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Birth weight has no influence on the morphology, digestive capacity and motility of the small intestine in suckling pigs

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ABSTRACT

Mortality and morbidity of newborn piglets are an economic burden and a threat to animal welfare. Perinatal mortality is particularly high in low birth weight (LBW) piglets. To unravel the possible underlying mechanisms, we investigated the effect of birth weight on morphology of the small intestine, *in vivo* motility, brush border enzyme activities (lactase, sucrase, maltase, aminopeptidase A, aminopeptidase N and dipeptidylpeptidase IV) and crypt cell proliferation (Ki-67 immunohistochemistry). This was performed in pairs of normal birth weight (NBW; 1.47 ± 0.29 kg) and LBW (0.84 ± 0.21 kg) piglets during the suckling period (0, 3, 10 and 28 d of age). Our study showed no effects on small intestinal morphology, except for an age-related increase in villus width ($P=0.02$), crypt depth ($P<0.05$) and thickness of the tunica muscularis ($P<0.001$). Regarding *in vivo* small intestinal motility, the peak distribution of the Evans bleu dye (geometric centre) was more progressed in NBW piglets of 28 d of age ($P=0.03$) compared to the other groups indicating more propulsive strength. Brush border enzyme activities were similar in NBW and LBW piglets. However, age- and region-related differences were present, except for the aminopeptidase A activity ($P=0.33$). Lactase activity was higher in the proximal ($P<0.001$) than in the distal part of the small intestine in all age groups and for both regions the lowest in piglets of 28 d old ($P<0.05$). In the proximal part of the small intestine, sucrase and maltase activities were increased in 10 and 28 d old piglets (sucrase: $P<0.001$; maltase: $P<0.001$), whereas in the distal part of the small intestine the highest activities were observed in 28 d old piglets (sucrase: $P<0.005$; maltase: $P<0.001$). No effect of birth weight ($P=0.74$) on the Ki-67 proliferation index was observed. However, 10 d old piglets had less proliferating cells compared to newborn and 28 d old piglets ($P=0.04$). Intestinal fatty acid binding protein (i-FABP), a marker of damage of the intestinal mucosa was undetectable in LBW and NBW piglets throughout the suckling period. To conclude, birth weight had no influence on small intestinal development and function. However, the results confirm that the development and function of the small intestine alters with age.

1. Introduction

Genetic selection and improved management techniques in pork production have resulted in larger litters but, as a consequence, also in higher birth weight variation and reduced mean piglet birth weight. LBW piglets are characterized by increased mortality and lower growth rates (Lay et al., 2002; Gondret et al., 2005). In the early postnatal period, the small intestine immediately needs to digest and absorb nutrients to sustain the high growth rate in the neonate. Consequently, the small intestine undergoes structural and functional changes (Pacha, 2000). In pigs this is illustrated by an alteration in activity of enterocyte brush border enzymes (Aumaitre and Corring, 1978; Huërou-Luron et al., 2000), a shift in villus shape and length, facilitated by altered apoptosis/mitosis ratios (Cera et al., 1988; Zhang et al., 1997; Zhang et al., 1998; Brown et al., 2006; Zabielski et al., 2008). Therefore these parameters are useful markers of intestinal differentiation. We hypothesized that LBW piglets have an altered digestive capacity. To this extend, the effect of birth weight, age and region on the morphology of the small intestine, activity of brush border enzymes and *in vivo* motility (gastric emptying, intestinal transit time, and peak distribution of Evans blue by means of the estimation of the geometric centre) is investigated in NBW and LBW

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piglets in the suckling period. In humans, it is demonstrated that gastrointestinal transit time, and especially the rate of gastric emptying, alters with age and most changes occur during suckling (Kaye, 2011). Whether similar changes occur in the pig and perhaps differ according to birth weight is unknown. Nonetheless this parameter is important since gastric emptying and transit time are key-factors in small intestinal digestive capacity. Thus gastric emptying, small intestinal transit time and geometric centre of small intestinal transit time, are measured to assess *in vivo* gastrointestinal motility. To our knowledge, this study reports for the first time *in vivo* intestinal motility in NBW and LBW piglets.

2. Material and methods

2.1. Animal selection

All experimental procedures involving animals were approved by the Ethical Committee of Animal Experimentation, University of Antwerp, Belgium. Sows (32, parity between 2 and 5, litter size 12 ± 0.9, mean litter birth weight 1.37 ± 0.25 kg) were kept under conventional Belgian commercial pig housing conditions.

A total of 64 crossbred piglets (Piétrain × (Finnish Yorkshire × Belgian Landrace)) obtained from a commercial farm were selected at birth and euthanized by severing the carotid arteries under deep barbiturate anaesthesia (intraperitoneal, sodium pentobarbital, 200 mg/kg, Kela Laboratoria, Hoogstraten, Belgium) either at d 0 (within 24 h after birth, after colostrum intake), d 3, d 10 or d 28 ($n=16$/age group). Piglets with a birth weight less than 1 kg and that deviated 1.5 SD from the mean birth weight of the litter were considered LBW (0.84 ± 0.21 kg), whereas piglets that deviated ± 0.5 SD from the mean birth weight of the litter were considered NBW (1.47 ± 0.29 kg). Each age-group consisted of 16 gender-matched piglets, i.e. 2 littermate piglets (LBW and NBW) from 8 different litters. Of each age group 10 piglets, i.e. 5 littermate LBW and NBW piglets were euthanized for small intestinal sampling. The remaining 6 piglets, i.e. 3 littermate LBW and NBW piglets were euthanized to assess *in vivo* gastro-intestinal motility. During the suckling period, piglets had free access to water and creep feed (starting at 10 d of age, Biggyfeed, AVEVE, Leuven, Belgium). Throughout the suckling period LBW piglets were not supported and all piglets survived.

