Galacto-oligosaccharides improve gut architecture and increase abundance of probiotic bacteria in poorly performing piglets

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Background & objectives: Poor performance and inability to thrive during lactation continues to be of concern in pig production. However, establishment and maintenance of beneficial gut microbiota during suckling is essential for future performance, growth, health and welfare of animals[1]. Microbiome manipulation through addition of pre- and/or probiotic feeds (without pharmaceutical zinc oxide), particularly in healthy, post-weaning pigs is established[2], but requires further investigation in pre-weaning piglets with below expected performance. Galacto-oligosaccharides (GOS) comprising 2 to 8 polymerised galactose units with terminal glucose moieties are functionally similar to those of mammalian milk and stimulate development of the immune system in neonates as well as modulating gut architecture and intestinal microbiota in healthy pigs[3]. The objectives of this study were to investigate the effects of GOS on gut architecture and the microbiome in poorly performing (non-thriving) piglets who may benefit from milk replacement feeding.

Methods: Poorly performing piglets potentially receiving sub-optimal nutrition from the sow were selected within the first seven days of life by visual assessment condition score and the appearance of “un-thriftiness”. Animals displayed no clinical symptoms of underlying disease, e.g. scour or lameness, but were considered to benefit from a complete milk replacement feeding program. Piglets were group housed in slatted plastic orphan pens heated by industry standard lamps, with water *ad libitum* through a nipple drinker and twice daily feeding to appetite with either complete porcine milk replacer (Faramate, Volac International Ltd) alone, n = 4, or supplemented with 1 % (w/w) DP2+ GOS (Nutrabiotic®, n = 4). Animals were euthanised at 23 – 26 d of age, the gastrointestinal tract (GIT) excised and samples of jejunum, ileum, colon and caecum fixed in 10% neutral buffered formalin for subsequent staining by haematoxylin and eosin and Periodic Acid Schiff staining for assessment of gut architecture and goblet cell enumeration respectively. Bacterial DNA was extracted from GIT luminal contents (MP Biomedicals Fast DNA Kit for Feces) and the V4 region of the bacterial 16S rRNA genes PCR amplified and sequenced on the Illumina MiSeq platform (Illumina Inc., US) with sequence analysis performed in Mothur v. 1.39 against the SILVA rRNA database reference alignment. Linear Discriminant Analysis Effect Size (LEfSe) was used to identify differentially abundant operational taxonomic units (OTUs) annotated to probiotic bacteria.

Results: Jejunal villus/crypt ratio (VCR), villus height, crypt area and width were greater in GOS fed piglets ($P < 0.05$) as were the number of goblet cells per mm^2 in villi and crypts. Caecal crypt area, depth, width and the number of goblet cells per crypt were higher in GOS fed pigs ($P < 0.05$) as were the numbers of goblet cells in ileal and colonic crypts. Supplementation with GOS increased the relative abundance of *Firmicutes* at phylum level in the ileum of animals ($P < 0.01$) in contrast to a greater abundance of *Bacteroides* in the ileum and colon of non-GOS pigs ($P< 0.01$). Feeding with GOS increased the differential abundance of OTUs annotated to probiotic bacteria at genus level (Table 1).

Table 1. Significant increases in OTUs annotated to probiotic bacteria in GOS fed piglets

	Duodenum	Jejunum	Ileum	Colon	Caecum	Rectum
Streptococcus	$P < 0.025$	$P < 0.05$	$P < 0.05$	-	$P < 0.05$	-
Lactobacillus	-	-	$P < 0.05$	$P < 0.025$	-	$P < 0.01$
Bifidobacterium	-	-	-	-	$P < 0.025$	-

Discussion & conclusion: Supplementation with GOS significantly increased jejunal VCR and GIT architectural features indicating a healthy well differentiated intestinal mucosa, with a potentially greater nutrient absorptive capacity. GOS animals significantly expressed more goblet cells known to protect the mucosa through secretion of mucins and regulation of the GIT immune system via exposure to luminal antigens[4]. Significant increases in probiotic bacteria allocated to GOS fed animals indicates possible long-term benefits in establishing a robust microbiome, growth, immunity and resistance to pathogens[2,5]. Given these results, GOS may have potential as a health promoter in poorly performing pigs and warrants further research as a pharmaceutical zinc oxide replacer in post-weaning animals.

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