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Intestinal immune cell quantification and gram type classification of the adherent microbiota in conventionally and artificially reared, normal and low birth weight piglets

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ABSTRACT

To be able to raise larger litters, the influences of alternative rearing strategies on piglets are currently assessed. This study compared the predominant Gram type of the adherent intestinal microbiota between conventionally and artificially reared piglets and quantified their major mucosal immune cell populations. In addition, the potential influence of the piglets' birth weights was examined. To this purpose, 40 neonatal piglets consisting of 20 normal birth weight (NBW) and 20 low birth weight (LBW) piglets suckled the sow for three days after which 20 piglets (10 NBW and 10 LBW) continued to suckle the sow and 20 piglets (10 NBW and 10 LBW) were transferred to brooders and raised on milk formula. At the age of 10 or 28 days, five piglets of each group (birth weight × rearing strategy) were euthanized and the jejunum was sampled. The presence of adherent Gram$^+$ and Gram$^-$ bacteria was scored and the volume densities of CD8$^+$ cells, CD4$^+$ cells and CD172a$^+$ myloid cells were determined. Irrespective of birth weight, sow-fed piglets possessed a predominant Gram$^+$ microbiome at 10 days of age, whereas formula-fed animals had more Gram$^-$ microbiota. With increasing age, however, Gram$^+$ microbiota took the upper hand in these animals. The volume densities of CD8$^+$ and CD4$^+$ cells rose with increasing age and were consistently lower in LBW piglets. Both rearing strategies had a similar influence on the volume densities of these cell populations. In contrast, the volume densities of CD172a$^+$ myloid cells did not differ significantly between the birth weight, rearing strategy and age groups. Three observations allow to conclude that artificial rearing could be a valuable alternative for suckling the sow. First, within each birth weight category, the mean weight of the 28-day-old piglets was similar for both rearing groups. Secondly, the effect of milk formula on the composition of the intestinal microbiome was only temporary since formula-fed piglets restored the more beneficial Gram$^+$ microbiome by the end of the artificial rearing period. Finally, artificial rearing did not influence, either positively or negatively, the volume densities of CD8$^+$, CD4$^+$ and CD172a$^+$ immune cells when compared to conventional rearing.

1. Introduction

Genetic selection for larger litters has substantially increased the number of live born piglets per litter (Su et al., 2007). However, larger litters are characterized by a decreased average birth weight (Bérard et al., 2010). Moreover, low birth weight (LBW) piglets are overrepresented in larger litters (Quesnel et al., 2008). These piglets in particular suffer from increased mortality and impaired growth, resulting in a higher slaughter age and poorer meat quality (Bérard et al., 2008; Kilbride et al., 2012). As a consequence, the genetic capacity of these more prolific sows is not fully exploited.

In order to improve the profitability of piglet production, pig farmers show interest in alternative rearing strategies. These most often include cross-fostering (Ferrari et al., 2014), supplementing the piglets with milk formula and split-weaning (Rutherford et al., 2011). The latter includes artificial rearing, consisting of weaning the piglets after they had been suckling colostrum, and subsequent feeding with milk formula (De Vos et al., 2014a; Levast et al., 2010).

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Since conventional weaning induces significant changes in gastrointestinal physiology (Lallès et al., 2007), it is expected that any change in rearing strategy also affects digestive functioning. The Gram$^+$ gut microbiota that, amongst others, provide the suckling piglet with a colonization resistance against pathogenic bacteria could thus be disturbed at weaning (Pluske et al., 2002). This results in a higher susceptibility to gastrointestinal infection and diarrhoea (Konstantinov et al., 2006). At the moment however, the effect of artificial rearing on the adherent intestinal microbiota has only been studied fragmentary (D'Inca et al., 2010; Van Haver et al., 2009).

In addition, weaning also profoundly influences the maturation of the intestinal immune system, which is shaped by a dynamic interplay between diet and the local microbiota (Juul-Madsen et al., 2010). First at birth and again at weaning the piglet's gut is exposed to foreign antigens and is colonized by specific bacterial populations, which play a major role in the trafficking of lymphoid cells to the intestinal mucosa (Pabst and Rothkötter, 1999; Rothkötter et al., 1991). The potential influence of artificial rearing on intestinal lymphocyte numbers is, however, ambiguous. Indeed, some authors have demonstrated an increase in the number of intestinal lymphocytes (Orgeur et al., 2001; Vega-López et al., 1995) after early weaning, whereas others observed smaller Peyer's patches (Helm et al., 2007). In contrast, there is consensus in literature that a low birth weight impairs the development of the immune system (D'Inca et al., 2011; Tuchscherer et al., 2000; Wang et al., 2008; Zhong et al., 2012). However, the potential influence of birth weight on the gut-associated lymphoid tissue (GALT) has not been determined. Furthermore, to our knowledge, this is the first study that focused on the presence of CD172a$^+$ myloid cells in the GALT of differently reared piglets of various birth weight categories, i.e. normal birth weight (NBW) piglets vs. LBW piglets.

