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Characterization of porcine milk oligosaccharides during early lactation and their relation to the fecal microbiome

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ABSTRACT

The composition of porcine milk oligosaccharides (PMO) was analyzed during early lactation and their relation to piglet gut microbiome was investigated. Pigs are considered ideal intestinal models to simulate humans because of the striking similarity in intestinal physiopathology to humans. The evolution of PMO was investigated in the milk from 3 healthy sows at prefarrowing, farrowing, and d 7 and 14 postpartum by Nano-LC Chip Quadrupole-Time-of-Flight mass spectrometer (Agilent Technologies, Santa Clara, CA). Previously sequenced metagenome libraries were reanalyzed to examine changes with specific gut bacterial populations. Over 30 oligosaccharides (OS) were identified in the milk, with 3′-sialyllactose, lacto-*N*-tetraose, α1–3,β1–4-D-galactotriose, 2′-fucosyllactose, and 6′-sialyllactose being the most abundant species (accounting for ~70% of the total OS). Porcine milk had lower OS diversity (number of unique structures) than human milk, and appeared closer to bovine and caprine milk. In agreement with previous studies, only 3 fucosylated OS were identified. Surprisingly, their contribution to total OS abundance was greater than in bovine milk (9 vs. 1%). Indeed, fucosylated PMO increased during lactation, mirroring a similar trend observed for neutral and type I OS content during early lactation. Taken together, these results suggest that, in terms of abundance, PMO are closer to human milk than other domestic species, such as bovine and caprine milks. Metagenomic sequencing revealed that fucose-consuming bacterial taxa in the gut microbiota of piglets were qualitatively but not quantitatively different between nursing and weaning stages, suggesting that both the composition and structure of dietary glycans may play a critical role in shaping the distal gut microbiome. The similarity of both intestinal physiopathology and milk OS composition in human and porcine species suggests similar effects on gastrointestinal development of early nutrition, reinforcing the use of the pig intestinal model to simulate human intestinal models in the clinical setting.

Key words: porcine milk, oligosaccharide, gut microbiota

INTRODUCTION

Breast milk is the optimal source of nutrition for the first few months of an infant's life, as it provides all the necessary nutrients for optimal growth and development. Its composition has been shaped by natural selection, which acts on both the infant and the mother, maximizing infant survival, growth, and development while minimizing the metabolic costs of lactation for the mother (Zivkovic et al., 2011). As a result of this natural selective pressure, breast milk evolved to have a unique composition where lipids and lactose are the most abundant components, followed by oligosaccharides (**OS**) and proteins (German et al., 2008). Despite OS being the third most abundant components in milk (Kunz et al., 2000), it was long thought they had no biological significance. Recent studies pointed out many and diverse biological activities afforded by milk OS, including prebiotic activity, antiadhesion effects, anti-inflammatory properties, glycome-modifying activity, and roles in brain development, as well as influencing the growth-related characteristics of intestinal cells (Hickey, 2012; Lane et al., 2012; ten Bruggencate et al., 2014). Taken together, these bioactivities favor the establishment of a protective and healthful intestinal environment (Smilowitz et al., 2014). Milk OS are typically composed of 3 to 10 repeating monosaccharide units, including glucose (Glc), galactose (Gal), *N*-acetyl-glucosamine (GlcNAc), *N*-acetyl-galactosamine (GalNAc), fucose (Fuc), and sialic acids (NeuAc/NeuGc). The core unit of bovine milk and other domestic milk OS can be either lactose [Gal(β1–4)Glc] or *N*-acetyl-lactosamine [Gal(β1–4)GlcNAc; Aldredge et al., 2013]. Based on their chemical composition, OS are

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classified as acidic (decorated by sialic acid, NeuAc/NeuGc) or neutral (containing GlcNAc, HexNAc, or fucose, lacking sialic acid).

