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**Effects of dietary sheep, cow and goat milk solids on colitis in the interleukin-10
gene deficient mouse model of Inflammatory Bowel Disease.**

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

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in

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Abstract

Inflammatory Bowel Disease (IBD) is a group of chronic, immunologically-mediated gastrointestinal disorders resulting from interactions between environmental influences, host genetic susceptibility, and the intestinal microbiota. Dietary factors can ameliorate symptoms, providing a rationale for using targeted nutrition to alleviate symptoms. Food components, including milk-derived oligosaccharides and conjugated linoleic acid, have shown anti-inflammatory effects in IBD patients or animal models of IBD. Additionally, some ruminant milks are perceived by some IBD patients to have more beneficial effects on their symptoms (goat, sheep) than others (cow). Soy-based milk substitutes are perceived to be more beneficial than milk. No reports describe the effects of milk solids from different species on molecular pathways in the intestine that might explain differential effects in IBD. This thesis aimed to investigate the effects of dietary intervention with milk solids on the severity of colitis (histology) and molecular pathways (microarrays and qPCR) in the interleukin-10 gene deficient (*Il10*^{-/-}) mouse model of IBD.

First, laser microdissection (LMD) combined with microarrays was used to analyse colon epithelium gene expression in 6 and 12 week old *Il10*^{-/-} mice fed a control diet. This indicated that intact colon was an appropriate tissue in which to study global changes in gene expression when colitis is established. It also showed that studying colon epithelium during the early stages of inflammation (6 weeks old) may identify molecular changes not seen in intact colon. Secondly, analysis of DNA methylation changes (both globally, and in specific inflammation-associated genes (*Ppara*, *Stat1* and *Tap2*)) in *Il10*^{-/-} mouse colon showed that changes in total DNA methylation were correlated with changes in global gene expression, and changes in *Stat1* methylation during inflammation correlated with *Stat1* gene expression. However, these techniques had limitations for obtaining a global overview of molecular changes (DNA methylation) in response to dietary intervention in established inflammation (LMD) and therefore were not applied in the dietary intervention study. Finally, diets containing goat and cow whole milk solids (40% w/w) fed for 6 weeks had anti-inflammatory effects in the colon of 11-12 week old *Il10*^{-/-} mice, shown by a reduction in colitis severity and immune-related gene expression. Further research is required to elucidate the physiological and molecular mechanisms of these anti-inflammatory effects.

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*May it be when darkness falls your heart will be true.
You walk a lonely road. Oh, how far you are from home!
May it be the shadow's call will fly away.
May it be your journey on to light the day.
When the night is overcome, you may rise to find the sun.*

*Selected lyrics from the song "May it be" by Enya.
Featured in the film "The Fellowship of the Ring".*

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List of abbreviations

ANOVA	analysis of variance
BCM7	β -casomorphin-7
C57BL/6J, C3H/HeJBir, BALBc, 129 SvEv, 129 Ola	various strains of inbred mice
CD	Crohn's disease
CIF	complex intestinal flora
CLA	conjugated linoleic acid
DSS	dextran sodium sulphate
EASE	expression analysis systematic explorer
EF	<i>E. faecalis/faecium</i> culture
EF.CIF	a 1:1 mixture of EF and CIF
FC	fold change
FDR	false discovery rate
GHS	general health score
GO	gene ontology
GSEA	gene set enrichment analysis
HIS	histological injury score
HPLC	high performance liquid chromatography
IBD	Inflammatory bowel disease
IEL	intraepithelial lymphocyte
Ig	immunoglobulin
IL	interleukin
IL10	interleukin-10
<i>Il10</i> ^{-/-}	interleukin-10 gene deficient (mouse)
IPA	Ingenuity pathway analysis
Limma	linear models for microarray analysis
LMD	laser microdissection
LSD	least significant difference
MALDI-TOF	matrix-assisted laser desorption ionisation-time of flight (mass spectrometry)
MCT	medium chain triglycerides
MDR1	multi-drug-resistant gene/protein
<i>Mdr1a</i> ^{-/-}	multi-drug-resistant gene deficient (mouse)
n-3/n-6	omega-3/6 (fatty acid)
NOD	nucleotide oligomerisation domain
PUFA	polyunsaturated fatty acids
qPCR	quantitative real-time polymerase chain reaction
REML	residual maximum likelihood
RIN	RNA integrity number
SPF	specific pathogen free
T _H	T-helper (cell)
TNBS	trinitrobenzenesulfonic acid
UC	ulcerative colitis