The aim of this study was to compare conventional rearing with artificial rearing on milk formula with regards to the adherent gastrointestinal microbiota and presence of the major mucosal immune cell populations in the jejunum. Both parameters where determined in the same intestinal region to shed light on the interplay between the microbiota and the local immune cells. Given the fact that formula is low in specific factors to adequately stimulate the immune system and gut microbiota (Helm et al., 2007; Li et al., 2012), it is hypothesized that the development of the different components of the GALT is impaired in artificially reared piglets. Therefore, a quantification of key-cells in the jejunal effector site of the GALT, i.e. the epithelium and lamina propria mucosae, and a Gram type classification of the intestinal adherent microbiota were performed. To examine whether piglets of distinct birth weight categories respond differently to artificial rearing, both NBW and LBW piglets were included.

2. Materials and methods

2.1. Animals

Forty neonatal piglets ((Finnish Yorkshire × Belgian Landrace) × Piétrain), born on a local farm, were designated as NBW or LBW piglets when their birth weights ranged within 0.5 standard deviation (SD) or were below 1.5 SD of the mean litter birth weight, respectively (Paredes et al., 2012; Willemen et al., 2014).

After three days of suckling the sow, 10 gender- and sow-matched pairs of NBW and LBW piglets were transferred to commercial brooders (Rescue Decks®, S&R Resources LLC, Mason, USA) where they were artificially reared on milk formula, which was *ad libitum* provided until the age of 10 days ($n=10$, viz. 5 NBW and 5 LBW piglets) or 28 days ($n=10$, viz. 5 NBW and 5 LBW piglets).

Table 1
Composition and nutritional value of sow milk and formulated milk.

	Sow milk[a]	Milk formula[b]
Composition		
Vitamin A (IU/kg)	3067	55,000
Vitamin D3 (IU/kg)	360	5500
Vitamin E (IU/kg)	3.80	300
Vitamin C (IU/kg)	906	110
Ca (%)	0.18	0.89
P (%)	0.14	0.73
Lysine (%)	7.0	1.70
Methionine+Cysteine (%)	3.1	0.80
Tryptophan (%)	1.6	0.30
Threonine (%)	4.1	1.10
Nutritional value		
Protein (g/L)	55	28
Lipid (g/L)	76	23
Lactose (g/L)	53	56
Gross Energy (kcal/L)	1290	590

[a] According to Xu (2003);
[b] as provided by the manufacturer.

The dietary control group, consisting of 10 gender- and sow-matched pairs of NBW and LBW piglets, suckled the sow on the farm until the age of 10 days ($n=10$, viz. 5 NBW and 5 LBW piglets) or 28 days ($n=10$, viz. 5 NBW and 5 LBW piglets). The composition and nutritional value of sow milk and the milk formula is presented in Table 1.

Piglets in both systems had free access to water and were maintained under standard environmental conditions (12 h/12 h light/dark cycle, temperature adjusted to age). Their body weights were recorded both at birth and before they were sacrificed for sampling. Animals were observed daily, with focus on general behavior, body condition and faecal composition, to document general health status. All experiments were approved by the Ethical Committee for Animal Experiments of the University of Antwerp, Belgium (2014-01).

2.2. Tissue samples

Piglets from the different experimental groups were euthanized at the age of 10 or 28 days by exsanguination via transection of the jugular veins and carotid arteries after intraperitoneal injection of sodium pentobarbital (200 mg/kg, Kela, Hoogstraten, Belgium). The intestinal tract was dissected and samples were taken at the distal part of the jejunum. Samples intended for (immuno)histochemistry on paraffin sections (analysis of adherent microbiota) were fixated for 2 h in 4% phosphate-buffered paraformaldehyde (pH 7.4) at room temperature. They were routinely processed to paraffin-embedded tissue blocks after rinsing in phosphate-buffered saline solution (PBS; 0.01 M, pH 7.4). Those samples intended for cryosection-immunohistochemistry (analysis of mucosal immune cells) were embedded in KP-Cryocompound® (Klinipath, Olen, Belgium) prior to snap-freezing in liquid nitrogen. All frozen samples were stored at −80 °C until further processing.

2.3. Adherent microbiota

To detect adherent Gram$^+$ bacteria in the intestinal samples, 4 μm thick paraffin sections were Gram-stained according to the modified Brown and Brenn stain described by Taylor (1966). Gram$^-$ bacteria were analysed by means of immunohistochemical staining for lipopolysaccharides (LPS) according to the protocol of Van Haver et al. (2009). In brief, tissue sections were treated with