An *in vivo* model to study mucin-bacterial interactions during early post-hatch development of broiler chickens

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Abstract

Mucins, synthesised and secreted by goblet cells, possess potential binding sites for both commensal and pathogenic organisms, and may perform a defensive role during establishment of the intestinal barrier in newly hatched chickens. Increasing interest has been directed toward bacterial interactions within the mucus layer, and the mechanisms by which bacterial colonisation can influence mucus composition during early development. This is important, firstly, as a means to understand initiation of infection and secondly, to optimise the gut microflora for enhanced animal production. Currently, information on mucosal-bacterial interactions in poultry is limited. In order to observe the effects of bacterial exposure on intestinal goblet cell mucin production during early development, differences in the small intestine of conventionally-raised (CV) and low bacterial load (LBL) broiler chicks were examined during the first 7 days post-hatch.

The initial aim of the study was to construct a small-scale, economical isolator system to hatch and raise chicks in a bacterial-free environment as a means to observe bacterial interactions with the intestinal mucosa in chickens exposed to normal environmental conditions. The design and construction of flexible plastic isolators for incubation and brooding are described, along with methodologies for preparation of eggs for entry into the isolators, incubation and hatching. Two trials were conducted, the first in August 2005 and the second in March 2006. It was found that the isolator system was successful in producing low bacterial load chicks for comparative studies with conventionally raised chicks, without compromising body weight.

A histological study was then conducted whereby ileal and jejunal goblet cells were stained with either periodic acid-Schiff or high iron diamine/alcian blue pH 2.5 to

discriminate between neutral, sulphated and sialyated acidic mucins. Total goblet cell numbers and goblet cell and villous/crypt morphology were also examined. Bacterial colonisation of CV animals induced an increase in sialic acid moieties in both ileal and jejunal goblet cell such that initiation of these changes occurred at day 3-4 post-hatch. Differences in intestinal morphology were also consistent with other germ-free animal studies.

In order to further understand the extent to which bacteria affected mucin composition, purified, isolated oligosaccharide fractions from ileal mucin at d 4 and 7 post-hatch were collected and analysed using mass spectrometry techniques to determine any structural differences in chain length or chain number between LBL and CV animals. No differences in chain length or number were observed between CV and LBL animals at either d 4 or 7 post-hatch with both groups equally displaying chain lengths of both low and high molecular weights.

Although structural differences in mucin oligosaccharides were not observed between LBL and CV animals, bacterial binding assays utilising whole ileal sections were employed to determine whether or not the differences in mucin composition between LBL and CV animals during early development may have deterred or enhanced binding of certain bacterial species. *Escherichia coli* and *Lactobacillus salivarius* were selected for the experiment. Binding of *L. salivarius* to ileal sections was very low whereas *E. coli* binding was greater, and more pronounced in LBL animals, especially at d 7 post-hatch. No statistically significant differences were observed in binding of *E. coli* to purified ileal mucin from LBL and CV animals at either d 4 or d 7 post-hatch. Correlations between *E. coli* and *L. salivarius* adherence to ileal tissue and mucin samples, and goblet cell parameters, were not statistically significant when fitted as co-variates. It was concluded

that the changes in mucin composition played a minor role in bacterial adhesion of *L. salivarius* and this *E. coli* serotype.

In summary, this thesis explores the physiological changes in goblet cell mucin production in response to bacterial exposure post-hatch. The thesis outlines the complexity of mucosal-bacterial interactions which would benefit from the employment of specialised techniques such as nuclear magnetic resonance spectroscopy and microarray technologies to examine a greater range of mucin structures and gene expression. This thesis provides support for future investigations into the influence of intestinal microflora on mucosal and mucin dynamics of poultry and the potential development of prebiotics for use in animal production.

Declaration

This thesis contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and to the best of my knowledge and belief contains no material previously published or written by another person except where due reference has been made in the text.

I give consent to this copy of my thesis being made available in the University of Adelaide Library.

Date

Rebecca Forder

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Abbreviations

°C	-	Degrees Celsius
AB	-	Alcian blue
ANOVA	-	Analysis of variance
BSA	-	Bovine serum albumin
CV	-	Conventionally reared
d	-	Day
DMSO	-	Dimethyl sulfoxide
DTT	-	Dithiotreitol
FITC	-	Fluorescein isothiocyanate
GF	-	Germ free
h	-	Hours
HCl	-	Hydrochloric acid
HID	-	High iron diamine
kDa	-	Kilodaltons
LBL	-	Low bacterial load
LPS	-	Lipopolysaccharide
min	-	Minutes
mL	-	Millilitres
MS	-	Mass spectrometry
MUC	-	Mucin core peptide gene
NFW	-	Nuclease free water
NMR	-	Nuclear magnetic resonance
OCT	-	Optimal cutting temperature
PAS	-	Periodic acid-schiff

PBS	-	Phosphate-buffered saline
PC2	-	Physical containment level 2
PLB	-	Protein loading buffer
PVC	-	Polyvinyl chloride
SDS-PAGE	-	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SHQ	-	Super high quality
SPF	-	Specific pathogen free
TGS	-	Tris-glycine-saline
TRITC	-	Tetramethylrhodamine B-isothiocyanate
WGA	-	Wheat germ agglutinin

Publications

Forder, R.E.A., Firth, G., Howarth, G.S., Tivey D.T. and Hughes, R.J. (2007) A small-scale, low-cost isolation system for the incubation and rearing of low bacterial load chicks as a model to study microbial-intestinal interactions. *Laboratory Animals*. In press

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