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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.

Key words: Low birth weight; piglet; small intestine; *in vivo* motility

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 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 ~~also~~ 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.

Material and methods

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 $\times$ (Finnish Yorkshire $\times$ 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.

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):

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

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:

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

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.

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**.

Ki-67 immunohistochemistry

After antigen retrieval with citrate buffer (pH 6, Dako, Glostrup, Denmark) paraffin sections were incubated with 3% H₂O₂ 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.

Enzyme activities

Intact frozen small intestinal tissue segments of the proximal and distal part of the small intestine were homogenized in 1% Triton X-100 solution. Lactase, maltase and sucrase activities were determined with lactose, maltose and sucrose as substrates. Aminopeptidase A, aminopeptidase N and dipeptidylpeptidase IV were kinetically measured by spectrophotometry using Glu-p-nitroaniline, L-alanine 4-nitro-anilide hydrochloride and H-glycyl-prolyl-p-nitroaniline p-tosylate (Sigma-Aldrich, Bornem, Belgium) as substrates, respectively (De Vos *et al.*, 2014). As the enzyme activity was measured in intact tissue segments, enzyme activities were expressed as units per gram wet tissue. Hence, one unit of activity was defined as the amount of enzyme that hydrolyses 1 μ mole substrate per min.

I-FABP

Intestinal fatty acid binding protein (i-FABP) can be used as a sensitive marker of damage of the intestinal mucosa in pigs (Niewold *et al.*, 2004). Using a commercial available ELISA kit (Human i-FABP, HyCult Biotechnology, Uden, The Netherlands), i-FABP was determined in serum according to the manufacturer's instructions.

Statistical analysis

A linear mixed model was fitted to model the data (for all parameters). The model included birth weight, age, and if applicable region, and their interaction as fixed effects. To account for the dependence between observations within the same litter and animal, random effect terms for litter and animal, nested within litter, were added to the model. Significance of the fixed effect terms was tested using an F-test with a Kenward-Roger correction for the number of degrees of freedom. If the effect of age was significant, a posthoc test was performed with a Tukey correction for multiple testing. Calculations were performed in the statistical package R, version 2.13.1. Mixed models were fitted using the lmer function in the lme4 package. Posthoc tests were completed using the multcomp package. The F-test with Kenward-Roger correction was carried out as implemented in the pbkrtest package.

Results

Daily growth

Daily growth was on average 80 g/d lower in LBW piglets compared to NBW piglets. In addition, the results of the body weight data at d 3 (LBW: 1.01 ± 0.35 kg; NBW: 1.73 ± 0.36 kg), at d 10 (LBW: 2.40 ± 0.47 kg; NBW: 3.77 ± 0.23 kg) and at d 28 (LBW: 5.3 ± 0.84 kg; NBW: 8.2 ± 0.95 kg) demonstrated that piglets born with lower weights remained lighter than those born with normal weights, indicating that the LBW piglets did not catch up with NBW piglets during the suckling period.

Small intestinal transit time and gastric emptying

Gastric emptying did not differ according to birth weight ($P = 0.55$) or age ($P = 0.77$). For intestinal transit time no effect of birth weight ($P = 0.53$) or age ($P = 0.77$) was

observed. However, a significant interaction between birth weight and age ($P = 0.02$) was observed for the geometric centre, i.e. NBW piglets of 28 d of age had a significantly higher geometric centre ($P = 0.03$). A high geometric centre implies a faster small intestinal transit (Table 1).

Small intestinal morphology

Piglets with a normal birth weight had heavier stomachs than LBW piglets ($P < 0.001$) and the weight of the stomach increased with age ($P < 0.001$) (Table 2). Notwithstanding that the relative weight of the stomach was higher in LBW piglets ($P < 0.001$) compared to their NBW littermates. No difference between the age groups could be observed ($P = 0.96$) (Table 2).

