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Growth and small intestine histomorphology of low and normal birth weight piglets during the early suckling period

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ABSTRACT

The present study compared growth performance and small intestinal histomorphological changes between low birth weight (LBW) and normal birth weight (NBW) piglets at day 0 and day 7 postpartum. The LBW and NBW piglets (Large White × Landrace × Duroc) from five multiparous crossbred sows were used. At birth, all piglets were weighed and classified into LBW (0.8–1.0 kg) or NBW (1.4–1.6 kg) piglets. At days 0 and 7 postpartum, one LBW and one NBW piglets per sow were randomly selected and euthanized for sample collection. The results showed that at day 7, the average daily gain and percentage change of body weight from day 0 of LBW piglets were not different from NBW piglets; however, their body weights were still lower than those of NBW piglets ($P < 0.01$). The organ weights including small intestine, large intestine, liver, spleen and kidney as normalized per 100 g of body weight showed no difference between LBW and NBW piglets at the same day; but they increased with age ($P < 0.05$). The histomorphological changes of small intestine revealed that the villus height and crypt depth were not different between groups at the same day; but they increased with age ($P < 0.01$). In terms of the enterocyte turnover rate as determined by the ratio of villus height to crypt depth, at day 7, the LBW piglets had a higher ratio of villus height to crypt depth with significant effect at the duodenum ($P < 0.05$). Additionally, the proliferative marker index (Ki-67) was also greater in LBW than NBW piglets at day 7 with significant effect at the ileum ($P < 0.05$). These results indicated that the LBW piglets grew at the same rate as NBW piglets with undisturbed organ developments as evidenced by the similar average daily gain, percentage change of body weight and normalized organ weights. Furthermore, the difference in enterocyte proliferation and turnover rate of the small intestine indicated a difference in small intestine development and function between LBW and NBW piglets. These data suggest that the LBW piglets were not only different from their NBW littermates in the body weight but also in small intestinal histomorphology.

1. Introduction

Over the last 20 years, the swine industry has focused on litter size as a key production indicator. Breeders have succeeded in improving the number of pigs per farrowing; thus, the number of piglets born is an important economic trait (Johnson et al., 1999). By selecting for greater litter size, the drawback is that the number of piglets born with a low birth weight increases. Studies have shown that increased litter size is associated with a reduction in the mean birth weight and therefore, an increase in the proportion of low birth weight (LBW) piglets (Quesnel et al., 2008; Quiniou et al., 2002). According to Morise et al. (2008), the LBW

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piglets referred to piglets weighing 0.8–1 kg, the normal birth weight (NBW) piglets referred to piglets weighing 1.4–1.6 kg and the high birth weight (HBW) piglets referred to piglets weighing more than 1.6 kg. The very low birth weight piglets or an intrauterine growth retardation (IUGR) piglet referred to piglets weighing less than 0.8 kg. LBW piglets are weak and often die before weaning resulting in a higher mortality rate of newborn piglets (Quiniou et al., 2002). In general husbandry practices, these LBW piglets are normally killed; however, there are questions concerning the necessity of this practice.

There is evidence that LBW piglets have lower postnatal growth rates reflected by lower average daily weight gain at suckling, post-weaning and during the growing-finishing period (Beaulieu et al., 2010; Berard et al., 2008; Rehfeldt and Kuhn, 2006; Quiniou et al., 2002). D'Inca et al. (2010) used an intrauterine growth retardation piglet (IUGR), which is a model for a very low birth weight piglet (BW $<$ 0.8 kg), and found that it had a slower growth rate compared to a normal pig at 5 day post-partum. This corresponds with the findings that the small intestine and other internal organs of the IUGR were small and did not function properly (Xu et al., 1994). Since the majority of nutrient absorption occurs in the small intestine of mammals, this organ typically undergoes rapid development in the early stage of life. Intestinal function can be estimated by observing villus height and crypt depth in LBW and NBW piglets during the early suckling period. Therefore, the aim of this study was to compare the growth performances along with the respective small intestinal histomorphological changes between LBW and NBW piglets at day 0 and day 7 postpartum.

