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**Effects of dietary caprine milk oligosaccharides enriched fraction  
on maternal large intestine and the consequences for the  
development of the offspring**

A thesis presented in partial fulfilment of the requirements for the degree of

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## Abstract

The colonisation of the neonate gastrointestinal tract by health-promoting microbiota is likely to improve the overall health of the infant and may also have health benefits in later life. Initial development and maturation of the foetal/neonatal gastrointestinal tract is heavily influenced by the *in utero* environment which itself, may be altered by the maternal diet and gastrointestinal tract microbiota composition. The maternal gastrointestinal tract microbiota can be altered by supplementation with synthetic oligosaccharides; however, positive effects on the health and well-being of the offspring have not been adequately established. Human milk contains natural oligosaccharides known to improve the gastrointestinal tract colonisation and the development and maturation of the infant gastrointestinal tract. Among domestic farm animals, caprine milk has oligosaccharides structurally similar to human milk and potentially similar beneficial effects for the infant. We hypothesised that feeding caprine milk oligosaccharide enriched product to pregnant and lactating mice would induce changes in the maternal large intestine microbiota and milk composition, accelerating the development and maturation of the offspring's large intestine tissue and altering the gastrointestinal tract microbiota composition. The aim of this project was to obtain bifidobacteria from the faeces of breast-fed human infants and determine which were of capable fermenting caprine milk oligosaccharide enriched product. Subsequently, the effects of the best strains on the morphology and metabolic pathways of the colonic mucosa of germ-free and conventionally raised mice, supplemented with dietary caprine milk oligosaccharide enriched product.

The present study is the first to report New Zealand Saanen caprine colostrum, milk and whey. An enrichment method previously described was used to produce a caprine milk oligosaccharide enriched product for *in vitro* and *in vivo* assessment of its health effects. Caprine milk oligosaccharide enriched product was shown to differentially stimulate the growth of bifidobacteria, commonly found in the gastrointestinal tract of breast-fed infants.

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Among the bifidobacterial species tested, *Bifidobacterium bifidum* utilised caprine milk oligosaccharide enriched product most efficiently when compared to *Bifidobacterium breve* and *Bifidobacterium longum* subsp. *longum*. *B. bifidum* (AGR2166) was shown to ferment the sialyloligosaccharides, 3'- and 6'-sialyl-lactose present in caprine milk oligosaccharide enriched product through cell-associated sialidase expression. Augmented microbial biomass associated with enhanced growth and *in vitro* fermentation of caprine milk oligosaccharide enriched product, increased the production of microbial fermentation end products such as acetate and lactate. These findings indicate that *in vivo* caprine milk oligosaccharide enriched product may stimulate the growth and fermentation of bifidobacteria within the gastrointestinal tract.

Germ-free mice or mice mono-associated with *B. bifidum* (AGR2166) were used to test the *in vivo* effects of maternal caprine milk oligosaccharide enriched product consumption during pregnancy and the effects on the foetus. Caprine milk oligosaccharide enriched product diet showed no effects on maternal gastrointestinal tract or foetal growth regardless of microbial status. Mice inoculated with *B. bifidum* (AGR2166) and fed caprine milk oligosaccharide enriched product diet, however, showed an increased bacterial translocation from maternal gastrointestinal tract to organs and placenta (inferred by the presence of the bifidobacteria 16S rRNA gene in the maternal organs). Increased translocation of commensal bacteria from maternal gastrointestinal tract to the foetus may have important effects on foetal immunological programming.

The consumption of caprine milk oligosaccharide enriched product, during gestation and lactation were also tested in conventional rodents and it had no effects on maternal gastrointestinal tract microbiota and morphology. Changes on maternal lipid metabolism and increased maternal milk protein, however, were observed. These modifications may have positively affected the development of the pups, relative abundance of gastrointestinal tract bifidobacteria and butyric acid production at weaning. Important changes in the plasma and

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urine metabolites involved in bile acid and fatty acid metabolism were also observed in the pups as a consequence of maternal caprine milk oligosaccharide-enriched diet. The effects of maternal caprine milk oligosaccharide enriched product diet on pups, were no longer apparent after 30 days of consuming a control diet post-weaning, however, detrimental physiological characteristics such as an increased body fat were observed. Further studies, are needed to understand the physiological effects of caprine milk oligosaccharides on the maternal/infant pair.