For absolute small intestinal length, effects of birth weight ($P < 0.001$) and age ($P < 0.001$) were observed (Table 2). Piglets with a normal birth weight had a longer small intestine compared to LBW piglets. An increase in length of the small intestine was observed with increasing age, except for newborns and piglets of 3 d of age where the length of the small intestine was comparable. An interaction for the relative small intestinal length (adjusted for body weight) was observed between age and birth weight ($P = 0.03$) (Table 2). Newborn and 28 d old NBW piglets had a relatively shorter small intestine compared to their age-matched LBW counterparts ($P < 0.001$). In the other age groups, the relative length of the small intestine between NBW and LBW piglets was not significantly different. An interaction for the absolute weight of the small intestine was observed ($P = 0.02$). The weight of the small intestine is higher in NBW piglets and this effect was more significant in piglets of 10 d of age ($P < 0.001$) than in the other age groups ($P = 0.001$). The relative weight of the small intestine is higher in LBW piglets ($P = 0.04$) and did not alter with age ($P = 0.73$).

Intestinal villus height did not differ with birth weight ($P = 0.20$), age ($P = 0.20$) or region ($P = 0.58$) (Table 3). Villus width did not alter according to birth weight ($P = 0.06$) or region ($P = 0.93$) (Table 3). However, villus width significantly differed in the different age groups ($P = 0.03$) (Table 3). Newborn piglets had significantly slender villi than 10 d ($P = 0.03$) and 28 d ($P = 0.003$) old piglets and piglets of 3 d of age had more slender villi than piglets aged 28 d ($P = 0.05$). No effect of birth weight on crypt depth was detected ($P = 0.07$) but an interaction between age and region ($P = 0.02$) was present (Table 3). In the proximal part of the small intestine, crypt depth significantly increased from d 3 onwards, with the deepest crypts measured in 28 d old piglets. In the distal region of the small intestine, the crypts of 28 d old animals were significantly deeper than crypts of newborn and 10 d old piglets ($P = 0.01$). No effect of birth weight ($P = 0.74$), age ($P = 0.28$) or region ($P = 0.53$) on the thickness of the tela submucosa was observed (Table 3). The thickness of the tunica muscularis did not differ with birth weight ($P = 0.47$) or the small intestinal region ($P = 0.42$) investigated (Table 3). Age had a significant effect on the thickness of the tunica muscularis as newborn and 3 d old piglets had a thinner tunica muscularis compared to piglets of 10 d or 28 d old (Table 3).

(Part of the results on small intestinal morphology were already reported (Huygelen *et al.* 2014)).

Ki-67 immunohistochemistry

Ki-67 immunoreactivity was predominantly located in the crypts. No effect of birth weight ($P = 0.74$) or region ($P = 0.80$) was observed but a significant effect of age (P

= 0.04) was detected. Piglets of 10 d of age had significantly less proliferating cells compared to 0 d and 28 d old piglets (Figure 1A and B).

Enzyme activities

Birth weight had no effect on lactase activity in the small intestine ($P = 0.51$). Lactase activity was highest in the proximal part ($P < 0.001$) of the small intestine and the activity decreased towards the end of the suckling period as, piglets of 28 d old had lowest lactase activities ($P < 0.05$; Figure 2A). Birth weight had no influence on the sucrase activity ($P = 0.93$). However, an interaction between age and region was detected ($P < 0.001$). In the proximal part of the small intestine, sucrase activity increased in piglets of 10 and 28 d old ($P < 0.001$), in the distal part 28 d old piglets had the highest sucrase activity compared to the other age groups ($P < 0.005$; Figure 2B). No differences in maltase ($P = 0.23$) activity were seen in LBW or NBW piglets. However, maltase activities varied among the different intestinal regions ($P < 0.001$). Maltase activity was increased in the proximal part of the small intestine in 10 and 28 d old piglets ($P < 0.001$), whereas in the distal part, 28 d old piglets had the highest maltase activity compared to the other age groups ($P < 0.001$; Figure 2C). Birth weight ($P = 0.86$) and age ($P = 0.33$) had no effect on aminopeptidase A activity (Figure 2D). Aminopeptidase N activity did not differ ($P = 0.16$) between LBW and NBW piglets. A significant interaction was observed between age and region for aminopeptidase N activity ($P = 0.01$). In the proximal part the highest activities were detected in newborn piglets ($P < 0.001$). In the distal part the activity of aminopeptidase N differed between 10 d and 28 d old piglets ($P = 0.007$, Figure 2E). No effect of birth weight on dipeptidylpeptidase IV ($P = 0.75$) activity was observed. A