2. Materials and methods

2.1. Animals

Five multiparous crossbred sows (Large White $\times$ Landrace) were randomly selected from a commercial farm in Nakornpathom province, Thailand. The sows used in this study were healthy, weighing between 190 and 250 kg, with an average parity of 5.8 $\pm$ 0.37. The average litter size was 14.8 $\pm$ 0.92 with the average numbers of LBW and NBW piglets per litter of 3.0 $\pm$ 0.32 and 7.4 $\pm$ 0.87, respectively. The sows' clinical signs were observed daily by attentive veterinarian and the blood was collected for hematological analysis on the farrowing day (data not shown). All sows received commercial gestation and lactation diets and had unlimited accessed to drinking water. The nutritional values of the diet were 17.5% crude protein, 5.4% crude fat, 5.0% crude fiber, 0.8% calcium, 0.6% phosphorus with ME of 3.2 Mcal/kg.

On the farrowing day (day 0) within 3 h after birth and before suckling, all piglets were weighed and classified into a LBW or a NBW group according to the criteria proposed by Morise et al. (2008). The LBW piglets referred to piglets weighing 0.8–1.0 kg and NBW piglets referred to piglets weighing 1.4–1.6 kg. At day 0 (before suckling), one LBW and one NBW piglets were randomly obtained from each litter and euthanized with an overdose of sodium pentobarbital (200 mg/kg body weight, intraperitoneal) (Buddington et al., 2001) for sample collection. The remaining piglets were nursed by their dam. On day 7, another LBW and NBW piglets were randomly obtained and euthanized for sample collections.

Animal procedures used in this study were done according to the guidelines for laboratory animals and were submitted to the Animal Care and Use Committee, Faculty of Veterinary Science, Chulalongkorn University (Protocol no. 0931026).

2.2. Tissues collection

2.2.1. Small intestinal tissues

The small intestinal tissues were collected from LBW and NBW piglets at day 0 (within 3 h after birth and before suckling) and day 7 (at 0700–0800 h). The small intestine, marked from the pyloric valve to the ileocecal valve, was quickly removed and weighed. It was divided into three segments: duodenum, jejunum and ileum. The duodenum extended from posterior of the pylorus to the junction with jejunum. The jejunum and the ileum were separated using an anatomical landmark, the ligament of Trietz. The three segments were weighed individually. Each segment was cut longitudinally, washed with ice-cold 0.9% NaCl to remove intestinal contents, patted dry with paper towels, and then weighed again. In addition, approximately 0.5–1.0 cm of each segment of small intestine was stored in 10% formalin and kept at room temperature for later histomorphology analysis and immunohistology.

2.2.2. Large intestine, liver, spleen and kidney

The large intestine, liver, spleen and kidney of each animal were removed and weighed. The large intestine was weighed after it was cut longitudinally, washed with ice-cold 0.9% NaCl and patted dry with paper towels. All lobes of the liver were dissected and weighed as a whole; while both kidneys were weighed and presented as average kidney weight per piglet. In order to facilitate the comparisons between experimental groups, the organ weights were normalized per 100 g of body weight.

2.3. Histomorphological study

To determine the histomorphological changes of small intestine in LBW and NBW piglets, the segments of duodenum, jejunum and ileum were sectioned and stained with hematoxylin and eosin and then the villus height and the crypt depth were measured. Briefly, the tissues were placed in 10% buffer neutral formalin, dehydrated and embedded in paraffin. Transverse sections were cut at 4–6 μm thickness and stained with Harris' Alum Hematoxylin and counterstained with eosin following standard protocols. Photomicrographs were taken using a light microscope at 200 $\times$ magnification. Height of villi and depth of crypts were measured using Scion Image Software (Scion image; Scion Corporation, Frederick, MD).

Measurements of villus height and crypt depth were taken followed the protocols described by Martins-Rodrigues et al. (2007). For villus height or crypt depth measurement, each histological section was divided into 4 quadrants and at least 4 villi or crypts were measured per quadrant. Villus height was measured from the tip to