	7.4	7.5	7.3	7.5	7.2	7.2	7.6	0.17	0.94	0.32	0.28	0.02
<b>Average weight gain (g/d)</b>													
Wean to d													
23	-214	-293	-236	-272	-301	-207	-373	-135	25.4	0.03	0.39	0.32	<0.01
Wean to d													
25	52	45	54	43	48	49	8	89	13.1	0.59	0.45	0.99	<0.01
Wean to d													
28	106	94	108	91	104	96	76	124	9.6	0.33	0.16	0.54	<0.01
<b>DM feed intake (g/d)</b>													
Wean to d													
23	369	374	397	346	367	376	51	692	16.7	0.70	0.05	0.77	<0.01
Wean to d													
25	342	349	371	320	351	341	91	601	12.1	0.59	0.01	0.67	<0.01
Wean to d													
28	343	350	369	324	352	341	131	561	11.2	0.58	0.02	0.54	<0.01

**Table 3.** Effects of oxytocin administration (OT), sex (S), supplemental milk feeding during lactation (L), and pellets supplemented with milk diet post-weaning (W), on growth and dry matter (DM) feed intake from 28 d of age (1 wk after weaning) to 140 d of age (market slaughter weight).

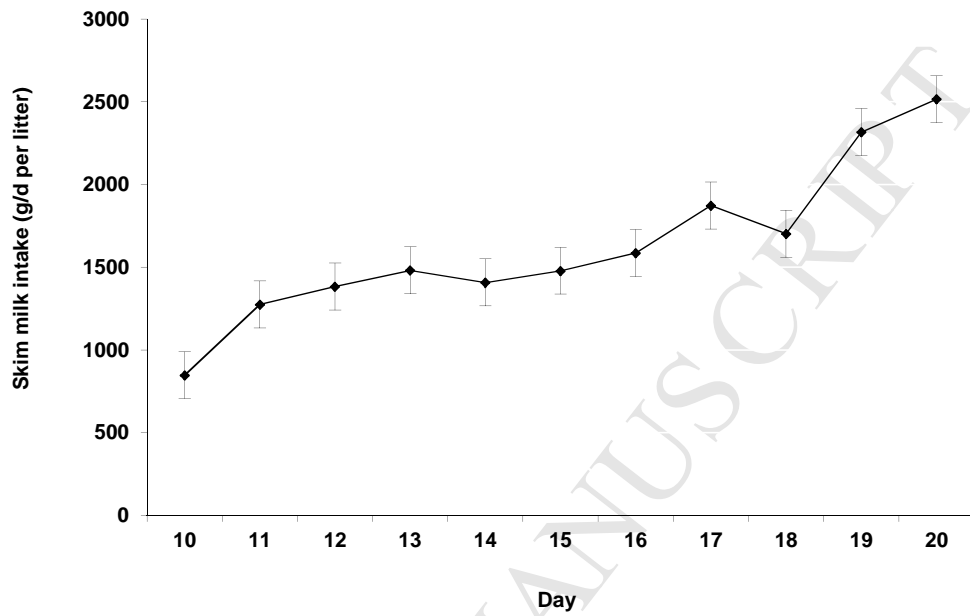
	<b>Administration</b>		<b>Sex</b>		<b>Pre-weaning</b>		<b>Post-weaning</b>			<b>Significance (P-values)</b>			
	Oxytocin	Saline	Female	Male	Milk	Unsupplemented	Unsupplemented	Pellets + milk	SED	OT	S	L	W
<b>Liveweight (kg)</b>													
d 28	7.4	7.4	7.5	7.3	7.5	7.2	7.2	7.6	0.18	0.94	0.32	0.28	0.02
d 35	9.7	9.4	9.8	9.2	9.9	9.2	9.4	9.7	0.29	0.39	0.04	0.07	0.42
d 42	12.7	12.4	12.9	12.2	13.0	12.1	12.5	12.6	0.37	0.42	0.04	0.08	0.88
d 49	17.4	16.5	17.4	16.5	17.6	16.4	17.0	16.9	0.43	0.04	0.04	0.02	0.88
d 56	22.4	21.6	22.4	21.6	22.6	21.4	21.9	22.1	0.52	0.12	0.08	0.05	0.68
d 140	90.0	87.0	87.9	89.3	86.4	90.5	87.6	89.3	1.72	0.11	0.44	0.05	0.39
<b>Average weight gain (g/d)</b>													
d28 to d35	340	325	350	315	349	316	337	328	23.0	0.54	0.13	0.20	0.63
d35 to d42	441	443	461	423	459	425	449	435	27.3	0.89	0.16	0.35	0.66
d42 to d49	672	593	640	625	655	610	638	627	29.4	0.01	0.57	0.20	0.65
d49 to d56	711	726	724	713	726	711	703	734	32.8	0.72	0.74	0.61	0.26
<b>DM feed intake (g/d)</b>													
d28 to d35	412	389	428	373	416	385	423	378	22.1	0.30	0.01	0.34	0.04
d35 to d42	710	711	728	693	726	694	705	716	32.3	0.92	0.30	0.45	0.70
d42 to d49	1060	1001	1064	997	1043	1018	1044	1017	39.8	0.13	0.09	0.63	0.47
d49 to d56	1284	1200	1257	1227	1277	1207	1217	1267	43.3	0.04	0.46	0.10	0.18

**Table 4.** Effects of oxytocin administration (OT), sex (S), supplemental milk feeding during lactation (L), and pellets supplemented with milk diet post-weaning (W), on gene expression according to the RT-PCR  $\Delta$ Ct of pigs slaughtered at 28 d of age.

	Administration		Sex		Pre-weaning		Post-weaning			Significance (P- values)			
	Oxytocin	Saline	Female	Male	Milk	Unsuppl- emented	Dry	Pellets +milk	SED	OT	S	L	W
<b>Stomach</b>													
Ghrelin	3.22	8.10	5.06	6.26	5.71	5.61	5.52	5.81	3.25	0.02	0.31	0.35	0.90
Leptin	6.17	8.89	7.24	7.82	8.35	6.72	9.21	5.86	1.49	0.02	0.93	0.20	0.02
<b>Duodenum</b>													
Leptin	3.91	5.58	4.85	4.64	5.44	4.04	4.89	4.60	1.48	0.61	0.73	0.43	0.94
GLP-1	7.00	7.99	8.93	6.06	7.91	7.08	6.36	8.63	1.5	0.36	0.03	0.55	0.08
GLP-2	5.02	6.99	5.78	6.27	6.22	5.79	5.61	6.41	1.02	0.06	0.50	0.98	0.51
<b>Ileum</b>													
Leptin	5.77	4.63	5.46	4.94	6.23	4.16	4.38	6.02	1.37	0.49	0.78	0.08	0.24
GLP-1	5.13	3.17	5.46	2.83	4.74	3.56	4.25	4.05	2.91	0.10	0.52	0.64	0.31
GLP-2	9.14	7.11	8.18	8.07	9.12	7.13	7.11	9.14	1.25	0.08	0.82	0.07	0.05



**Figure 1.** Liquid skim milk intake of nursing litters offered supplemental milk between 10 and 20 d of age (pre-weaning).



## Highlights

- Weaning is a multi-factorial stressor associated with a major diet change
- Neonatal oxytocin and milk supplemented post-weaning reduced weight loss
- Neonatal oxytocin increased stomach ghrelin and leptin expression
- Milk supplemented post-weaning increased stomach leptin and GLP-2 expression
- Neonatal oxytocin or post-weaning milk supplementation improve gut health at weaning