significant interaction was observed between age and region for dipeptidylpeptidase IV ($P < 0.001$). In the proximal part of the small intestine the highest activities were detected in piglets of 0 d of age ($P = 0.002$) whereas in the distal part the highest dipeptidylpeptidase IV activity was noticed in piglets of 10 d of age ($P < 0.001$; Figure 2F).

I-FABP

Throughout the suckling period i-FABP levels were undetectable (detection limit of the assay 47 pg/ml) in both LBW and NBW piglets (results not shown).

Discussion

The present study investigated the effect of low birth weight on intestinal morphology, brush border hydrolases and *in vivo* motility in naturally suckled piglets of 0, 3, 10 and 28 d of age. The weight of the stomach and the small intestine was higher in NBW piglets compared to their LBW littermates throughout the suckling period. However, the relative weight of the stomach and small intestine was higher in LBW piglets, as previously reported (Xu *et al.*, 1994, D'Inca *et al.*, 2011). Birth weight had no effect on villus height, villus width, crypt depth or on the thickness of the investigated intestinal layers (tela submucosa and tunica muscularis). Accordingly, no detectable i-FABP levels could be measured throughout the suckling period in LBW or NBW piglets, which is in line with a previous study in neonatal piglets (Cheung *et al.*, 2012). This suggests that the mucosa was not damaged in these piglets, as

302 already suggested by the unaltered villus height. Furthermore, no effect of birth
303 weight on brush border disaccharidases and peptidases was observed. This is in line
304 with a previous study in which no difference in lactase activity was observed in LBW
305 and NBW piglets of 7 d of age (Boudry *et al.*, 2011), but contrasts with other reports
306 that suggested that a low birth weight changes the maturation pattern of lactase (Xu
307 *et al.*, 1994). With regard to the other disaccharidases, reports in literature are
308 contradictory (Xu *et al.*, 1994, Che *et al.*, 2010, D'Inca *et al.*, 2010, D'Inca *et al.*,
309 2011). In our study we were not able to show that sucrase and maltase differ in
310 activity according to birth weight. However, it has been shown that in non-suckled
311 piglets a low birth weight was associated with precocious occurrence of maltase and
312 sucrase activity (Xu *et al.*, 1994), whilst other studies reported no differences in
313 sucrase activity according to birth weight (D'Inca *et al.*, 2010, D'Inca *et al.*, 2011) and
314 others reported a lower sucrase activity in LBW piglets, 2 d after birth, compared to
315 NBW piglets (Che *et al.*, 2010). Inconsistency in sampling segments throughout the
316 small intestine and ages that are examined can contribute to these observed
317 differences in enzyme activities. As previously reported, aminopeptidase N and
318 dipeptidylpeptidase IV activities were not altered in LBW piglets compared to NBW
319 piglets (Boudry *et al.*, 2011).