2.2. Small intestinal transit time and gastric emptying

In vivo gastrointestinal motility was evaluated using Evans blue (Sigma-Aldrich, Bornem, Belgium). Piglets were fasted for 1 h and *in vivo* transit of a liquid bolus marker was assessed (De Winter et al., 2002; Seerden et al., 2005) by intra-gastrical dosing of 1 ml Evans blue (50 mg/ml in 0.9% NaCl) per kg BW. After 30 min piglets were euthanized and the small intestine was dissected. To prevent leakage of the Evans blue solution, the stomach was clamped proximal to the lower oesophageal sphincter and distal to the pylorus. The stomach and small intestine were resected and the distance from the pylorus to the most distal point of migration of Evans blue was measured. This was expressed as a percentage of Evans blue migration compared to the total length of the small intestine and used as a measurement of small intestinal transit time. The small intestine was divided into 10 segments of equal length. The amount of Evans blue in the stomach and the intestinal segments were measured spectrophotometrically to assess gastric emptying and geometric centre of small intestinal transit. The latter is the standard technique to quantify distributional changes in transit in the gastrointestinal tract. It represents a weighed mean of the distribution of Evans blue within the small intestine and is sensitive to both changes in distribution and the distance travelled by Evans blue and is thus a more reliable marker for intestinal transit (Miller et al., 1981). The geometric centre was calculated using the following equation, with A being the absorbance measured at a wavelength of 565 nm, the number of segment referring to a figure between 1 and 10 that were given to the equally in length different segments of the small intestine (the entire small intestine was divided in 10 parts):

$$\text{Geometric centre} = \sum [(A \text{ Per segment}) \times \text{number of segment})]/\text{total A}$$

Gastric emptying was calculated using the following equation, with A being the absorbance measured at a wavelength of 565 nm, A(small intestine 1–10) refers to the sum of the absorbance in the 10 different small intestinal segments, A(stomach + small intestine 1–10) refers to the sum of the absorbance measured in the stomach added to the absorbance measured in the small intestine:

$$\%\ \text{Gastric emptying} = [\Sigma A(\text{small intestine } 1\text{–}10)/\Sigma A(\text{stomach} + \text{small intestine } 1 - 10)] \times 100$$

2.3. Sample collection

After euthanasia, the gastrointestinal tract was removed, rinsed and its length and wet weight were recorded. The small intestine was divided into a proximal (at 25% of total small intestinal length) and distal part (at 75% of total small intestinal length) and multiple 2–3 cm tissue samples were snap frozen in liquid nitrogen and stored at −80 °C until further analysis or immersed in 4% (wt/vol) paraformaldehyde solution for 2 h. After fixation, samples were rinsed with PBS (pH 7.4) for 24 h and paraffin embedded. From these tissue blocks, 5 μm sections were made and either conventionally stained, with haematoxylin-eosin or immunohistochemically (see below), after dewaxing and rehydrating with distilled water.

2.4. Small intestinal morphology

Samples of fixed small intestinal tissue from the proximal and distal part were embedded in paraffin, sectioned (5 μm) and stained with haematoxylin-eosin. Crypt depth, villus length, villus width, thickness of the tela submucosa and tunica muscularis were measured (Olympus BX 61, analySIS Pro®, Olympus Belgium, Aartselaar, Belgium) in 30 longitudinally villus-crypt columns and associated small intestinal wall component per region, per piglet in 10 sections per tissue sample.

2.5. Ki-67 immunohistochemistry

After antigen retrieval with citrate buffer (pH 6, Dako, Glostrup, Denmark) paraffin sections were incubated with 3% H_2O_2 and 20% normal goat serum. Subsequently, sections were incubated overnight at 4 °C with monoclonal mouse anti-human Ki-67 antibody (1:25, Dako, Glostrup, Denmark) diluted in 50 mM Tris(hydroxymethyl) aminomethane-buffered saline (TBS) solution enriched with 1% bovine serum albumin (BSA). After rinsing in 50 mM TBS (pH 7.4), sections were incubated with biotinylated goat anti-mouse antibody (Dako, Glostrup, Denmark) for 30 min at room temperature. Following rinsing with TBS, sections were incubated for 30 min with streptavidin-conjugated horseradish peroxidase (1:200 diluted in TBS/BSA, Dako, Glostrup, Denmark) at room temperature. After rinsing with TBS and demineralized water proliferating cells were visualized with 3,3′-diaminobenzidinetetrahydrochloride (DAB) and sections were counterstained with haematoxylin. The number of immunoreactive cells were quantified in 30 well-oriented crypt-villus units per region, per piglet in 10 sections per tissue sample and expressed as the number of Ki-67 positive nuclei per 100 cells, i.e. the proliferation index.