Breast milk composition is not static but changes during lactation. The OS content can be as high as 23 g/L in colostrum and rapidly decrease during lactation to values a low as 5 to 10 g/L late in nursing (Newburg, 2013). Human milk OS (**HMO**) have been widely studied, with more than 200 structures partially characterized (Wu et al., 2010, 2011) displaying a predominance of neutral fucosylated (50–70%) compared with sialylated OS (10–25%; Niñonuevo et al., 2008). The concentration of OS in milk from other mammalian species is lower than that in human milk, with a concentration of 1 g/L in bovine colostrum and even lower concentrations in mature bovine milk (Fong et al., 2011); however, a great diversity of OS structures has been reported for domestic animals. A bioinformatic library of 55 structures was assembled for bovine milk OS (**BMO**; Aldredge et al., 2013), whereas 39 OS were described in porcine milk (Tao et al., 2010; Albrecht et al., 2014), 20 OS in caprine milk (Meyrand et al., 2013; Albrecht et al., 2014), and only 12 OS in camel milk (Alhaj et al., 2013; Albrecht et al., 2014). Conversely, the ratio of fucosylated to sialylated OS in milk from domestic animals is shifted toward sialylated OS, which predominate the overall composition (70%; Urashima et al., 2013).

Swine are important agricultural species and are considered to be an excellent model for nutritional studies because their digestive system, immune system physiology, and anatomical structure are similar to those of humans (Guilloteau et al., 2010). Only a few studies to date have evaluated porcine milk oligosaccharides (**PMO**) changes during early lactation (Tao et al., 2010; Albrecht et al., 2014). The present study characterized the composition and variation of PMO during the first 2 wk of lactation and examined how PMO composition and of the gut metagenome of suckling pigs interact to shape the nursing pig microbiome.

MATERIALS AND METHODS

Materials

Sodium borohydride was from Sigma-Aldrich (St. Louis, MO). Commercial OS standards of lacto-*N*-tetraose (**LNT**), lacto-*N*-neotetraose (**LNnT**), lacto-*N*-hexaose (**LNH**), and lacto-*N*-neohexaose (**LNnH**) were purchased from V-Labs (Covington, LA). All solvents used for sample preparation were HPLC-MS grade (Fisher Scientific, Fair Lawn, NJ). The DNA was extracted from feces using the Zymo Research Fecal DNA kit (Zymo Research, Irvine, CA) according to the manufacturer's instructions, with modifications as described previously (Frese et al., 2015). Nonporous graphitized carbon solid-phase extraction (**GCC-SPE**; 2,000 μg binding capacity) was from Glygen Corp. (Columbia, MD). Nanopure water (18.2 MΩ/cm, 25°C) was used for the analytical work.

Collection of Porcine Samples

The University of California, Davis Institutional Animal Care and Use Committee approved all animal experiments under study before beginning the study, and this study has been described in detail previously (Frese et al., 2015). Three healthy Yorkshire or Hampshire adult pregnant multiparous sows from the University of California, Davis swine herd were selected for this study. Infant pigs were allowed to nurse freely until 21 d of age and did not consume sow feed. After 21 d, half the nursing pigs were moved to separate housing (away from the sow) and fed a standard starter feed (Hubbard Feeds, Mankato, MN). Animals were given ad libitum access to water and feed. Milk samples (2 mL) were collected by manual expression into a sterile collection container 1 d before farrowing (precolostrum), at farrowing (d 0), and at d 7 and 14 postfarrowing. Samples were immediately frozen at −80°C until analysis.

To avoid differences in sample preparation and analytical techniques that often hinder comparison of OS structures and abundances with published literature values, 2 separate samples of pooled human colostrum and pooled bovine colostrum were obtained at the University of California, Davis from other ongoing studies and analyzed simultaneously using the same analytical procedure.

OS Isolation and Purification

Milk OS were isolated and purified as previously described, with minor modification (Barile et al., 2010). Briefly, frozen milk samples were completely thawed, and a 0.5-mL aliquot of each sample was mixed with an equal volume of nanopure water and centrifuged at 14,000 × *g* in a microfuge for 30 min at 4°C to remove lipids. The top fat layer was removed, and 4 volumes of chloroform:methanol (2:1, vol/vol) were added, vigorously mixed, and the resulting emulsion was centrifuged at 4,000 × *g* for 30 min at 4°C. The upper methanol layer containing OS was transferred to a tube, 2 volumes of cold ethanol were added, and the solution was frozen for 1 h at −30°C, followed by centrifugation for 30 min at 4,000 × *g* and 4°C to precipitate the denatured protein. The supernatant (OS-rich fraction) was collected and freeze-dried using a speed vacuum centrifuge. Native milk OS were reduced to alditol forms using 2.0

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