320 In contrast to the lack of differences between LBW and NBW piglets, the
321 morphometric parameters changed with age. Intestinal crypts elongated and villus
322 width increased during the suckling period, whereas villus length remained constant
323 throughout the suckling period. This apparently contrasts with findings of other
324 authors who showed that villus length, in addition to villus width and crypt depth,
325 increased until 3 d after birth (Zhang *et al.*, 1997, Zhang *et al.*, 1998, Skrzypek *et al.*,
326 2010). After this initial period, characterized by elongating villi, the villi shorten again

327 giving rise to the leaf-like shaped villi seen in older pigs (Cera *et al.*, 1988, Brown *et*
328 *al.*, 2006). In this respect it needs to be mentioned that in our study we could have
329 missed the early increase and subsequent decrease and return to the initial villus
330 height since only samples on d 0, d 3, d 10 and d 28 were analysed. The increased
331 villus width that was observed corresponds to the change in villus shape from finger
332 to leaf-like that was reported by others (Cera *et al.*, 1988, Brown *et al.*, 2006).
333 Nevertheless, these light microscopic morphometric measurements do not fully
334 consider differences in the three-dimensional structure of villi and crypts. Also, the
335 number and density of the villi have to be taken into account to accurately estimate
336 the area available for absorption.

337 In our study, the proliferation index of the small intestine was highest in newborn and
338 piglets of 28 d of age. In the eldest piglets, deepening of the crypts reflected this
339 increase in proliferating cells. The increase in crypt depth might be associated with
340 creep feed intake, as it has been shown that creep feeding results in longer villi and
341 deeper crypts (van der Meulen *et al.*, 2010). However, previous studies have shown
342 that creep feed consumption prior to weaning is highly variable and that intake can
343 be considered as negligible before 4 weeks of age (Pluske *et al.*, 1995, Bruininx *et*
344 *al.*, 2002). This may explain why other authors reported no effect of creep feeding on
345 crypt-villus architecture (Kelly *et al.*, 1990).

346 Reports on *in vivo* motility in pigs during the suckling period are scarce (Wright *et al.*,
347 1998) and to our knowledge this is the first study that investigates *in vivo* motility with
348 a liquid marker in suckling LBW piglets. Earlier reports on *in vivo* gastrointestinal
349 motility focused more on characterizing fasting and non-fasting myoelectrical activity
350 in pigs of a few days old up to adulthood (Ruckebush *et al.*, 1981, Burrows *et al.*,
351 1986, Groner *et al.*, 1990, Laerke *et al.*, 2000, Naughton *et al.*, 2008). In order to

detect an effect of birth weight and age and to correlate with the migratory motor complex (MMC) and myoelectrical activity recordings, we opted for studying the fasting in vivo motility. No differences in gastric emptying and intestinal transit time and geometric centre of small intestinal transit could be observed in LBW and NBW piglets, nor was an age dependency detected. Indeed, in neonatal piglets the pattern of motility does not alter in piglets from 2 to 6 d after parturition (Wright *et al.*, 1998), nor does the underlying myoelectrical migratory activity changed in fasted piglets during the suckling period (Ruckebush *et al.*, 1981, Burrows *et al.*, 1986, Groner *et al.*, 1990, Laerke *et al.*, 2000, Naughton *et al.*, 2008). Additionally, previous descriptions of the neuro-endocrine system during the suckling period of the NBW piglet could not show changes regarding the density of cell populations containing neuroendocrine substances (i.e. neuronal nitric oxide synthase, gastric inhibitory peptide and glucagon-like peptide-1) that play a pivotal role in gastrointestinal motility (Knapper *et al.*, 1995, Van Ginneken *et al.*, 1998, Van Ginneken *et al.*, 2001, Van Ginneken and Weyns, 2004). The only difference that was observed was the finding that the geometric centre of small intestinal transit was more progressed in NBW piglets of 28 d old. This observation might be associated with the increased tunica muscularis thickness - and thus the propulsive capacity/strength of a 'contraction - seen in 28 d old piglets.