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## List of abbreviations

<b>afu</b>	Absolute fluorescent units
<b>ANOVA</b>	Analysis of variance
<b>AOAC</b>	Association of official analytical chemistry
<b>ARDRA</b>	Amplified ribosomal DNA restriction analysis
<b>BMI</b>	Body mass index
<b>BMO</b>	Bovine milk oligosaccharides
<b>CD4+ T cells</b>	T helper cells expressing the surface protein CD4
<b>cfu</b>	Colony forming units
<b>CMO</b>	Caprine milk oligosaccharide
<b>CMOP</b>	Caprine milk oligosaccharide enriched product
<b>CNS</b>	Central nervous system
<b>CpG</b>	Cytosine phosphate guanine
<b>CRM</b>	Certified reference material
<b>CRAMP</b>	Cathelicidin-related antimicrobial peptide
<b>DC</b>	Dendritic cells
<b>DGGE</b>	Denaturing gradient gel electrophoresis
<b>DNA</b>	Deoxyribonucleic acid
<b>DP</b>	Degree of polymerisation
<b>ENS</b>	Enteric nervous system
<b>FDR</b>	False discovery rate
<b>FL</b>	Fucosyllactose
<b>FOS</b>	Fructo-oligosaccharides
<b>Fuc</b>	Fucose
<b>FUT</b>	Fucosyltransferase
<b>GAL</b>	Galactose
<b>GALT</b>	Gut-associated lymphoid tissue

## List of abbreviations

<b>GC-FID</b>	Flame ionisation detector gas chromatography
<b>GF</b>	Germ-free
<b>GHS</b>	General health score
<b>GIT</b>	Gastrointestinal tract
<b>GLC</b>	Glucose
<b>GLCNac</b>	N-acetyl-glucosamine
<b>GOD</b>	Glucose oxidase
<b>GOS</b>	Galactooligosaccharides
<b>Hib</b>	<i>Haemophilus influenzae</i>
<b>HILIC</b>	Hydrophilic interaction liquid chromatography
<b>HLA</b>	Human leukocyte antigen
<b>HMO</b>	Human milk oligosaccharides
<b>HPLC</b>	High performance liquid chromatography
<b>TICP-OS</b>	Inductively coupled plasma atomic emission spectroscopy
<b>ICP-MS</b>	Inductively coupled plasma mass spectroscopy
<b>IECs</b>	Intestinal epithelial cells
<b>IELs</b>	Intraepithelial lymphocytes
<b>Ig</b>	Immunoglobulin
<b>I<math>\kappa</math>B</b>	Nuclear factor kappa inhibitor
<b>IL</b>	Interleukin
<b>ILFs</b>	Isolated lymphoid follicles
<b>INF<math>\gamma</math></b>	Interferon gamma
<b>LacNac</b>	N-acetyllactosamine
<b>LC-MS</b>	Liquid chromatography-mass spectrometry
<b>LDLs</b>	Lamina propria lymphocytes
<b>Le</b>	Lewis group
<b>LNB</b>	Lacto-N-biose

## List of abbreviations

<b>LPLs</b>	Lamina propria lymphocytes
<b>LPS</b>	Lipopolysaccharide
<b>LSD</b>	Least significant difference
<b>LTi</b>	Lymphoid tissue inducer
<b>MHC</b>	Major histocompatibility complex
<b>MLN</b>	Mesenteric lymph nodes
<b>MTPY</b>	Modified TPY agar
<b>m/z</b>	Mass to charge ratio
<b>NCC</b>	Neural crest cells
<b>NeuAc</b>	N-acetylneuraminic acid
<b>NF-<math>\kappa</math>B</b>	Nuclear factor Kappa-light-chain-enhancer of activated B cells
<b>NGS</b>	Next generation sequencing
<b>OD</b>	Optical density
<b>OTU</b>	Operational taxonomic unit
<b>PBS</b>	Phosphate buffered saline
<b>PCA</b>	Principal component analysis
<b>PCoA</b>	Principal coordinate analysis
<b>PCR</b>	Polymerase chain reaction
<b>POD</b>	Catalysis of peroxidase
<b>PUFA</b>	Polyunsaturated fatty acids
<b>RISA</b>	Ribosomal intergenic spacer analysis
<b>RNA</b>	Ribonucleic acid
<b>rRNA</b>	Ribosomal RNA
<b>SCFA</b>	Short chain fatty acids
<b>SI</b>	Small intestine
<b>SL</b>	Sialyllactose
<b>spp.</b>	Species



## List of abbreviations

<b>TGF</b>	Transforming growth factor beta
<b>Th</b>	Cellular T helper
<b>TLR</b>	Toll-like receptor
<b>TNF</b>	Tumour necrosis factor
<b>TReg</b>	Lymphocyte regulatory T cell
<b>TTGE</b>	Temperature gradient gel electrophoresis
<b>VIP</b>	Variable importance in projection
<b>4Mu-Neu5Ac</b>	4-methylubelliferyl-a-D- <i>N</i> -acetylneuraminic acid