Brush border hydrolases are responsible for the final stages of macronutrient digestion. Changes in their activities could result in changes in absorption of nutrient and concomitant the growth of the piglets. Particularly, the brush border glycohydrolases (such as, lactase, maltase and sucrase) are responsible for the degradation of di- and oligosaccharides into monosaccharides, and are thus crucial for the energy intake. Lactase activity was the highest in the proximal part of the

small intestine and declined towards the end of the suckling period, as previously reported (Manners and Stevens, 1972, Kelly *et al.*, 1991b). Sucrase and maltase activities increased towards the end of the suckling period as previously described (Kidder and Manners, 1980, Kelly *et al.*, 1991a). On the other hand, peptidases (such as aminopeptidase A, aminopeptidase N and dipeptidylpeptidase IV) are responsible for the degradation of peptides and enable the digestion of proteins in food. Age had no effect on the aminopeptidase A activity in the small intestine but age-related differences were seen regarding dipeptidylpeptidase IV and aminopeptidase N activities. Aminopeptidase N activity was the highest in the proximal part of the small intestine in piglets of 0 d of age whereas in the distal part of the small intestine the activity of aminopeptidase N differed significantly between 10 d and 28 d old piglets. Dipeptidylpeptidase IV activity in the proximal part of the small intestine was highest in newborn piglets. In the distal part dipeptidylpeptidase IV activity was highest in 10 d old piglets. It is generally accepted that peptidase activities are high at birth and decrease during suckling, with a typical drop between 2 and 3 weeks of age (Huërou-Luron *et al.*, 2000). Factors other than age also influence the activity of brush border hydrolases for example creep feeding (Hampson and Kidder, 1986) and growth performance of different breeds could explain differences in enzyme activities (Miao *et al.*, 2009).

In conclusion, the findings of the current study illustrate that *in vivo* intestinal motility, using Evans blue as a liquid bolus marker, and the structural and functional development of the small intestine are not altered in LBW piglets throughout the suckling period, suggesting that LBW piglets are not immature in regard to small intestinal development. Moreover these findings illustrate that co-ordinated gastrointestinal functioning is equally possible in LBW as in NBW pigs and cannot

explain suboptimal gastrointestinal functioning translated into impaired growth performance of LBW piglets. Nevertheless, the presented results confirm that the small intestinal structure and function of piglets alter with age.

Conclusions

Due to genetic selection and improved management techniques in pork industry, litter sizes have increased, resulting in a higher birth weight variation and reduced mean piglet birth weight. The latter are adversely correlated with piglet survival and weight gain in later stages of production. This study investigated the effect of birth weight on *in vivo* gastric emptying, *in vivo* small intestinal motility, the activity of brush border enzymes and intestinal morphology throughout the suckling period. These results illustrate the intestinal tract of LBW piglets is equally well equipped for nutrient intake and processing as of their NBW littermates.

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Figure captions

Figure 1 Proliferation index of low birth weight (LBW) and normal birth weight (NBW) piglets at birth and 3, 10 and 28 d after birth. Symbols represent mean $\pm$ SEM. Grey and dark symbols represent LBW piglets; and NBW piglets respectively (age $\times$ region: $P = 0.17$). ^{a-c} Different letters indicate differences between age groups. No effect of birth weight was observed (A)($P = 0.74$). Immunohistochemical staining of Ki-67. Ki-67 proliferating cells (red-brown) are predominantly seen in the crypts of the small intestine (arrow) besides in the lamina propria mucosae, in the submucosal layer and little in the intestinal epithelium (scale bar = 50 μ m) (B).

Figure 2 Small intestinal enzyme activities (lactase (A)(age $\times$ region: $P=0.39$; age: $P<0.001$; region: $P<0.001$), sucrase (B)(age $\times$ region: $P<0.001$), maltase (C))(age $\times$ region: $P<0.001$), aminopeptidase A (D))(age $\times$ region: $P<0.001$), aminopeptidase N (E))(age $\times$ region: $P<0.001$) and dipeptidylpeptidase IV (F)) of low birth weight (LBW) and normal birth weight (NBW) piglets at birth and 3, 10 and 28 d after birth. Grey and dark symbols represent LBW piglets; and NBW piglets respectively. ^{a-c} Different letters indicate differences between age groups. No effect of birth weight was observed.